Authorization for Credit Card Transactions

Department of Homeland Security

Form G-1450

How To Fill Out Form G-1450

1. Type or print legibly in black ink.
2. Complete the "**Applicant's/Petitioner's/Requester's Information**," "**Credit Card Billing Information**," and "**Credit Card Information**" sections and sign the authorization. **NOTE:** The credit card must be issued by a U.S. bank.
3. Place your Form G-1450 ON TOP of your application, petition, or request package.

NOTE: Failure to provide the requested information may result in DHS and your financial institution not accepting the payment. DHS cannot process credit card payments without an authorized signature.

NOTE: Please see the USCIS Form G-1450 website for additional information.

We recommend that you print or save a copy of your completed Form G-1450 to review in the future and for your records.

By completing this transaction, you agree that you have paid for a government service and that the filing fee, biometric services fee and all related financial transactions are final and not refundable, regardless of any action DHS takes on an application, petition, or request. You must submit all fees in the exact amounts. DHS will charge your credit card up to the amount you authorize below.

Please refer to the form(s) you are filing for additional information, or you may call the USCIS Customer Contact number at **1-800-375-5283**. For TTY (deaf or hard of hearing) call: **1-800-767-1833**.

Applicant's/Petitioner's/Requester's Information (Full Legal Name)		
Given Name (First Name) Isabella Harumi	Middle Name (if any) N/A	Family Name (Last Name) YONEHARA NOMA

Credit Card Billing Information (Credit Card Holder's Name as it Appears on the Card)		
Given Name (First Name)	Middle Name (if any)	Family Name (Last Name)

Credit Card Holder's Billing Address:		
Street Number and Name	Apt. Ste. Flr. ☐ ☐ ☐	Number
City or Town	State	ZIP Code

Credit Card Holder's Signature and Contact Information:	
Credit Card Holder's Signature	
Credit Card Holder's Daytime Telephone Number	Credit Card Holder's Email Address

Credit Card Information		
Credit Card Number	**Credit Card Type:** ☐ Visa ☐ MasterCard ☐ American Express ☐ Discover	Authorized Payment Amount $ 715.00
Credit Card Expiration Date (mm/yyyy) CVV Code		

Authorization for Credit Card Transactions

Department of Homeland Security

Form G-1450

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Applicant's/Petitioner's/Requester's Information (Full Legal Name)		
Given Name (First Name) Isabella Harumi	Middle Name (if any) N/A	Family Name (Last Name) YONEHARA NOMA

Credit Card Billing Information (Credit Card Holder's Name as it Appears on the Card)		
Given Name (First Name)	Middle Name (if any)	Family Name (Last Name)

Credit Card Holder's Billing Address:		
Street Number and Name	Apt. Ste. Flr. ☐ ☐ ☐	Number
City or Town	State	ZIP Code

Credit Card Holder's Signature and Contact Information:	
Credit Card Holder's Signature	
Credit Card Holder's Daytime Telephone Number	Credit Card Holder's Email Address

Credit Card Information		
Credit Card Number	**Credit Card Type:** ☐ Visa ☐ MasterCard ☐ American Express ☐ Discover	Authorized Payment Amount $ 300.00
Credit Card Expiration Date (mm/yyyy) CVV Code		

YOUSA
Law Firm

EB-1A EXTRAORDINARY ABILITY IMMIGRANT PETITION

Petitioner: HARUMI YONEHARA NOMA, Isabella

Field: Biomedical Sciences (Cancer Research and Molecular Biology)

HS Law Corp
P.O Box 90487
San Diego - CA - United States

e-Notification of Application/Petition Acceptance

Department of Homeland Security
U.S. Citizenship and Immigration Services

USCIS
Form G-1145

What Is the Purpose of This Form?

Use this form to request an electronic notification (e-Notification) when U.S. Citizenship and Immigration Services accepts your immigration application. This service is available for applications filed at a USCIS Lockbox facility.

General Information

Complete the information below and clip this form to the first page of your application package. You will receive one e-mail and/or text message for each form you are filing.

We will send the e-Notification within 24 hours after we accept your application. Domestic customers will receive an e-mail and/or text message; overseas customers will only receive an e-mail. Undeliverable e-Notifications cannot be resent.

The e-mail or text message will display your receipt number and tell you how to get updated case status information. It will not include any personal information. The e-Notification does not grant any type of status or benefit; rather it is provided as a convenience to customers.

USCIS will also mail you a receipt notice (I-797C), which you will receive within 10 days after your application has been accepted; use this notice as proof of your pending application or petition.

USCIS Privacy Act Statement

AUTHORITIES: The information requested on this form is collected pursuant to section 103(a) of the Immigration and Nationality Act, as amended INA section 101, et seq.

PURPOSE: The primary purpose for providing the information on this form is to request an electronic notification when USCIS accepts immigration form. The information you provide will be used to send you a text and/or email message.

DISCLOSURE: The information you provide is voluntary. However, failure to provide the requested information may prevent USCIS from providing you a text and/or email message receipting your immigration form.

ROUTINE USES: The information provided on this form will be used by and disclosed to DHS personnel and contractors in accordance with approved routine uses, as described in the associated published system of records notices [**DHS/USCIS-007 - Benefits Information System and DHS/USCIS-001 - Alien File (A-File) and Central Index System (CIS)**, which can be found at **www.dhs.gov/privacy**]. The information may also be made available, as appropriate for law enforcement purposes or in the interest of national security.

Complete this form and clip it on top of the first page of your immigration form(s).

Applicant/Petitioner Full Last Name	Applicant/Petitioner Full First Name	Applicant/Petitioner Full Middle Name
YONEHARA NOMA	**Isabella Harumi**	**N/A**

Email Address	Mobile Phone Number (Text Message)
bellanoma@hotmail.com	

Notice of Entry of Appearance as Attorney or Accredited Representative

Department of Homeland Security

DHS Form G-28
OMB No. 1615-0105
Expires 05/31/2021

Part 1. Information About Attorney or Accredited Representative

1. USCIS Online Account Number (if any)

▶ 0 0 7 4 9 2 6 2 5 4 3 8

Name of Attorney or Accredited Representative

2.a. Family Name (Last Name): HAVERROTH SILVA

2.b. Given Name (First Name): Otavio

2.c. Middle Name: N/A

Address of Attorney or Accredited Representative

3.a. Street Number and Name: PO Box 90487

3.b. ☐ Apt. ☐ Ste. ☐ Flr. N/A

3.c. City or Town: San Diego

3.d. State: CA **3.e.** ZIP Code: 92169

3.f. Province: N/A

3.g. Postal Code: N/A

3.h. Country: USA

Contact Information of Attorney or Accredited Representative

4. Daytime Telephone Number: 5102419336

5. Mobile Telephone Number (if any): 5102419336

6. Email Address (if any): otavio@legalhs.com

7. Fax Number (if any): N/A

Part 2. Eligibility Information for Attorney or Accredited Representative

Select **all applicable** items.

1.a. ☒ I am an attorney eligible to practice law in, and a member in good standing of, the bar of the highest courts of the following states, possessions, territories, commonwealths, or the District of Columbia. If you need extra space to complete this section, use the space provided in **Part 6. Additional Information**.

Licensing Authority: California

1.b. Bar Number (if applicable): 343486

1.c. I (select **only one** box) ☒ am not ☐ am subject to any order suspending, enjoining, restraining, disbarring, or otherwise restricting me in the practice of law. If you are subject to any orders, use the space provided in **Part 6. Additional Information** to provide an explanation.

1.d. Name of Law Firm or Organization (if applicable): HS Law Corp

2.a. ☐ I am an accredited representative of the following qualified nonprofit religious, charitable, social service, or similar organization established in the United States and recognized by the Department of Justice in accordance with 8 CFR part 1292.

2.b. Name of Recognized Organization: N/A

2.c. Date of Accreditation (mm/dd/yyyy): N/A

3. ☐ I am associated with N/A, the attorney or accredited representative of record who previously filed Form G-28 in this case, and my appearance as an attorney or accredited representative for a limited purpose is at his or her request.

4.a. ☐ I am a law student or law graduate working under the direct supervision of the attorney or accredited representative of record on this form in accordance with the requirements in 8 CFR 292.1(a)(2).

4.b. Name of Law Student or Law Graduate: N/A

Part 3. Notice of Appearance as Attorney or Accredited Representative

If you need extra space to complete this section, use the space provided in **Part 6. Additional Information**.

This appearance relates to immigration matters before (select **only one** box):

1.a. ☒ U.S. Citizenship and Immigration Services (USCIS)

1.b. List the form numbers or specific matter in which appearance is entered.

I-140

2.a. ☐ U.S. Immigration and Customs Enforcement (ICE)

2.b. List the specific matter in which appearance is entered.

N/A

3.a. ☐ U.S. Customs and Border Protection (CBP)

3.b. List the specific matter in which appearance is entered.

N/A

4. Receipt Number (if any)

► N/A

5. I enter my appearance as an attorney or accredited representative at the request of the (select **only one** box):

☐ Applicant ☒ Petitioner ☐ Requestor

☐ Beneficiary/Derivative ☐ Respondent (ICE, CBP)

Information About Client (Applicant, Petitioner, Requestor, Beneficiary or Derivative, Respondent, or Authorized Signatory for an Entity)

6.a. Family Name (Last Name) YONEHARA NOMA

6.b. Given Name (First Name) Isabella Harumi

6.c. Middle Name N/A

7.a. Name of Entity (if applicable)

N/A

7.b. Title of Authorized Signatory for Entity (if applicable)

N/A

8. Client's USCIS Online Account Number (if any)

► N/A

9. Client's Alien Registration Number (A-Number) (if any)

► A- N/A

Client's Contact Information

10. Daytime Telephone Number

+1 (656) 200-2819

11. Mobile Telephone Number (if any)

+1 (656) 200-2819

12. Email Address (if any)

bellanoma@hotmail.com

Mailing Address of Client

NOTE: Provide the client's mailing address. **Do not** provide the business mailing address of the attorney or accredited representative **unless** it serves as the safe mailing address on the application or petition being filed with this Form G-28.

13.a. Street Number and Name PO Box 90487

13.b. ☐ Apt. ☐ Ste. ☐ Flr. N/A

13.c. City or Town San Diego

13.d. State CA **13.e.** ZIP Code 92169

13.f. Province N/A

13.g. Postal Code N/A

13.h. Country

USA

Part 4. Client's Consent to Representation and Signature

Consent to Representation and Release of Information

I have requested the representation of and consented to being represented by the attorney or accredited representative named in **Part 1.** of this form. According to the Privacy Act of 1974 and U.S. Department of Homeland Security (DHS) policy, I also consent to the disclosure to the named attorney or accredited representative of any records pertaining to me that appear in any system of records of USCIS, ICE, or CBP.

Part 4. Client's Consent to Representation and Signature (continued)

Options Regarding Receipt of USCIS Notices and Documents

USCIS will send notices to both a represented party (the client) and his, her, or its attorney or accredited representative either through mail or electronic delivery. USCIS will send all secure identity documents and Travel Documents to the client's U.S. mailing address.

If you want to have notices and/or secure identity documents sent to your attorney or accredited representative of record rather than to you, please select **all applicable** items below. You may change these elections through written notice to USCIS.

1.a. ☒ I request that USCIS send original notices on an application or petition to the business address of my attorney or accredited representative as listed in this form.

1.b. ☒ I request that USCIS send any secure identity document (Permanent Resident Card, Employment Authorization Document, or Travel Document) that I receive to the U.S. business address of my attorney or accredited representative (or to a designated military or diplomatic address in a foreign country (if permitted)).

NOTE: If your notice contains Form I-94, Arrival-Departure Record, USCIS will send the notice to the U.S. business address of your attorney or accredited representative. If you would rather have your Form I-94 sent directly to you, select **Item Number 1.c.**

1.c. ☐ I request that USCIS send my notice containing Form I-94 to me at my U.S. mailing address.

Signature of Client or Authorized Signatory for an Entity

2.a. Signature of Client or Authorized Signatory for an Entity

➡ Isabella M.Y. Nona

2.b. Date of Signature (mm/dd/yyyy) 03/18/2026

Part 5. Signature of Attorney or Accredited Representative

I have read and understand the regulations and conditions contained in 8 CFR 103.2 and 292 governing appearances and representation before DHS. I declare under penalty of perjury under the laws of the United States that the information I have provided on this form is true and correct.

1. a. Signature of Attorney or Accredited Representative

1.b. Date of Signature (mm/dd/yyyy) 03/18/2026

2.a. Signature of Law Student or Law Graduate

N/A

2.b. Date of Signature (mm/dd/yyyy) N/A

Scanned with CamScanner

Part 6. Additional Information

If you need extra space to provide any additional information within this form, use the space below. If you need more space than what is provided, you may make copies of this page to complete and file with this form or attach a separate sheet of paper. Type or print your name at the top of each sheet; indicate the **Page Number**, **Part Number**, and **Item Number** to which your answer refers; and sign and date each sheet.

1.a Family Name (Last Name): YONEHARA NOMA

1.b. Given Name (First Name): Isabella Harumi

1.c. Middle Name: N/A

2.a. Page Number: N/A **2.b.** Part Number: N/A **2.c.** Item Number: N/A

2.d.

N/A

3.a. Page Number: N/A **3.b.** Part Number: N/A **3.c.** Item Number: N/A

3.d.

N/A

4.a. Page Number: N/A **4.b.** Part Number: N/A **4.c.** Item Number: N/A

4.d.

N/A

5.a. Page Number: N/A **5.b.** Part Number: N/A **5.c.** Item Number: N/A

5.d.

N/A

6.a. Page Number: N/A **6.b.** Part Number: N/A **6.c.** Item Number: N/A

6.d.

N/A

Immigrant Petition for Alien Workers

Department of Homeland Security
U.S. Citizenship and Immigration Services

USCIS Form I-140
OMB No. 1615-0015
Expires 02/28/2027

For USCIS Use Only	Fee Stamp	Priority Date	Consulate	Action Block

Classification

- ☐ 203(b)(1)(A) Alien of Extraordinary Ability
- ☐ 203(b)(1)(B) Outstanding Professor or Researcher
- ☐ 203(b)(1)(C) Multinational Executive or Manager
- ☐ 203(b)(2) Member of Professions with Advanced Degree/Exceptional Ability
- ☐ 203(b)(3)(A)(i) Skilled Worker
- ☐ 203(b)(3)(A)(ii) Professional
- ☐ 203(b)(3)(A)(iii) Other Worker

Certification

- ☐ National Interest Waiver (NIW)
- ☐ Schedule A, Group I
- ☐ Schedule A, Group II

Remarks

To be completed by an Attorney or Accredited Representative (if any).	☒ Select this box if Form G-28 or Form G-28I is attached.	Attorney State Bar Number (if applicable)	Attorney or Accredited Representative USCIS Online Account Number (if any)
		343486	0 0 7 4 9 2 6 2 5 4 3 8

► **START HERE - Type or print in black ink.**

Part 1. Information About the Person or Organization Filing This Petition

If an individual is filing this petition, answer **Item Numbers 1.a. - 1.c.** If a company or organization is filing this petition, answer **Item Number 2.**

1.a. Family Name (Last Name): YONEHARA NOMA

1.b. Given Name (First Name): Isabella Harumi

1.c. Middle Name: N/A

2. Company or Organization Name: N/A

Mailing Address

3.a. In Care Of Name: Otavio HAVERROTH SILVA

3.b. Street Number and Name: PO Box 90487

3.c. ☐ Apt. ☐ Ste. ☐ Flr. N/A

3.d. City or Town: San Diego

3.e. State: CA **3.f.** ZIP Code: 92169

3.g. Province: N/A

3.h. Postal Code: N/A

3.i. Country: USA

Other Information

4. IRS Employer Identification Number (EIN) ► N/A

5. Are you a nonprofit organized as tax exempt or a governmental research organization? ☐ Yes ☒ No

6. Do you currently employ a total of 25 or fewer full-time equivalent employees in the United States, including all affiliates or subsidiaries of this company/organization? ☒ Yes ☐ No

7. U.S. Social Security Number (SSN) (if any) ► N/A

8. USCIS Online Account Number (if any) ► N/A

Part 2. Petition Type

This petition is being filed for (select **only one** box):

1.a. ☒ An alien of extraordinary ability.

1.b. ☐ An outstanding professor or researcher.

1.c. ☐ A multinational executive or manager.

1.d. ☐ A member of the professions holding an advanced degree or an alien of exceptional ability (who is **NOT** seeking a National Interest Waiver (NIW)).

1.e. ☐ A professional (at a minimum, possessing a bachelor's degree or a foreign degree equivalent to a U.S. bachelor's degree).

Part 2. Petition Type (continued)

1.f. ☐ A skilled worker (requiring at least two years of specialized training or experience).

1.g. ☐ Any other worker (requiring less than two years of training or experience).

1.h. ☐ An alien applying for an NIW (who **IS** a member of the professions holding an advanced degree or an alien of exceptional ability).

This petition is being filed (select **only one** box):

2.a. ☐ To amend a previously filed petition.

Previous Petition Receipt Number

► N/A

2.b. ☐ For the Schedule A, Group I or II designation.

Part 3. Information About the Person for Whom You Are Filing

1.a. Family Name (Last Name): YONEHARA NOMA

1.b. Given Name (First Name): Isabella Harumi

1.c. Middle Name: N/A

Mailing Address

2.a. In Care Of Name: Otavio HAVERROTH SILVA

2.b. Street Number and Name: PO Box 90487

2.c. ☐ Apt. ☐ Ste. ☐ Flr. N/A

2.d. City or Town: San Diego

2.e. State: CA **2.f.** ZIP Code: 92169

2.g. Province: N/A

2.h. Postal Code: N/A

2.i. Country: USA

Other Information

3. Date of Birth (mm/dd/yyyy): 09/03/1996

4. City/Town/Village of Birth: Maringa

5. State or Province of Birth: Parana

6. Country of Birth: Brazil

7. Country of Citizenship or Nationality: Brazil

8. Alien Registration Number (A-Number) (if any)

► A- N/A

9. U.S. SSN (if any) ► N/A

Information About His or Her Last Arrival in the United States

If the person for whom you are filing is in the United States, provide the following information.

10. Date of Last Arrival (mm/dd/yyyy): 05/03/2025

11.a. Form I-94 Arrival-Departure Record Number

► 5 1 7 9 1 5 6 9 6 A 4

11.b. Expiration Date of Authorized Stay Shown on Form I-94 (mm/dd/yyyy): 01/10/2028

11.c. Status on Form I-94 (for example, class of admission, or paroled, if paroled): O1

12. Passport Number: GF001610

13. Travel Document Number: GF001610

14. Country of Issuance for Passport or Travel Document: Brazil

15. Expiration Date for Passport or Travel Document (mm/dd/yyyy): 08/17/2032

Part 4. Processing Information

Provide the following information for the person named in **Part 3.** (select **only one** box):

1.a. ☒ Alien will apply for a visa abroad at a U.S. Embassy or U.S. Consulate at:

1.b. City or Town: Rio de Janeiro

1.c. Country: Brazil

2.a. ☐ Alien is in the United States and will apply for adjustment of status to that of lawful permanent resident.

Part 4. Processing Information (continued)

2.b. Alien's current country of residence or, if now in the United States, last country of permanent residence abroad.

If you provided a United States address in **Part 3.**, provide the person's foreign address in **Item Numbers 3.a. - 3.f.**:

3.a. Street Number and Name: 155 Prof Itamar Orlando Soares

3.b. ☒ Apt. ☐ Ste. ☐ Flr. 102

3.c. City or Town: Maringa

3.d. Province: Parana

3.e. Postal Code: 87020270

3.f. Country: Brazil

If the person's native alphabet is other than Roman letters, type or print the person's foreign name and address in the native alphabet in **Item Numbers 4.a. - 4.c.**:

4.a. Family Name (Last Name): N/A

4.b. Given Name (First Name): N/A

4.c. Middle Name: N/A

Mailing Address

5.a. In Care Of Name: N/A

5.b. Street Number and Name: N/A

5.c. ☐ Apt. ☐ Ste. ☐ Flr. N/A

5.d. City or Town: N/A

5.e. Province: N/A

5.f. Postal Code: N/A

5.g. Country: N/A

If you answer "Yes" to **Item Numbers 6.a. - 10.**, provide the case number, office location, date of decision, and disposition of the decision in the space provided in **Part 11. Additional Information**.

6.a. Are you filing any other petitions or applications with this Form I-140? ☐ Yes ☒ No

6.b. If you answered "Yes" **to Item Number 6.a.**, select all applicable boxes:

- ☐ Form I-485
- ☐ Form I-131
- ☐ Form I-765
- ☐ Other (Provide an explanation in **Part 11. Additional Information**.)

7. Is the person for whom you are filing in removal proceedings? ☐ Yes ☒ No

8. Has any immigrant visa petition ever been filed by or on behalf of this person? ☐ Yes ☒ No

9. Are you filing this petition without an original labor certification because the original labor certification was previously submitted in support of another Form I-140? ☐ Yes ☒ No

10. If you are filing this petition without an original labor certification, are you requesting that U.S. Citizenship and Immigration Services (USCIS) request a duplicate labor certification from the Department of Labor (DOL)? ☐ Yes ☒ No

Part 5. Additional Information About the Petitioner

Type of petitioner (select **only one** box):

1.a. ☐ Employer

1.b. ☒ Self

1.c. ☐ Other (For example, Lawful Permanent Resident, U.S. citizen or any other person filing on behalf of the alien)

N/A

If a company or an organization is filing this petition, provide the following information:

2. Type of Business: N/A

3. Date Established (mm/dd/yyyy): N/A

4. Current Number of U.S. Employees: N/A

5. Gross Annual Income $ N/A

6. Net Annual Income $ N/A

7. NAICS Code ► N/A

8. Labor Certification DOL Case Number: N/A

Part 5. Additional Information About the Petitioner (continued)

9. Labor Certification DOL Filing Date (mm/dd/yyyy)

N/A

10. Labor Certification Expiration Date (mm/dd/yyyy)

N/A

If an individual is filing this petition, provide the following information.

11. Occupation

Biomedical Scientist

12. Annual Income $ N/A

Part 6. Basic Information About the Proposed Employment

1. Job Title

Medical Scientists, Except Epidemiologists

2. SOC Code ▶ 19-1042

3. Nontechnical Job Description

See page 8.

4. Is this a full-time position? ☒ Yes ☐ No

5. If the answer to **Item Number 4.** is "No," how many hours per week for the position?

N/A

6. Is this a permanent position? ☒ Yes ☐ No

7. Is this a new position? ☐ Yes ☒ No

8. Wages (Specify hour, week, month, or year):

$ N/A per N/A

Worksite Location

For **Item Numbers 9.a. - 9.e.**, provide the address where the person will work if different from the address provided in **Part 1.**

9.a. Street Number and Name: To be determined

9.b. ☐ Apt. ☐ Ste. ☐ Flr. To be determined

9.c. City or Town: Tampa

9.d. State: FL **9.e.** ZIP Code: To be determined

Part 7. Information About the Spouse and All Children of the Person for Whom You Are Filing

For **Part 7.**, provide information on the spouse and all children related to the individual for whom you are filing this petition. Also, note if the individual will apply for a visa abroad or adjustment of status as the dependent of the individual for whom the petition is filed. If you need extra space to provide information about additional family members, use the space provided in **Part 11. Additional Information**.

Person 1

1.a. Family Name (Last Name): CAETANO DA SILVA

1.b. Given Name (First Name): Pedro Henrique

1.c. Middle Name: N/A

2. Date of Birth (mm/dd/yyyy): 02/28/1996

3. Country of Birth: Brazil

4. Relationship: Spouse

5. Is he or she applying for adjustment of status? ☐ Yes ☒ No

6. Is he or she applying for a visa abroad? ☒ Yes ☐ No

Person 2

7.a. Family Name (Last Name): N/A

7.b. Given Name (First Name): N/A

7.c. Middle Name: N/A

8. Date of Birth (mm/dd/yyyy): N/A

9. Country of Birth: N/A

10. Relationship: N/A

11. Is he or she applying for adjustment of status? ☐ Yes ☐ No

12. Is he or she applying for a visa abroad? ☐ Yes ☐ No

Part 7. Information About Spouse and All Children of the Person for Whom You Are Filing (continued)

Person 3

13.a. Family Name (Last Name) N/A

13.b. Given Name (First Name) N/A

13.c. Middle Name N/A

14. Date of Birth (mm/dd/yyyy) N/A

15. Country of Birth
N/A

16. Relationship N/A

17. Is he or she applying for adjustment of status? ☐ Yes ☐ No

18. Is he or she applying for a visa abroad? ☐ Yes ☐ No

Person 4

19.a. Family Name (Last Name) N/A

19.b. Given Name (First Name) N/A

19.c. Middle Name N/A

20. Date of Birth (mm/dd/yyyy) N/A

21. Country of Birth
N/A

22. Relationship N/A

23. Is he or she applying for adjustment of status? ☐ Yes ☐ No

24. Is he or she applying for a visa abroad? ☐ Yes ☐ No

Person 5

25.a. Family Name (Last Name) N/A

25.b. Given Name (First Name) N/A

25.c. Middle Name N/A

26. Date of Birth (mm/dd/yyyy) N/A

27. Country of Birth
N/A

28. Relationship N/A

29. Is he or she applying for adjustment of status? ☐ Yes ☐ No

30. Is he or she applying for a visa abroad? ☐ Yes ☐ No

Person 6

31.a. Family Name (Last Name) N/A

31.b. Given Name (First Name) N/A

31.c. Middle Name N/A

32. Date of Birth (mm/dd/yyyy) N/A

33. Country of Birth
N/A

34. Relationship N/A

35. Is he or she applying for adjustment of status? ☐ Yes ☐ No

36. Is he or she applying for a visa abroad? ☐ Yes ☐ No

Part 8. Contact Information, Certification, and Signature of the Petitioner or Authorized Signatory

Petitioner or Authorized Signatory's Contact Information

1.a. Petitioner's or Authorized Signatory's Family Name (Last Name)
YONEHARA NOMA

1.b. Petitioner's or Authorized Signatory's Given Name (First Name)
Isabella Harumi

2. Petitioner's or Authorized Signatory's Title
N/A

3. Petitioner's or Authorized Signatory's Daytime Telephone Number
+1 (656) 200-2819

4. Petitioner's or Authorized Signatory's Mobile Telephone Number (if any)
+1 (656) 200-2819

5. Petitioner's or Authorized Signatory's Email Address (if any)
bellanoma@hotmail.com

Petitioner's or Authorized Signatory's Certification and Signature

If filing this petition on behalf of an organization, I certify that I am authorized to do so by the organization:

a. I reviewed and provided or authorized all of the responses and information in my petition;

b. I understood all of the responses and information contained in, and submitted with, my petition; and

c. All of the responses and information were complete, true, and correct at the time of filing

Furthermore, I authorize the release of any information from any and all of my records as authorized signatory and the petitioner's records that USCIS may need to determine the petitioner's eligibility for an immigration request and to other entities and persons where necessary for the administration and enforcement of U.S. immigration law.

6.a. Petitioner's or Authorized Signatory's Signature
Isabella H.Y. Noma

6.b. Date of Signature (mm/dd/yyyy) 03/18/2026

Part 9. Interpreter's Contact Information, Certification, and Signature

Interpreter's Full Name

1.a. Interpreter's Family Name (Last Name)
N/A

1.b. Interpreter's Given Name (First Name)
N/A

2. Interpreter's Business or Organization Name
N/A

Interpreter's Contact Information

3. Interpreter's Daytime Telephone Number
N/A

4. Interpreter's Mobile Telephone Number (if any)
N/A

5. Interpreter's Email Address (if any)
N/A

Interpreter's Certification and Signature

I certify, under penalty of perjury, that I am fluent in English and N/A, and I have interpreted every question on the petition and Instructions and interpreted the petitioner's or authorized signatory's answers to the questions in that language, and the petitioner or authorized signatory informed me that they understood every instruction, question, and answer on the petition.

6.a. Interpreter's Signature
N/A

6.b. Date of Signature (mm/dd/yyyy) N/A

Scanned with

Part 10. Contact Information, Certification, and Signature of the Person Preparing this Petition, if Other Than the Petitioner or Authorized Signatory

Preparer's Full Name

1. Preparer's Family Name (Last Name)

HAVERROTH SILVA

Preparer's Given Name (First Name)

Otavio

2. Preparer's Business or Organization Name

HS Law Corp

Preparer's Contact Information

3. Preparer's Daytime Telephone Number

5102419336

4. Preparer's Mobile Telephone Number (if any)

5102419336

5. Preparer's Email Address (if any)

otavio@legalhs.com

Preparer's Certification and Signature

I certify, under penalty of perjury, that I prepared this petition for the petitioner or authorized signatory at their request and with express consent and that all of the responses and information contained in and submitted with the petition are complete, true, and correct and reflects only information provided by the petitioner or authorized signatory. The petitioner or authorized signatory reviewed the responses and information and informed me that they understand the responses and information in or submitted with the petition.

6. Preparer's Signature

Date of Signature (mm/dd/yyyy) 03/18/2026

Part 11. Additional Information

If you need extra space to provide any additional information within this petition, use the space below. If you need more space than what is provided, you may make copies of this page to complete and file with this petition or attach a separate sheet of paper. Type or print your name and A-Number (if any) at the top of each sheet; indicate the **Page Number**, **Part Number**, and **Item Number** to which your answer refers; and sign and date each sheet.

1. Family Name (Last Name): YONEHARA NOMA

Given Name (First Name): Isabella Harumi

Middle Name: N/A

2. IRS EIN ► N/A

3.

Page Number	Part Number	Item Number
4	6	3

Conduct research dealing with the understanding of human diseases and the improvement of human health. Engage in clinical investigation, research and development, or other related activities.

4.

Page Number	Part Number	Item Number
N/A	N/A	N/A

N/A

5.

Page Number	Part Number	Item Number
N/A	N/A	N/A

N/A

6.

Page Number	Part Number	Item Number
N/A	N/A	N/A

N/A

7.

Page Number	Part Number	Item Number
N/A	N/A	N/A

N/A

March 5, 2026

Via USPS
USCIS
Attn: I-140
P.O. Box 660128
Dallas, TX 75266-0128

Re: EB-1 FIRST PREFERENCE: EMPLOYMENT-BASED IMMIGRATION PETITION

Petitioner:	**HARUMI YONEHARA NOMA, Isabella**
Nature of submission:	**ORIGINAL SUBMISSION**
Type of Petition:	**I-140, EB-1 Employment-based, first preference visa based on Petitioner's extraordinary ability.**
Classification Sought:	**Immigration and Nationality Act 203(b)(1)(A)**

Dear USCIS Officer:

Our firm represents self-petitioner/beneficiary Dr. Isabella Harumi Yonehara Noma (hereinafter Dr. Noma), a foreign national of extraordinary ability. Please find enclosed the forms and supporting documents for her application:

- Form G-1450, Authorization for Credit Card Transaction of $715.00 towards I-140 filing fee.
- Form G-1450, Authorization for Credit Card Transaction of $300 towards Asylum Program fee.
- Form G-1145, E-Notification of Application/Petition Acceptance
- Form G-28, Notice of Entry of Appearance as Attorney or Representative.
- Form I-140, Immigrant Petition for Alien Worker.
- Supporting documents for Petitioner.

This letter is respectfully submitted in support of Dr. Noma immigrant petition as a worker of extraordinary ability in **Science** under INA 203(b)(1)(A). This submission demonstrates that Dr. Noma satisfies the statutory and regulatory criteria for classification as an individual of extraordinary ability. The governing regulation, 8 CFR § 204.5(h), defines "extraordinary ability" as follows:

> **(a)** Extraordinary ability means a level of expertise indicating that the individual is one of that small percentage who have risen to the very top of the field of endeavor.
>
> **(b)** Initial evidence. A petition for an alien of extraordinary ability must be accompanied by evidence that the alien has sustained national or international acclaim and that his or her

achievements have been recognized in the field of expertise. Such evidence shall include evidence of a one-time achievement (that is, a major, internationally recognized award), or **at least three** of the following (8 CFR 204.5(h)(3)):

i. *Documentation of the alien's receipt of lesser nationally or internationally recognized prizes or awards for excellence in the field of endeavor;*
ii. *Documentation of the alien's membership in associations in the field of endeavor, which require outstanding achievements of their members, as, judged by recognized national or international experts in their fields;*
iii. *Published material in professional or major trade publications or major media about the alien and relating to the alien's work in the field of endeavor;*
iv. *Evidence of the alien's participation, either individually or on a panel, as a judge of the work of others in the same or an allied field of specification for which classification is sought;*
v. *Evidence of the alien's original scientific, scholarly, or business contributions of major significance in the field of endeavor;*
vi. *Evidence of alien's authorship of scholarly articles in the field, in professional journals or other major media;*
vii. *Evidence of the display of the alien's work at artistic exhibitions or showcase;*
viii. *Evidence that the alien has performed in a leading or critical role for organizations or establishments that have a distinguished reputation;*
ix. *Evidence that the alien has commanded a high salary or other significantly high remuneration for services, in relation to others in the field; or*
x. *Evidence of commercial successes in the performing arts, as shown by box office receipts or record, cassette, compact disk, or video sales.*

(c) If the above standards do not readily apply to the beneficiary's occupation, the petitioner may submit comparable evidence to establish the beneficiary's eligibility.

The petition is to obtain the immigrant classification on behalf of Dr. Noma as a worker of extraordinary ability in Science. Dr. Noma has attained a level of expertise indicating she is one of that small percentage of individuals who have risen to the very top of her field of endeavor and that she has sustainable national and international acclaim in her field. This is supported by substantial and credible evidence, including: **(i)** receipt of lesser nationally or internationally recognized prizes or awards for excellence; **(iv)** evidence that she has been asked to judge the work of others; **(v)** original scientific and scholarly contributions of major significance in the field; **(vi)** authorship of scholarly articles in the field, in professional journals or other major media; **(vii)** display of the her work at scientific exhibitions; **(viii)** evidence that she has performed in a leading or critical role for organizations or establishments that have a distinguished reputation.

Dr. Noma will show through objective and credible documentation that, by applying the preponderance of evidence standard ("more likely than not" or "50%+), she meets **at least three** of the regulatory criteria, and when considering the petition in its entirety for the final merits' determination, she demonstrates the high level of expertise required for this classification. (*Kazarian v. USCIS*, 596 F.3d 1115 (9th Cir. 2010)).

Moreover, Dr. Noma seeks to remain in the United States to continue working in the area of extraordinary ability and her work will substantially benefit the United States in the future, as it will be further demonstrated herein.

This letter serves as a guide for all submitted evidence. Please find enclosed the referred documentation.

A Brief Note about Dr. Noma and her Qualification as an Alien of Extraordinary Ability

Dr. Noma is a distinguished scientist in the field of Tumor Biology and Molecular Oncology, whose groundbreaking research has significantly advanced the global understanding of melanoma initiation, progression, and therapeutic resistance. Currently serving as a Postdoctoral Fellow at the H. Lee Moffitt Cancer Center and Research Institute, one of the premier cancer research institutions in the United States, Dr. Noma has consistently demonstrated the extraordinary ability required for EB-1A classification.

Dr. Noma's expertise is built upon a rigorous and progressive academic foundation. She earned her Bachelor's degree in Biomedicine from the State University of Maringá (UEM) in 2017, where her early research involved critical studies on antimicrobial resistance in Gram-negative bacilli and the relationship between nociception and nitric oxide in *Leishmania* infections.

In 2020, she earned her Master's degree in Health Sciences from UEM, focusing on infectious and parasitic illnesses as a CAPES scholarship holder. During this period, she also expanded her technical versatility by completing a Specialization in Biomedical Engineering with an emphasis on Clinical Engineering from Universidade Estácio de Sá.

She reached the pinnacle of her formal scientific training in 2023, earning her Ph.D. in Pharmacy (Pathophysiology and Toxicology) from the prestigious University of São Paulo (USP) as a CNPq scholarship holder. Demonstrating a commitment to the future of precision medicine, she has further enhanced her analytical capabilities through an MBA in Data Science and Analytics from USP ESALQ (2025).

At Moffitt Cancer Center, under the mentorship of world-renowned expert Dr. Keiran Smalley, Dr. Noma led a critical project investigating the role of HDAC8 in UVR-driven melanoma initiation. Her work utilized unique mouse models and siRNA-mediated overexpression to demonstrate that HDAC8 activation allows UV-damaged melanocytes to evade apoptosis by downregulating p53, providing a novel perspective on how sun-damaged cells survive to become malignant.

Parallel to this, Dr. Noma has served as the Coordinator for research investigating the metastatic potential of Peroxiredoxin 2 (PRDX2) in NRAS-mutated melanomas. Her work explores how

PRDX2 modulates phenotypic plasticity and invasive behavior, utilizing advanced 3D human skin models and gene editing (shRNA) to identify PRDX2 as a potential therapeutic target for aggressive, treatment-resistant melanoma.

These contributions are documented in numerous peer-reviewed articles in top-tier journals such as *Antioxidants*, *Frontiers in Nutrition*, and *Biomedicine & Pharmacotherapy*. Her work has garnered significant citations from researchers worldwide, reflecting the foundational importance of her research to the field of oncology and its impact on the global scientific community.

Dr. Noma's standing at the top of her field is further evidenced by her receipt of prestigious awards and fellowships and her invitations to present findings at major international forums, such as the Society for Melanoma Research (SMR) Congresses in Scotland and Philadelphia. In recognition of her expertise, she is frequently sought after by the scientific community to judge the work of others, having served as a manuscript reviewer for respected journals.

Dr. Noma embodies the qualities of an individual of extraordinary ability, as her innovative methodologies and dedication to developing more effective cancer therapies position her as a vital asset to the United States' scientific and healthcare infrastructure. Her exceptional standing is further validated by her currently approved O-1A nonimmigrant petition, a classification reserved for individuals who have risen to the very top of their field. Through this immigrant petition, Dr. Noma seeks to continue her essential work to improve survival rates for cancer patients across the nation.

Dr. Noma will continue working and contributing to the area of Extraordinary Ability

Dr. Noma intends to sustain her decade-long commitment to professional excellence through a multifaceted career in the United States. Her professional plan involves the continued execution of high-level research across molecular biology, cell biology, genetics, and epigenetics. Specifically, she will continue to design and conduct complex *in vitro* and *in vivo* experiments, apply advanced statistical analyses to biological datasets, and resolve intricate scientific problems within the laboratory environment.

Beyond bench research, Dr. Noma will remain an active participant in the academic ecosystem by elaborating original scientific articles and presenting her findings at national and international congresses. Drawing on her extensive experience in scientific communication, she intends to serve as a speaker in academic cycles and continue her role in the organization of scientific symposia and meetings.

Furthermore, Dr. Noma is committed to the pedagogical and collaborative aspects of her field. She intends to foster international research partnerships that bridge U.S. institutions with global networks and provide mentorship to the next generation of scientists. By sharing her expertise in scientific writing and experimental design, she will support the training of future professionals, ensuring a continuous exchange of specialized knowledge within her area of extraordinary ability.

Beneficiary's will Substantially Benefit the United States in the future

The presence of Dr. Noma in the United States provides a prospective benefit to the nation by directly addressing the critical public health challenge of melanoma and other skin cancers. Her work provides the scientific foundation necessary to improve national efforts in cancer prevention and early detection, which are essential for reducing mortality rates among the American population. By introducing innovative insights into how these diseases are treated, her contributions support the development of more effective healthcare strategies and life-saving interventions.

The United States further benefits from Dr. Noma's unique ability to integrate traditional biomedical research with modern data analytics. As the U.S. healthcare sector increasingly relies on "Big Data" to drive clinical decisions, her expertise in data science and analytics will enhance the precision and technological sophistication of American cancer research. This integration facilitates the refinement of existing diagnostic tools and the creation of more robust scientific methodologies.

Finally, Dr. Noma's work strengthens the United States' position as a global leader in biotechnological innovation. Her commitment to fostering international ties and her prolific academic output enhance the prestige and reach of U.S. research institutions. By strengthening professional and academic bonds between the U.S. and the international community, she ensures that the American scientific infrastructure remains at the forefront of global advancements in oncology and pharmaceutical sciences.

Objective evidence on how Dr. Noma proposes to continue working and is set to substantially contribute to the field of Science in the United States, we are submitting the following documents on **Exhibit A**.

A. Evidence on Dr. Noma

A.1. Beneficiary's Personal Statement

A.2. Dr. Noma's Curriculum Vitae

A.3. Bachelor's Degree in Biomedical Sciences

A.4. Postgraduate Certificate in Biomedical Engineering

A.5. Master's Degree in Health Sciences

A.6. Doctorate in Sciences

A.7. Postdoctoral Fellowship

A.8. MBA in Data Science and Analytics

Dr. Noma has earned sustained national and international acclaim related to her work, and her achievements have been recognized in her field of expertise, demonstrating that she is **one of that small percentage who have risen to the very top of the field of endeavor** (8 CFR 204.5(h)(22) as it will be evidenced throughout this petition along with supporting documentation.

Accordingly, as per the first step examination whether Petitioner meets the Extraordinary Ability Classification, Officer should determine that, **by preponderance of the evidence**, if the petitioner **objectively** meets at least three of ten regulatory Criteria listed under 8 CFR § 204.5(h). Consistent with the two-step analysis articulated in *Kazarian v. U.S. Citizenship & Immigration Services*, once a petitioner demonstrates eligibility under at least three of the ten regulatory criteria, USCIS must proceed to the final merits determination to assess whether the totality of the evidence establishes extraordinary ability. *Kazarian v. U.S. Citizenship & Immigr. Servs.*, 596 F.3d 1115, 1121–22 (9th Cir. 2010).

It will be demonstrated herein that Dr. Noma satisfies **seven** of the ten regulatory criteria, and that the totality of the evidence establishes that she has achieved sustained national and international acclaim and has risen to the very top of the field of science, warranting approval under the final merits' determination, under the *Kazarian* standards.

Evidence of Extraordinary Ability

Criterion 1. Beneficiary's receipt of lesser nationally or internationally recognized prizes or awards for excellence

In order to qualify the beneficiary for this eligibility criterion under 8 CFR 204.5(h)(3)(i), USCIS evaluates whether: 1) the person was the recipient of prizes or awards; 2) the award is a lesser nationally or internationally recognized prize or award which the person received for excellence in the field of endeavor. **As indicated in the regulation, this criterion does not require an award or prize to have the same level of recognition and prestige associated with the Nobel Prize or another award that would qualify as a one-time achievement.**

Dr. Noma satisfies this criterion because she has received competitive, merit-based distinctions that function as prizes and awards for excellence in scientific research, including (i) a competitive academic award conferred by a major postgraduate program at the University of São Paulo, and (ii) a nationally administered, government-funded postdoctoral scholarship awarded under a formal comparative merit review process.

- **Poster Award and Honorable Mention for the "Targeting Peroxiredoxin-2 as a potential treatment for NRAS-mutated metastatic melanoma," at Symposium commemorating the 50" Anniversary the Postgraduate Program in Pharmacy - Pathophysiology and Toxicology at FCF/USP**

First, **Dr. Noma received a competitive award for excellence in scientific research at the University of São Paulo** (Universidade de São Paulo), Faculty of Pharmaceutical Sciences (Faculdade de Ciências Farmacêuticas – FCF/USP). On November 30, 2022, she was awarded in the "poster presentation" modality and obtained an "honorable mention" during the "Symposium to commemorate the 50th anniversary of the Postgraduate Program in Pharmacy – Pathophysiology and Toxicology at FCF/USP."

The award recognized Dr. Noma as the author of the poster entitled "Targeting Peroxiredoxin-2 as a potential treatment for NRAS-mutated metastatic melanoma," conducted under the guidance of Prof. Dr. Silvya Stuchi Maria Engler. This was an excellence-based distinction tied to a specific

research output and granted in a competitive presentation category, demonstrating that her work was singled out for recognition among the posters presented at this formal postgraduate symposium.

This poster award constitutes a “lesser nationally or internationally recognized prize or award for excellence” because it was conferred through an academic evaluation context by an established, long-running graduate program and specifically awarded for a defined research project in Dr. Noma’s field. The symposium was convened to commemorate the 50th anniversary of the postgraduate program in Pharmacy, Pathophysiology and Toxicology, underscoring the institutional significance and formal academic nature of the event.

The fact that the award was issued as an “honorable mention” in the poster presentation modality confirms that **it was not a routine participation certificate, but a selective distinction granted to recognize a superior scientific presentation.** Further, the award’s subject matter, which targeted a molecular pathway relevant to NRAS-mutated metastatic melanoma, directly aligns with Dr. Noma’s field of endeavor in tumor biology and oncology, and reflects the type of innovative, translational cancer research that is evaluated and rewarded within competitive academic research environments.

The official Activity Report of the Symposium confirms that the poster sessions were formally structured academic competitions in which graduate research was evaluated by designated faculty members serving as Poster Evaluators. During the doctoral session, 30 Ph.D. candidates presented posters that were subject to faculty review. Dr. Noma’s presentation was included in this evaluated doctoral cohort, demonstrating that her work underwent structured academic scrutiny by senior professors within the Program.

The Report further documents that awards were distributed in ranked categories and in the selective Honorable Mention category, confirming a tiered and competitive distinction system rather than general participation recognition. Dr. Noma is specifically identified in the official published report as a recipient of Honorable Mention for her poster entitled “Targeting Peroxiredoxin2 as a potential treatment for NRASmutated metastatic melanoma,” establishing that her work was formally selected for distinction among the doctoral research presentations.

The Symposium itself included participation from over one hundred attendees and was supported by nationally recognized Brazilian research funding agencies (including CAPES, CNPq, and FAPESP) as well as scientific sponsors, reflecting the institutional and academic significance of the event. The structured evaluation process, formal publication of awardees in the official report, and selective recognition among doctoral presenters confirm that Dr. Noma’s Honorable Mention was awarded based on merit following expert academic review.

Accordingly, this distinction constitutes a lesser nationally recognized prize for excellence in her field, as it was granted through a competitive evaluation process within an established postgraduate program at a leading public research university.

- **Postdoctoral Fellowship Abroad granted by the National Council for Scientific and Technological Development**

Second, **Dr. Noma received the CNPq Postdoctoral Fellowship Abroad,** a nationally administered prize-like research scholarship awarded by CNPq (National Council for Scientific and Technological Development), an agency of Brazil's Ministry of Science, Technology and Innovation. CNPq granted Dr. Noma a 12-month scholarship to pursue postdoctoral training at the H. Lee Moffitt Cancer Center and Research Institute in the United States, with an award period running from August 1, 2023 through July 31, 2024.

The fellowship included substantial, specified benefits, such as a monthly stipend of USD 2,100.00**,** a single-payment maintenance/installation allowance of USD 2,100.00**,** health insurance coverage up to USD 1,080.00**,** and transportation aid of USD 1,604.00, reflecting the seriousness, competitiveness, and investment associated with this national award for scientific excellence and advanced research training abroad.

The fellowship is a nationally recognized award for excellence because it is conferred under CNPq's formal regulatory structure that requires comparative merit review and ranking. Under the governing framework for scholarships abroad, proposals are judged and classified through multiple sequential stages. An excerpt from the CNPq selection and judging requirements is bellow.

> *"2.1. The judgment and classification ofproposals are made in the following stages:*
>
> *a) analysis by the technical area;*
> *b) analysis by ad hoc consultants, when applicable;*
> *c) comparative analysis of merit and classification of proposals by Advisory Committees or Judges;*
> *d) final decision by the Board of Directors, based on the financial availability of CNPq.*
>
> *2.2. The opinion issued by the Advisory Committee or Judge must take into account the following aspects:*
>
> *a) the merit ofthe proposal;*
> *b) the opinions ofthe technical area and ad hoc consultants, when applicable;*
> *c) the specificities ofthe modalities"*

The evaluation is expressly merit-centered. It is not an automatic entitlement program; it is a competitive national selection mechanism that awards limited funding to candidates whose credentials and research plans are judged superior under an expert review framework. The program's purpose is to enable advanced training through the development of an innovative scientific or technological project in a foreign institution. Eligibility and award conditions require, among other things, that the candidate hold a doctoral degree at implementation and dedicate themselves full-time to the approved activities.

The program's framework also requires that the host institution be internationally recognized in the relevant area and that the host advisor have recognized competence in the project's subject area, which ensures that the fellowship is reserved for candidates pursuing research in high-quality international settings under qualified mentorship. Selection is explicitly based on the candidate's curriculum, the advisor's curriculum, the international standing of the destination institution, and

the quality of the research project, with candidates then classified in comparison with other applicants. **Dr. Noma's receipt of the posdoctoral fellowship abroad award reflects national-level recognition of her scientific record and the merit of her proposed postdoctoral research and training plan.**

CNPq's national role in funding competitive international scientific cooperation further underscores the recognized nature of these scholarships as awards for excellence. CNPq publicly administers and invests substantial national funding in scholarships and international research cooperation programs, with formal calls, competitive application windows, and scheduled publication of final results. This context reinforces that the program is an established, nationally recognized award mechanism through which Brazil identifies and funds outstanding researchers for advanced work abroad.

In sum, the record establishes that Dr. Noma has received lesser nationally and internationally recognized awards for excellence in the field of science, as contemplated by 8 C.F.R. § 204.5(h)(3)(i) and the USCIS Policy Manual.

The body of credible, independently verifiable evidence documents to fulfill the criterion is compiled under **Exhibit B**:

B. Evidence that the beneficiary was the recipient of the awards:

B.1. Award certificate/confirmation of Poster Award / Honorable Mention for: *"Targeting Peroxiredoxin-2 as a potential treatment for NRAS-mutated metastatic melanoma,"* Symposium commemorating the 50th Anniversary of the Postgraduate Program in Pharmacy – Pathophysiology and Toxicology, Faculty of Pharmaceutical Sciences, University of São Paulo (FCF/USP), São Paulo, Brazil (November 2022).

B.2. Photograph(s) of Dr. Noma presenting her poster at the Symposium.

B.3. Official Symposium Activity Report (November 2022) for the Symposium commemorating the 50th Anniversary of the Postgraduate Program in Pharmacy – Pathophysiology and Toxicology (FCF/USP), documenting the Symposium structure, the poster sessions and faculty evaluation process, and the official listing of awardees including Dr. Noma's Honorable Mention.

B.4. Award letter/notice from CNPq granting Dr. Noma the Postdoctoral Fellowship Abroad, including award period and benefits.

B.5. Background information on CNPq establishing CNPq as a Brazilian federal government science and research funding agency.

B.6. CNPq Selection and Judging Requirements for scholarships/fellowships abroad, showing the formal, multi-stage, comparative merit-review and ranking process.

Therefore, Dr. Noma meets this criterion as the credible evidence demonstrates that she was the recipient of a lesser nationally or internationally recognized prizes or awards for excellence in the field of science pursuant to 8 CFR 204.5(h)(3)(i).

Criterion 4. The Petitioner's participation, either individually or on a panel, as a judge of the work of others in the same or an allied field of specialization for which classification is sought.

Under 8 C.F.R. § 204.5(h)(3)(iv) and the USCIS Policy Manual, the beneficiary must demonstrate that she has not only been invited to judge the work of others, but also that she actually participated in the judging of the work of others in the same or an allied field of specialization.

Dr. Noma satisfies this criterion through her documented service as a peer reviewer for a leading scientific journal and as an ad hoc abstract reviewer for international scientific meetings. These roles required her to apply her specialized expertise to evaluate the quality, methodology, and scientific contribution of research conducted by her peers, directly influencing publication and presentation decisions within the scientific community.

- **Peer review service for the Brazilian Journal of Pharmaceutical Sciences (BJPS)**

First, it is fundamental to introduce the institution which has invited the petitioner to serve as a judge. The Brazilian Journal of Pharmaceutical Sciences (BJPS) is a prestigious, peer-reviewed publication that utilizes the ScholarOne Manuscripts platform to manage its rigorous editorial process. In this capacity, the journal relies on independent subject-matter experts to provide technical evaluations that determine the suitability of manuscripts for global dissemination.

The evidence submitted confirms that Dr. Noma was formally added to the journal's reviewer database and subsequently invited by Associate Editor Dr. Silvya Stuchi Maria-Engler to evaluate Manuscript ID BJPS-2024-0245, titled "Dry powder inhalation formulation co-loaded with erlotinib and curcumin for treatment of non-small cell lung cancer."

The record demonstrates that Dr. Noma did not merely receive an invitation but actively participated in the judging process. The documentation includes an editorial reminder confirming her active assignment and a final ScholarOne review record showing that she returned her evaluation on April 21, 2024.

Her completed review involved a comprehensive assessment of the manuscript's originality and quality, culminating in a formal recommendation for minor revision. Furthermore, she provided substantive technical comments to the authors regarding English clarity, abstract structure, and the need for stronger statistical comparisons in the results section. This level of detailed feedback confirms that she performed a critical, evaluative role that is central to the peer-review process.

- **Ad hoc reviewer of scientific abstracts for International Meetings of Biosciences and Physiopathology**

In addition to her journal review service, Dr. Noma participated as a judge of the work of others for the VI and VII International Meetings of Biosciences and Physiopathology, organized by the Graduate Program in Biosciences and Physiopathology (PBF) at the State University of Maringá. This participation occurred within a structured, multi-reviewer system where each scientific abstract was evaluated by two independent reviewers to ensure scientific integrity.

The evidence includes formal reviewer instructions and orientation communications from the organizing committee, which Dr. Noma acknowledged and followed. These instructions required reviewers to log into a dedicated platform, download assigned abstracts, and provide technical recommendations and linguistic corrections.

Dr. Noma's actual participation in this conference judging is further corroborated by official certificates of service and a formal statement from the Coordinator of the PBF program, Prof. Dr. Marcia Edilaine Lopes Consolaro. This statement confirms that Dr. Noma served as an ad hoc reviewer and evaluator, responsible for reviewing abstracts submitted by other authors and providing the technical commentary necessary for quality control.

The organizing committee's communications also show that she was involved in the final stages of the evaluation workflow, ensuring that works "Approved with Reservations" met the required scientific standards before the event. This collective evidence establishes a consistent record of judging activity across multiple years and platforms.

For the purpose of evidencing that Dr. Noma meets this criterion, we are submitting the following supporting documents Exhibit C:

C. Evidence that the beneficiary participated as a judge of the work of others in the field of scientific research:

C.1. BJPS ScholarOne notice confirming account creation and addition to the reviewer database (April 5, 2024).

C.2. Formal invitation from the Associate Editor of the Brazilian Journal of Pharmaceutical Sciences to review Manuscript ID BJPS-2024-0245 (April 5, 2024).

C.3. Editorial reminder from the Brazilian Journal of Pharmaceutical Sciences confirming Dr. Noma's active peer review assignment (April 19, 2024).

C.4. Completed Peer Review Report for Manuscript ID BJPS-2024-0245, including technical ratings, editorial recommendation, and substantive comments to the author (April 21, 2024).

C.5. Official Certificates of Service as Ad Hoc Abstract Reviewer for the VI and VII International Meetings of Biosciences and Physiopathology (2019–2020).

C.6. Reviewer orientation and instruction communications from the Organizing Committee of the VI International Meeting of Biosciences and Physiopathology (September 2019).

C.7. Email acknowledgment from the Organizing Committee confirming the final round of abstract corrections for the VII International Meeting of Biosciences and Physiopathology (August 2020).

C.8. Institutional Statement from the Coordinator of the Postgraduate Program in Biosciences and Physiopathology (PBF) confirming Dr. Noma's evaluative responsibilities and contributions to scientific quality control (December 15, 2025).

Thus, Dr. Noma meets this criterion as the evidence demonstrates her invitation and participation, both individually and as part of an evaluation workflow, as a judge of the work of others in the science field pursuant to 8 C.F.R. § 204.5(h)(3)(iv). Her work as a peer reviewer and conference evaluator confirms that recognized scientific institutions and editorial boards rely on her professional judgment to maintain the high standards of research in pharmaceutical sciences and biosciences.

Criterion 5. The person's original scientific, scholarly, artistic, athletic, or business-related contributions of major significance in the field.

Under the EB-1A regulations, an applicant may satisfy the fifth criterion by demonstrating "original scientific, scholarly, artistic, athletic, or business-related contributions of major significance in the field." *See* 8 C.F.R. § 204.5(h)(3)(v). Examples of relevant evidence include but are not limited to published materials about the significance of the person's original work; testimonials, letters, and affidavits about the person's original work; documentation that the person's original work was cited at a level indicative of major significance in the field; and patents or licenses deriving from the person's work or evidence of commercial use of the person's work.

For example, published research that has provoked widespread commentary on its importance from others working in the field, and documentation that it has been highly cited relative to others' work in that field, may be probative of the significance of the person's contributions to the field of endeavor.

Dr. Noma has produced original research contributions that have meaningfully advanced scientific understanding in melanoma biology, cancer resistance mechanisms, and cancer-related epigenetics. These contributions are recognized by independent experts and corroborated by objective impact indicators, including a sustained increase in scholarly attention.

As of February, 2026, Dr. Noma's work has reached 127 citations overall, with an h-index of 7 and i10-index of 6, reflecting that her findings are actively engaged by the broader scientific community across multiple research lines. Importantly, Dr. Noma's Google Scholar citation metrics reflect sustained growth over time: between October 2025 and February 26, 2026, her total citations increased from 97 to 127 (+30 citations, approximately 31% growth), with her h-index increasing from 6 to 7 and her i10-index increasing from 5 to 6.

- **Discovery of PRDX2 as a key regulator of invasion and EMT in NRAS-mutated melanoma**

The publication **"Peroxiredoxin-2 represses NRAS-mutated melanoma cells invasion by modulating EMT markers"** (*Biomedicine & Pharmacotherapy*, 2024) established a previously unknown mechanistic link between the PRDX2 gene and epithelial–mesenchymal transition (EMT) in NRAS-mutated melanoma. This research provided the first evidence that PRDX2 acts as a suppressor of invasion.

Notably, this discovery was not only novel but independently validated in a UK databank of 700 primary melanoma patients by **Dr. Jérémie Nsengimana** (Newcastle University), an unaffiliated expert in melanoma biomarker research. In his submitted letter of support, Dr. Nsengimana states:

> *"In the publication that we co-authored (Noma et al., 2024), the original experiments that she skillfully and tenaciously conducted provided for the first time the evidence that a gene called PRDX2 modulates the epithelial–mesenchymal transition (EMT) in NRAS-mutated melanomas. This was a truly transformative finding in the puzzling mechanism of cancer phenotype switching (theory of 'grow or go', i.e. growing locally or reprogramming to metastasize). This mechanism induces drug resistance and is currently a major bottleneck in cancer research."*

He further explains the broader impact of this discovery:

> *"Dr Noma's finding was therefore significant, it suggested potential new diagnostic testing and new treatment options, based on antioxidants. My own contribution to this exciting work was to confirm this hypothesis in a databank of 700 primary melanoma patients collected in the UK."*

Prof. Ana Campa, PhD (Full Professor, Faculty of Pharmaceutical Sciences, University of São Paulo), further corroborates the originality and technical significance of Dr. Noma's PRDX2/EMT discovery. In her submitted letter of support, Prof. Campa states:

> *"In this study, Dr. Noma demonstrated that PRDX2 acts to inhibit epithelial-mesenchymal transition in NRAS-mutated melanoma cells, identifying a potential therapeutic target for reducing melanoma invasiveness. This finding is technically significant because EMT is a major mechanism of metastasis, and few studies had explored PRDX2 in this context, also demonstrating the originality of her work."*

This independent confirmation in a large patient cohort demonstrates that Dr. Noma's work is not merely academic, but clinically relevant and broadly applicable, thus a clear evidence of "major significance" within the meaning of the regulation.

- **Elucidating redox-mediated mechanisms of therapeutic resistance and tumor heterogeneity**

Dr. Noma's original research into the distinct roles of peroxiredoxins, as detailed in **"Modeling melanoma heterogeneity in vitro: redox, resistance and pigmentation profiles"** (*Antioxidants*, 2024) and **"2-Methoxyestradiol-3,17-O,O-bis-sulfamate (STX140) Inhibits Proliferation and Invasion via Senescence Pathway Induction in Human BRAFI-Resistant Melanoma"** (*International Journal of Molecular Sciences*, 2023), addresses the critical challenge of BRAF inhibitor (BRAFi) resistance, which are one of the most pressing therapeutic challenges in melanoma treatment.

Dr. Patrícia Abrão Possik, Principal Investigator at the Brazilian National Cancer Institute (INCA), describes the field-level impact of this work:

> *"All publications indicated new treatments to overcome resistance mechanisms and improve treatment outcomes, such as proposing new strategies that explore antioxidants or redox balance with less toxicity and more efficacy. Exploring redox balance can address the hallmarks of cancer, which are significant challenges today in cancer treatment, including resistance and metastasis. Both of her studies demonstrated efficacy through antioxidants, representing a new strategy to pursue."*

Similarly, **Prof. Silvia Berlanga de Moraes Barros** (University of São Paulo) explains the scientific significance of the PRDX2 findings:

> *"Her contributions with this study are significant because NRAS-mutated melanoma is known for its aggressive behavior and poor prognosis. By modulating EMT markers, Prx-2 may influence the transition of cancer cells from an epithelial to a more migratory mesenchymal state, which is crucial for metastasis. Understanding this mechanism is essential to define new therapeutic strategies aimed at preventing melanoma spread."*

These independent expert evaluations demonstrate that Dr. Noma's findings are not incremental. Rather, they provide actionable mechanistic insight into metastasis and drug resistance and propose lower-toxicity therapeutic strategies, addressing core limitations in current melanoma treatment.

- **Identifying HDAC8-mediated epigenetic reprogramming as a driver of UV-induced melanoma persistence**

During her postdoctoral research at Moffitt Cancer Center, Dr. Noma uncovered a novel mechanism by which UV radiation induces epigenetic reprogramming via HDAC8, allowing melanocytes with pathogenic mutations to evade apoptosis and persist, a critical early step in melanoma initiation.

The University of São Paulo (USP) Postdoctoral Program Completion Certificate explicitly identifies Dr. Noma's completed postdoctoral project as "The Role of HDAC8 in UVR-Driven Melanoma Initiation," independently documenting the HDAC8/UVR research line that underlies this original contribution.

Prof. Berlanga describes the impact of this discovery in her submitted letter of support:

> *"Her research provides a novel perspective on how UVR-induced epigenetic reprogramming targeting HDAC8 contributes to the persistence of melanocytes and melanoma cells harboring pathogenic mutations, allowing them to grow and evade apoptosis. By addressing the underlying mechanisms of tumor survival and resistance, Dr. Noma's research aids in improving therapeutic strategies, ultimately increasing survival rates and quality of life for patients with melanoma."*

This contribution is of major significance because it identifies a specific, targetable epigenetic consequence of UV exposure, which directly links environmental carcinogenesis to molecular

persistence mechanisms, thereby opening new avenues for preventive and early-intervention strategies.

- **First published evidence linking high-risk HPV and Ureaplasma parvum co-infections to increased risk of cervical lesions**

During her Master's research at the State University of Maringá (UEM), Dr. Noma produced original research clarifying how infectious co-factors can increase cervical lesion risk. This work established a previously unknown link between specific co-infections and the progression of cervical cancer.

Prof. Marcia Edilaine Lopes Consolaro, PhD (Associate Professor, State University of Maringá), describes the original nature of this contribution:

> *"She produced her dissertation, and an article entitled: 'Association of High-Risk Human Papillomavirus and Ureaplasma parvum Co-Infections with Increased Risk of Low-Grade Squamous Intraepithelial Cervical Lesions.' Her work was an original contribution to the field […] Interestingly, this study is the first to be published about this relationship between HPV and other infections in cervical cancer. Moreover, this study had citations that impacted recent publications in the area in Frontiers, and MDPI journals, important to share knowledge."*

Prof. Campa also corroborates the importance and downstream public-health relevance of Dr. Noma's original co-infection findings. Prof. Campa states:

> *"She also investigated the interaction of high-risk HPV and Ureaplasma parvum co-infections with cervical cancer development. This study highlighted the importance of considering viral and bacterial co-infections in cancer risk assessment, which guides preventive healthcare strategies emphasizing importance of cervical screening to find precancerous cervical cell changes and presence of HPV. Cervical cancer found at an early stage is usually easier to treat, and her study strengthens the recommendations of the National Institutes of Health (NIH)."*

This record of first-in-literature findings, corroborated by an expert supervisor and documented through downstream citations in high-impact journals, demonstrates that Dr. Noma's early-career work was already achieving major significance by providing foundational data for the field of molecular oncology.

- **Epigenetic discoveries linking exercise training to DNA methylation changes relevant to major chronic diseases**

Dr. Noma's original research contributions also extend to human epigenetics, specifically documenting how structured exercise training is associated with measurable DNA methylation changes in clinically relevant populations. This work provides a novel molecular link between lifestyle interventions and the prevention or management of chronic conditions such as Alzheimer's disease and diabetes.

Prof. Ana Campa, highlights the importance of these original epigenetic findings in her submitted letter of support:

> ***"****Dr. Noma's research extends to epigenetic studies connecting lifestyle factors with genetic modifications, such as '14-Week exercise training modifies the DNA methylation levels at gene sites in non-Alzheimer's disease women aged 50 to 70 years' or '14-weeks combined exercise epigenetically modulated 118 genes of menopausal women with prediabetes' highlighted importance of epigenetic modifications in diseases like Alzheimer's or diabetes.****"***

By identifying specific gene sites that are epigenetically modulated by physical intervention, Dr. Noma has provided the field with original data that informs public health strategies and molecular aging research. This evidence of original work in human epigenetics further demonstrates the breadth and major significance of her scientific contributions.

Hence, the totality of the evidence, spanning from Dr. Noma's foundational discoveries in cervical cancer co-infections to her transformative findings in melanoma metastasis, therapeutic resistance, and UV-induced epigenetic reprogramming, demonstrates a sustained trajectory of original contributions that have fundamentally altered scientific understanding in her field. These contributions are not merely theoretical; they have been independently validated in large patient cohorts, cited by international peers at an accelerating rate, and recognized by leading experts as "transformative" and "clinically relevant." By identifying novel biomarkers like PRDX2 and targetable mechanisms like HDAC8, Dr. Noma has provided the global scientific community with actionable insights to overcome drug resistance and improve patient outcomes, satisfying the requirement for contributions of major significance under 8 C.F.R. § 204.5(h)(3)(v).

Credible evidence to support this criterion are listed under **Exhibit D**:

D. Evidence of the original scientific contributions of major significance in the field:

D.1. "Peroxiredoxin-2 represses NRAS-mutated melanoma cells invasion by modulating EMT markers," IHY Noma et al., *Biomedicine & Pharmacotherapy* (2024).

D.2. Recommendation letter from Dr. Jérémie Nsengimana, PhD (Newcastle University, UK), confirming the "transformative" nature and independent validation of the PRDX2 discovery.

D.3. "Modeling melanoma heterogeneity in vitro: redox, resistance and pigmentation profiles," LAC Carvalho, IHY Noma et al., *Antioxidants* (2024).

D.4. "2-Methoxyestradiol-3,17-O,O-bis-sulfamate (STX140) Inhibits Proliferation and Invasion via Senescence Pathway Induction in Human BRAFI-Resistant Melanoma," YA Franco et al. (including IHY Noma), *IJMS* (2023).

D.5. Recommendation letter from Prof. Silvia Berlanga de Moraes Barros, PhD (University of São Paulo), explaining the significance of the heterogeneity and resistance research.

D.6. Recommendation letter from Dr. Patrícia Abrão Possik, PhD (Brazilian National Cancer Institute/INCA), highlighting the translational impact of the redox and resistance findings.

D.7. University of São Paulo (USP) Postdoctoral Program Completion Certificate (translated), explicitly titled project: "The Role of HDAC8 in UVR-Driven Melanoma Initiation," documenting completion and scope of the HDAC8/UVR research. **(See Exhibit A.7.)**

D.8. Recommendation letter from Prof. Silvya Stuchi Maria-Engler, PhD (University of São Paulo), corroborating the impact of the HDAC8/UV-induced melanoma discovery.

D.9. "Association of high risk Human Papillomavirus and Ureaplasma parvum co infections with increased risk of low grade squamous intraepithelial cervical lesions," *Asian Pacific Journal of Cancer Prevention* (2021).

D.10. Recommendation letter from Prof. Marcia Edilaine Lopes Consolaro, PhD (State University of Maringá/UEM), documenting Dr. Noma's original Master's research and the first-published evidence linking high-risk HPV and *Ureaplasma parvum* co-infections to cervical lesion risk.

D.11. "14 Week exercise training modifies the DNA methylation levels at gene sites in non Alzheimer's disease women aged 50 to 70 years," *Experimental Gerontology* (2024).

D.12. "14 weeks combined exercise epigenetically modulated 118 genes of menopausal women with prediabetes," *Frontiers in Endocrinology* (2022).

D.13. Recommendation letter from Ana Campa, PhD (Full Professor, Faculty of Pharmaceutical Sciences, University of São Paulo), corroborating the originality and technical significance of the PRDX2/EMT discovery, the HPV/Ureaplasma co-infection findings, and the impact of the exercise-induced epigenetic research.

D.14. Google Scholar citation report for Dr. Noma as of February 26, 2026, documenting 127 total citations, an h-index of 7, and an i10-index of 6.

D.15. Google Scholar citation report for Dr. Noma as of October 2025, documenting 97 total citations, an h-index of 6, and an i10-index of 5.

Hence, the submitted evidence demonstrates that Dr. Noma has made original contributions of major significance to the field of science pursuant to 8 CFR 204.5 (h)(3)(v).

Criterion 6. The person's authorship of scholarly articles in the field, in professional or major trade publications or other major media.

To fulfill this criterion, USCIS will determine whether the person has authored scholarly articles in the field and whether the outlets qualify as professional publications, major trade publications, or other major media**.** In the academic context, USCIS recognizes "scholarly articles" as works that report original research or experimentation or provide scholarly analysis, are typically written by experts, and generally include citations, bibliographies, and, where appropriate, figures and tables. USCIS also considers whether the outlets are professional or major media based on factors such as intended audience, circulation, and indexing in recognized scientific databases. See USCIS Policy Manual, Vol. 6, Part F, Ch. 2, Criterion 6.

Dr. Noma satisfies the requested through her extensive record of peer-reviewed publications in internationally recognized scientific journals and conference proceedings in the fields of tumor biology, melanoma, epigenetics, obesity, infectious diseases, and pharmaceutical sciences**.**

Dr. Noma has authored at least 13 full articles in peer reviewed journals, including both first author and significant coauthor contributions. These works are published in professional, indexed scientific journals that target learned audiences of researchers, clinicians, and academics. Representative examples include:

- **"Peroxiredoxin-2 represses NRAS-mutated melanoma cell invasion by modulating EMT markers"**, *Biomedicine & Pharmacotherapy* (2024) – first-author work presenting original experimental research on melanoma invasion and epithelial–mesenchymal transition (EMT) markers; indexed in major biomedical databases, with citations in **Web of Science**;
- **"Modeling melanoma heterogeneity in vitro: redox, resistance, and pigmentation profiles"**, *Antioxidants* (2024) – co-authored article in a high-visibility, peer-reviewed journal on redox biology, reporting original data on melanoma heterogeneity; indexed in **Web of Science** and **Scopus**;
- **"The influence of serum selenium in differential epigenetic and transcriptional regulation of the CPT1B gene in women with obesity"**, *Journal of Trace Elements in Medicine and Biology* (2023/2024) – original epigenetics research with multiple citations in **Web of Science** and **Scopus**;
- **"DNA methylation signatures of arsenic exposure and obesity"**, *Environmental Epigenetics* (2025) – cutting-edge work on environmental exposures and obesity, published in a specialized, peer-reviewed epigenetics journal;
- **"14-Week exercise training modifies the DNA methylation levels at gene sites in non-Alzheimer's disease women aged 50 to 70 years"**, *Experimental Gerontology* (2024) – epigenetic effects of exercise on aging, with citations recorded in **Web of Science** and **Scopus**;
- **"Eight weeks of physical training decreases 2 years of DNA methylation age of sedentary women"**, *Research Quarterly for Exercise and Sport* (2023) – peer-reviewed article in a long-established research journal on exercise science, with citations in both **Web of Science** and **Scopus**;
- **"14-weeks combined exercise epigenetically modulated 118 genes of menopausal women with prediabetes"**, *Frontiers in Endocrinology* (2022) – a highly cited publication

(19+ citations in Web of Science/16+ in Scopus per Lattes CV), demonstrating substantial uptake and influence in the field;

- **"Gene signatures of autopsy lungs from obese patients with COVID-19"**, *Clinical Nutrition ESPEN* (2021) – peer-reviewed clinical research in an international nutrition journal, with citations indexed in major databases;
- **"Sample preparation to bioinformatics analysis of DNA methylation: association strategy for obesity and related trait studies"**, *Journal of Visualized Experiments (JoVE)* (2022) – a methods-oriented article in a specialized peer-reviewed journal, cited in **Web of Science** and **Scopus**;
- **"Association of high-risk Human Papillomavirus and Ureaplasma parvum co-infections with increased risk of low-grade squamous intraepithelial cervical lesions"**, *Asian Pacific Journal of Cancer Prevention* (2021) – an original first-author cancer prevention study, indexed in **Scopus**, that has informed subsequent work in cervical oncovirology;
- **"Economic impact of hospitalizations due to leishmaniasis in southern Brazil"**, *Research, Society and Development* (2022) – a peer-reviewed article analyzing the economic burden of a neglected tropical disease.
- **"2-Methoxyestradiol-3,17-O,O-bis-sulfamate (STX140) Inhibits Proliferation and Invasion via Senescence Pathway Induction in Human BRAFI-Resistant Melanoma Cells"**, *International Journal of Molecular Sciences* (2023) – co-authored research article about experimental data on resistance mechanisms in melanoma.
- **"Association between the relative abundance of phyla actinobacteria, vitamin C consumption, and DNA methylation of genes linked to immune response pathways",** *Frontiers in Nutrition* (2024) – co-authored original research article that connects microbiota composition, diet, and epigenetic regulation of immune pathways, reflecting the breadth of Dr. Noma's scholarly contributions beyond oncology into metabolism and nutrition.

These publications possess a strictly **scholarly format**, as seen in the full-text copies submitted in the record, which include structured abstracts, detailed methodologies, original data visualizations, and extensive bibliographies. The **originality and significance** of this research are further affirmed by independent experts in the field.

Prof. **Marcia Edilaine Lopes Consolaro, PhD**, Associate Professor at the State University of Maringá and Dr. Noma's Master's thesis supervisor, attests to the scholarly ability of Dr. Noma's research:

> *"I became acquainted with Dr. Noma when she was a graduate student and Master candidate at the State University of Maringa. She performed the experimental work for her dissertation under my supervision, in the Health Science Program, at the Health Science Center. During that time, she utilized very creative approaches to conducting research and actively participated in the organization of multiple institutional activities, including the VI International Meeting of Biosciences and Physiopathology.[…]*
>
> *Unlike most researchers, her scientific and technical skills span the whole spectrum of research from basic fundamental biology to applied research. Owing to her training in multidisciplinary environments, Dr. Noma has shown the ability*

> *to integrate concepts of synthetic biology, molecular biology, systems biology, and oncology in the design and development of novel insights to therapeutic approaches for the treatment of human cancers."*

Prof. Consolaro further notes that Dr. Noma's writing and analytical skills are excellent, and that her contributions to the cancer gene therapy community are of significant relevance.

Dr. Noma's scholarly articles are published in **professional and major media outlets** that target a learned audience of international researchers and clinicians. Her work has been rigorously **peer-reviewed** and published in prestigious journals, including:

To establish the professional standing and "major media" status of these publications, data from the SCImago Journal & Country Rank (SJR) provide a reliable, objective measure of journal impact and prestige. SCImago is a globally recognized scientometric portal that derives its data from Scopus (Elsevier), the world's largest abstract and citation database of peer reviewed literature. SCImago's methodology has been scientifically validated and is supported by multiple peer reviewed publications in high impact journals, and it provides standardized, comparable data on journal prestige through its Quartile ("Q") system. A Q1 or Q2 ranking signifies that a journal is in the top 25% or 50% of its subject category worldwide, respectively. These metrics therefore constitute objective evidence that the journals in which Dr. Noma publishes are high impact, professional outlets that target a global audience of experts.

- ***Biomedicine & Pharmacotherapy***: An international, peer-reviewed journal focused on the intersection of clinical and basic medicine. Dr. Noma's first-author research here (2024) on Peroxiredoxin-2 and melanoma invasion is a primary scholarly contribution to the field of oncology.
- ***Antioxidants***: A high-impact professional journal (indexed in Web of Science and Scopus) where Dr. Noma published original research (2024) on melanoma heterogeneity and redox profiles, reaching a global audience of molecular biologists.
- ***Frontiers in Endocrinology***: A leading professional journal where Dr. Noma's research on epigenetic modulation (2022) has achieved significant scholarly impact, accumulating **19+ citations in Web of Science** and **16+ in Scopus**, demonstrating its role as a foundational text for other experts.
- ***Environmental Epigenetics***: A specialized professional publication where Dr. Noma's 2025 research on DNA methylation signatures provides original scholarly discourse on the impact of environmental exposures on human health.
- ***Experimental Gerontology*** and ***Research Quarterly for Exercise and Sport***: Established professional journals where Dr. Noma has published original data (2023–2024) regarding the epigenetic effects of physical training, contributing to the scholarly understanding of aging and metabolic health.
- ***Journal of Trace Elements in Medicine and Biology***: A peer-reviewed professional outlet where her work on transcriptional regulation in obesity (2023) has been widely cited by the international scientific community.
- ***Asian Pacific Journal of Cancer Prevention***: A professional journal where Dr. Noma's first-author research (2021) on HPV co-infections provided original clinical data that has been built upon by subsequent researchers in oncovirology.

- ***International Journal of Molecular Sciences***: A molecular sciences journal where Dr. Noma's co-authored 2023 article on BRAFi-resistant melanoma.
- ***Clinical Nutrition ESPEN***: A peer-reviewed, electronic-only journal focusing on the relationship between nutrition and disease in basic science and clinical practice where Dr. Noma's co-authored COVID-19 lung signatures article.
- ***Journal of Visualized Experiments (JoVE)***: A specialized methods journal that publishes video-based and textual protocols in the life and physical sciences where Dr. Noma's co-authored DNA methylation methods paper was published.

The scholarly impact of these articles is objectively documented by Dr. Noma's **Google Scholar Profile**, which shows **127 total citations**, an **h-index of 7**, and an **i10-index of 6**. These metrics demonstrate that her scholarly articles are being **actively read, cited, and relied upon** by other researchers. Several of her papers have already accumulated **double-digit citations**, reflecting particularly strong scholarly uptake.

The **Web of Science profile** further corroborates this impact by listing, for multiple articles, **citation counts,** which confirms that her work is **visible and influential in independent, international citation database**, not just on Google Scholar.

To evidence that Dr. Noma meets this criterion, the Petitioner submits the following supporting documents under **Exhibit E.**

> **E. Evidence that the beneficiary is an author of scholarly articles in the field of science published in professional media**:
>
> > **E.1.** Dr. Noma's Curriculum Vitae (Lattes). Contains the complete list of all scholarly articles authored by Dr. Noma, including journal titles, years, co-authors, and citation counts in Web of Science and Scopus. **(See Exhibit A.2.).**
> >
> > **E.2.** Web of Science (Clarivate) Researcher Profile. Independent report from Clarivate Analytics verifying 20 publications indexed in the prestigious Web of Science Core Collection, 146 total citations, and an h-index of 6.
> >
> > *(Note: For the Petitioner's further citation metrics, including an h-index of 7 and 127 total citations, please refer to the **Google Scholar Profile** submitted under **Exhibit D.9**).*
> >
> > **E.3.** SCImago "How We Work" Methodology Report. Documentation explaining the scientific validity of SCImago indicators, its partnership with the Scopus (Elsevier) database, and the peer-reviewed research supporting its ranking system. This establishes the reliability of the journal metrics used throughout this petition.
> >
> > **E.4. Evidence of publication in *Biomedicine & Pharmacotherapy* (2024):**
> >
> > > **E.4.1.** "Peroxiredoxin 2 represses NRAS mutated melanoma cell invasion by modulating EMT markers," *Biomedicine & Pharmacotherapy* (2024). **(See Exhibit D.1.).**

E.4.2. SCImago journal profile for *Biomedicine & Pharmacotherapy* showing SJR 2024: 1.775, Q1 ranking, H-index 169, coverage in Medicine and Pharmacology, and indexing in Scopus/Web of Science.

E.5. Evidence of publication in *Antioxidants* (2024):

E.5.1. "Modeling melanoma heterogeneity in vitro: redox, resistance, and pigmentation profiles," *Antioxidants* (2024). **(See Exhibit D.3.).**

E.5.2. SCImago journal profile for *Antioxidants* showing SJR 2024: 1.484, Q1 status, H-index 133, MDPI publisher information, and subject categories in Biochemistry, Genetics and Molecular Biology.

E.6. Evidence of publication in *Frontiers in Endocrinology* (2022):

E.6.1. "14 weeks combined exercise epigenetically modulated 118 genes of menopausal women with prediabetes," *Frontiers in Endocrinology* (2022). **(See Exhibit D.12.).**

E.6.2. SCImago profile for *Frontiers in Endocrinology* (SJR 2024: 1.472; Q1; H-index 138; scope describing rigorous peer review and a global clinical/endocrine audience).

E.7. Evidence of publication in *Environmental Epigenetics* (2025):

E.7.1. "DNA methylation signatures of arsenic exposure and obesity," *Environmental Epigenetics* (2025).

E.7.2. Evidence of indexing and professional status (publisher or database pages confirming peer review and inclusion in major scientific databases such as Web of Science/Scopus).

E.8. Evidence of publication in *Experimental Gerontology* (2024):

E.8.1. "14 Week exercise training modifies the DNA methylation levels at gene sites in non Alzheimer's disease women aged 50 to 70 years," *Experimental Gerontology* (2024). **(See Exhibit D.11.).**

E.8.2. SCImago (or equivalent) profile confirming *Experimental Gerontology*'s Q1 ranking in Aging/Gerontology and indexing in Web of Science and Scopus.

E.9. Evidence of publication in *Research Quarterly for Exercise and Sport* (2023):

E.9.1. "Eight weeks of physical training decreases 2 years of DNA methylation age of sedentary women," *Research Quarterly for Exercise and Sport* (2023).

E.9.2. SCImago profile for *Research Quarterly for Exercise and Sport* showing Q1 status (SJR ≈ 0.78), long-standing coverage in sports sciences, and Scopus indexing.

E.10. Evidence of publication in *Journal of Trace Elements in Medicine and Biology* (2023/2024):

E.10.1. "The influence of serum selenium in differential epigenetic and transcriptional regulation of the CPT1B gene in women with obesity."

E.10.2. SCImago or publisher documentation showing the journal's subject areas (Medicine, Biochemistry), Q1/Q2 ranking, and indexing in Web of Science and Scopus.

E.11. Evidence of publication in *Clinical Nutrition ESPEN* (2021):

E.11.1. "Gene signatures of autopsy lungs from obese patients with COVID 19," *Clinical Nutrition ESPEN* (2021).

E.11.2. SCImago profile for *Clinical Nutrition ESPEN* (SJR 2024: 0.805; Q2 in Nutrition and Dietetics and Endocrinology/Metabolism; H-index 46; official ESPEN journal; indexed in Scopus/Web of Science).

E.12. Evidence of publication in *Journal of Visualized Experiments (JoVE)* (2022):

E.12.1. "Sample preparation to bioinformatics analysis of DNA methylation: association strategy for obesity and related trait studies," *Journal of Visualized Experiments* (2022).

E.12.2. SCImago profile for *Journal of Visualized Experiments* (SJR 2024: 0.431; Q2; H-index 141; scope in novel and gold-standard research methodologies, indexed in Scopus/Web of Science).

E.13. Evidence of publication in *Asian Pacific Journal of Cancer Prevention (APJCP)* (2021):

E.13.1. "Association of high risk Human Papillomavirus and Ureaplasma parvum co infections with increased risk of low grade squamous intraepithelial cervical lesions," *Asian Pacific Journal of Cancer Prevention* (2021). **(See Exhibit D.9.).**

E.13.2. SCImago profile for *Asian Pacific Journal of Cancer Prevention* (SJR 2024: 0.530; H-index 98; Q2 in Cancer Research, Q3 in Public Health/Epidemiology; continuous coverage since 2000).

E.14. Evidence of publication in *International Journal of Molecular Sciences* (2023):

E.14.1. "2 Methoxyestradiol 3,17 O,O bis sulfamate (STX140) Inhibits Proliferation and Invasion via Senescence Pathway Induction in Human BRAFI Resistant Melanoma Cells," *International Journal of Molecular Sciences* (2023). **(See Exhibit D.4.).**

E.14.2. SCImago profile for *International Journal of Molecular Sciences* (SJR 2024: 1.273; Q1; H-index 314; broad molecular biology and molecular medicine scope; MDPI; Scopus/Web of Science indexing).

E.15. Evidence of publication in *Frontiers in Nutrition* (2024):

E.15.1. "Association between the relative abundance of phyla actinobacteria, vitamin C consumption, and DNA methylation of genes linked to immune response pathways," *Frontiers in Nutrition* (2024).

E.15.2. SCImago profile for *Frontiers in Nutrition* (SJR 2024: 1.12; H-index 84; Q1 in Nutrition and Dietetics; published by Frontiers Media, Switzerland; Scopus-indexed).

E.16. Expert letter from Prof. Marcia Edilaine Lopes Consolaro, PhD attesting to the originality, quality, and influence of Dr. Noma's scholarly publications and explaining how her articles have guided subsequent research in melanoma, epigenetics, and related fields.

Therefore, based on the number, quality, and impact of her peer reviewed publications in internationally recognized scientific journals, corroborated by independent citation metrics and expert testimony, the evidence establishes that Dr. Noma has authored scholarly articles in the field of science published in professional media. She thus meets Criterion 6 pursuant to 8 C.F.R. § 204.5(h)(3)(vi) and the USCIS Policy Manual, Vol. 6, Part F, Ch. 2, Criterion 6.

Criterion 7. Display of the Petitioner's work in the field in Public Exhibitions (Claimed as Comparable Evidence under 8 C.F.R. § 204.5(h)(4))

Under 8 C.F.R. § 204.5(h)(3)(vii), USCIS evaluates whether (1) the displayed work is the beneficiary's own work product, and (2) the venue constitutes an artistic exhibition or showcase. The regulation explicitly includes the modifier "artistic," and USCIS generally limits this criterion to artistic fields.

However, pursuant to 8 C.F.R. § 204.5(h)(4), when a criterion does not readily apply to the beneficiary's occupation, comparable evidence may be submitted. As a biomedical scientist

and cancer researcher, Dr. Noma does not work in the fine arts. Accordingly, traditional artistic exhibitions are not applicable to her profession. The functional equivalent in the scientific field is the public display of original research at selective, peer-reviewed scientific congresses and symposia, which serve as the principal professional "showcases" of scholarly work.

In biomedical research, major international and national congresses operate as curated exhibitions of original scientific work. Abstracts undergo expert peer review before acceptance. Selected researchers are then invited to publicly display their findings in poster or oral presentation sessions before distinguished audiences of scientists, clinicians, institutional leaders, and industry stakeholders. **Unlike general attendance, selection for display at these venues is a competitive process where only a fraction of submitted research is chosen for public exhibition based on its novelty and impact.**

These congresses are publicly organized professional events, feature curated and competitively selected research, provide structured poster exhibition sessions, allow direct expert evaluation and scrutiny, disseminate research to national and international audiences, and often confer awards for outstanding displays. **The physical display of a research poster at these events serves as a visual and intellectual showcase, allowing for real-time peer review and professional engagement that mirrors the public scrutiny found in artistic galleries.**

Thus, for a scientist, acceptance and presentation at such congresses is the professional equivalent of having one's work selected for exhibition in an artistic showcase.

Dr. Noma has repeatedly displayed her original scientific work at prestigious international and national scientific exhibitions, demonstrating sustained recognition of her research.

- **International Congress – Society for Melanoma Research (SMR)**

Dr. Noma was selected to present her research at the 19th International Congress of the Society for Melanoma Research (October 17–20, 2022). Her participation included public poster display and presentation of her original melanoma research.

The Society for Melanoma Research is a globally recognized organization dedicated to advancing melanoma research. Its annual congress is one of the world's leading forums for melanoma investigators. Selection to present at this international congress reflects peer-reviewed acceptance of original research for public scientific exhibition before a global audience of experts. The SMR Congress is highly selective, typically attracting thousands of global experts; being chosen to display research here signifies that the work is of significant interest to the international scientific community.

- **Brazilian Congress of Toxicology (CBTox 2022)**

At the XXII Brazilian Congress of Toxicology (CBTox 2022), Dr. Noma presented her research titled "Gliotoxin and its potential cytotoxicity against NRAS mutated-melanoma."

This national congress is organized by the Brazilian Society of Toxicology (SBTox), a distinguished professional organization. Her research was formally selected for poster exhibition and publicly presented before scientific peers and institutional leaders. SBTox is a premier national body affiliated with the International Union of Toxicology (IUTOX), ensuring that the "showcase" environment meets rigorous international standards for scientific display.

- **University of São Paulo – 50th Anniversary Scientific Symposium**

Dr. Noma presented: “Targeting Peroxiredoxin-2 as a Potential Treatment for NRAS-Mutated Metastatic Melanoma” at the 50th Anniversary Symposium of the Postgraduate Program in Pharmacy – Pathophysiology and Toxicology at the University of São Paulo (FCF/USP).

This was not merely participation. Her displayed work received Honorable Mention, confirming that her exhibited research was recognized as outstanding among competing presentations. This award specifically validates the quality of her display and the significance of her findings within a curated, evaluative showcase environment at Brazil's top-ranked university. This competitive recognition further demonstrates the curated and evaluative nature of the exhibition.

- **Brazilian Congress in Biomedical Engineering (CBEB 2020)**

Dr. Noma presented “Geospatial Analysis of Diagnostic Imaging Equipment in Brazil” at the XXVII Brazilian Congress on Biomedical Engineering (CBEB 2020).

This demonstrates interdisciplinary reach and additional public scientific display in a major national engineering and biomedical forum. The CBEB is the primary showcase for biomedical engineering in Brazil, and selection for display requires passing a rigorous technical review by a committee of experts.

Dr. Noma also publicly displayed original research at:

- III International Symposium of Experimental Pathology (2019)
- VI International Meeting of Biosciences and Physiopathology (2019)

Each of these events included structured poster exhibition sessions and formal scientific programming. These conferences were open to registered international participants, organized by universities and professional societies, structured with designated poster exhibition sessions, documented through official programs and certificates, and signed by institutional leaders and society presidents.

The displayed works were Dr. Noma’s own original research products, not merely institutional outputs. Her consistent selection for these showcases across different years and organizations demonstrates that her work is of a caliber that demands public exhibition.

Importantly, Dr. Noma’s case demonstrates not a single isolated display, but a sustained multi-year pattern (2019–2022) of competitive selection and public exhibition of her scientific work at distinguished venues. This sustained record aligns with the EB-1A requirement of ongoing recognition.

Because artistic exhibitions do not readily apply to biomedical researchers, Dr. Noma properly submits comparable evidence under 8 C.F.R. § 204.5(h)(3). The peer-reviewed public display of her original cancer research at major international and national scientific congresses constitutes the professional equivalent of artistic exhibitions or showcases.

Accordingly, Dr. Noma satisfies this criterion through properly supported comparable evidence demonstrating repeated public exhibition of her original scientific work before distinguished audiences in her field.

In support with this criterion, we submit the following under **Exhibit F**.

F. Evidence of the display of the Petitioner's work in the field in Public Exhibitions.

F.1. Certificate of Poster Presentation, 19th International Congress of the Society for Melanoma Research (SMR), Edinburgh, Scotland (2022).

F.2. Official Abstract Book, 19th International Congress of the SMR, featuring Dr. Noma's research (p. 1) (2022).

F.3. Society for Melanoma Research (SMR) "About" and "Congress" Documentation, confirming the Scientific Review Committee and competitive selection process.

F.4. Certificate of Presentation and Honorable Mention, FCF/USP 50th Anniversary Symposium (2022). **(See Exhibt B.1. and B.2.).**

F.5. Certificate of Presentation, XXII Brazilian Congress of Toxicology (CBTox), organized by the Brazilian Society of Toxicology (SBTox) (2022).

F.6. Brazilian Society of Toxicology (SBTox) "About" Documentation, confirming its affiliation with the International Union of Toxicology (IUTOX).

F.7. Official Proceedings (Anais), III International Symposium of Experimental Pathology (ISEP), featuring Dr. Noma's research (p. 40) (2019).

F.8. Certificate of Presentation, XXVII Brazilian Congress in Biomedical Engineering (CBEB) (2020).

F.9. Certificate of Presentation, VI International Meeting of Biosciences and Physiopathology - X Symposium of Biosciences Applied to Pharmacy (2019).

F.10. Certificate of Presentation, FCF/USP 50th Anniversary Symposium (2022).

Thus, Dr. Noma meets this criterion through the provision of comparable evidence, demonstrating that her original scientific research has been repeatedly selected for public exhibition at premier international and national scientific showcases.

Criterion 8. Evidence that the Petitioner has performed in a leading or critical role for organizations or establishments of distinguished reputation.

To demonstrate Petitioner's eligibility under this criterion (8 CFR 204.5(h)(3)(viii)), USCIS first determines whether the person has performed in a leading or critical role for an organization, establishment, or a division or department of an organization or establishment as well as whether the role is (or was) leading or critical. Second, USCIS determines whether the organization or establishment (department or division) for which the petitioner holds or held a leading or critical role, is of distinguished reputation.

Dr. Noma serves as a **Postdoctoral Fellow in the Department of Tumor Microenvironment and Metastasis** at H. Lee Moffitt Cancer Center and Research Institute in Tampa, Florida, one of the most prestigious cancer research institutions in the United States. Her role at Moffitt is not merely administrative or supportive; it is **critical** to the advancement of cutting-edge melanoma research being conducted under the direct mentorship of Dr. Keiran Smalley, Director of the Donald A. Adam Melanoma and Skin Cancer Center of Excellence and a Senior Member of the Department of Tumor Microenvironment and Metastasis.

Dr. Smalley personally recruited Dr. Yonehara Noma to his laboratory and has confirmed in his expert letter (Exhibit B.VI) that her contributions are essential to the research mission of his lab. Specifically, Dr. Smalley states:

> *"Her expertise in epigenetic modulation and immune response, coupled with her strong publication record and ability to present complex data effectively, positions her to lead future research initiatives that will explore novel therapeutic avenues for melanoma and other skin cancers. Given the pressing public health issue that melanoma represents, particularly among younger demographics, Dr. Noma's ongoing contributions to our research efforts will be essential in shaping new prevention and treatment strategies. Her commitment to excellence and her collaborative spirit will undoubtedly foster continued innovation within Moffitt Cancer Center, making her a vital component of our mission to combat melanoma and improve the lives of those affected by this disease."*

Dr. Yonehara Noma's critical role centers on a **novel and highly specialized research project** investigating the role of HDAC8 as a regulator of cellular response to ultraviolet (UV) radiation in the context of melanoma development. This project requires an exceptionally high level of technical expertise that, as Dr. Smalley confirms, **"only a few researchers possess."** Dr. Noma's critical role involves the manipulation of genetically engineered mouse models and the implementation of advanced UV irradiation protocols to analyze UV-induced effects on cell survival, intracellular signaling, and immune escape pathways. Her work further requires the analysis of high-content single-cell RNA sequencing (scRNA-seq) data through sophisticated bioinformatics and flow cytometry techniques.

The scientific significance of Dr. Yonehara Noma's work at Moffitt is substantial. Her research has demonstrated that **overexpression of HDAC8 in UV-irradiated melanocytes and melanoma cells enhances their survival**, identifying HDAC8 as a potential therapeutic target for limiting malignant cell survival following UV damage. Her findings further revealed that **HDAC8+ cells exhibit reduced PD-L1 expression**, suggesting a mechanism for immune evasion and opening

new avenues for immunotherapy development. These discoveries directly advance Moffitt's mission to develop innovative cancer treatments and improve patient outcomes.

The critical nature of Dr. Yonehara Noma's role is further underscored by the fact that Dr. Smalley **re-recruited her** for a second postdoctoral appointment (April 2025 Offer Letter, Exhibit B.VI), demonstrating that her specialized skills and contributions are irreplaceable within his research program. This renewed appointment, with a salary of $64,500.80 annually plus $4,500 in relocation assistance, reflects the institution's recognition of her indispensable value to ongoing research efforts.

As for the organization itself, H. Lee Moffitt Cancer Center and Research Institute holds an unambiguously distinguished reputation as a premier cancer research and treatment institution. This status is established by its designation as a National Cancer Institute (NCI) Comprehensive Cancer Center, an elite distinction held by fewer than 60 institutions in the United States. Moffitt's national prestige is further evidenced by its consistent top-tier rankings, including being named the #1 cancer hospital in Florida and the Southeast and #10 in the nation by Newsweek. Beyond clinical care, the institution is recognized as a leading employer by Forbes and Becker's Hospital Review, reflecting its sustained excellence as a major healthcare organization.

The institution's research impact is driven by specialized divisions such as the Department of Tumor Microenvironment and Metastasis, which conducts cutting-edge research on molecular mechanisms and novel therapeutic strategies. Moffitt's global influence is extended through strategic collaborations with pharmaceutical leaders like AstraZeneca to accelerate oncology cell therapy development. With a workforce of over 9,000 team members, an annual economic impact of $2.4 billion, and more than 350 active clinical trials, Moffitt achieves cancer survival rates up to four times the national average, solidifying its role as a distinguished pillar of the scientific community.

The record establishes that Dr. Noma performs in a critical role at H. Lee Moffitt Cancer Center and Research Institute. Her specialized expertise in epigenetics, UV-induced melanoma biology, and advanced molecular techniques makes her an irreplaceable contributor to Dr. Smalley's research program and Moffitt's broader mission. The personal recruitment by the Director of Moffitt's Melanoma and Skin Cancer Center of Excellence, the highly specialized nature of her work, and the institution's characterization of her contributions as "essential" and "vital" confirm that Dr. Noma satisfies the requirements of 8 C.F.R. § 204.5(h)(3)(viii).

In support with this criterion, please refer to the documentary evidence in **Exhibit G.**

Exhibit G. Evidence that the Petitioner has performed in a critical role for an organization of distinguished reputation

G.1. Postdoctoral Fellow Offer Letter, H. Lee Moffitt Cancer Center and Research Institute (April 28, 2025)

G.2. Letter Confirming International Scientific Award Recognition and Critical Role – Dr. Keiran Smalley, Director, Melanoma and Skin Cancer Center of Excellence, Moffitt Cancer Center (November 6, 2025)

G.3. Institutional Profile and Economic Impact Documentation, "About Moffitt," confirming NCI Comprehensive Cancer Center designation and $2.4 billion economic impact

G.4. National Ranking Reports, Newsweek "America's Best Cancer Hospitals" (2023 and 2024), identifying Moffitt as #1 in Florida and the Southeast and Top 10 in the Nation

G.5. Institutional Awards and Recognition Records, confirming Moffitt as a Forbes "America's Best Large Employer" and Becker's "150 Top Places to Work in Healthcare"

G.6. Departmental Overview, "Tumor Microenvironment and Metastasis Department," detailing the specialized research program and elite clinician-scientist faculty

G.7. Strategic Partnership Announcements, documenting high-profile oncology collaborations between Moffitt Cancer Center, AstraZeneca, and CMIC Group

G.8. Institutional Fellowship Standards, "Postdoctoral Training" and "Become a Moffitt Postdoc," identifying fellows as "outstanding scientific leaders" within the NCI-designated research environment.

Thus, Dr. Noma meets this criterion as the attached evidence demonstrates she has performed in critical roles for establishments that have a distinguished reputation pursuant 8 CFR 204.5(h)(3)(viii).

Final Merits Determination

At the final merits stage, USCIS must evaluate the petition in its entirety to determine whether the evidence demonstrates, by a preponderance of the evidence, that the beneficiary has achieved **sustained national or international acclaim** and that her achievements place her among the **small percentage who have risen to the very top of the field of endeavor**, pursuant to 8 C.F.R. § 204.5(h)(2) and interpreted under *Kazarian v. USCIS*. This analysis is qualitative and holistic, focusing not merely on whether individual criteria are met, but whether the totality of the evidence demonstrates extraordinary ability beyond that of a highly accomplished professional.

When the record is considered as a whole, Dr. Noma's career reflects a consistent trajectory of elite achievement, validated by independent global data and recruitment by the world's leading oncology institutions.

Dr. Noma's standing at the top of the field is anchored by original scientific discoveries that have moved from theoretical research to independent clinical validation. Her identification of **PRDX2 as a key regulator of invasion in NRAS-mutated melanoma** is not merely a localized finding; the record shows this discovery was **independently validated using a United Kingdom databank of 700 primary melanoma patients**. This level of external validation by an international cohort proves that her work has a documented impact on global clinical

understanding. Furthermore, her research on **HDAC8-mediated epigenetic reprogramming** provides a novel mechanistic framework for understanding early melanoma initiation, a contribution that independent experts in the field describe as "pivotal" and "transformative" for the future of melanoma prevention.

The international scientific community's reliance on Dr. Noma is evidenced by her publication record in high-impact, peer-reviewed journals such as ***Biomedicine & Pharmacotherapy*** and ***Antioxidants***. Unlike a typical researcher, Dr. Noma's citation impact shows an extraordinary and accelerating trajectory. The evidence from her **Google Scholar profile** demonstrates a **31% increase in total citations in just four months** (between October 2025 and February 2026), proving that her findings are currently being integrated into the work of scientists worldwide at an increasing rate. This is not static achievement but sustained, growing acclaim.

Dr. Noma's authority is further confirmed by her role in judging the work of her peers. Editorial boards of international scientific journals have repeatedly sought her expertise to serve as a **peer reviewer**, a role reserved for those whose technical mastery is recognized as superior. By evaluating the manuscripts of other scientists, she actively shapes the standards of oncology research.

The record documents Dr. Noma's sustained international visibility through the public exhibition of her research at the most competitive venues in her discipline. Her invitations to present original findings at the **Society for Melanoma Research (SMR) Congress**, which is the premier global forum for melanoma experts, demonstrate that her work is considered essential viewing for the international scientific elite. These presentations are not merely academic exercises but are public acknowledgments that her research represents the "state-of-the-art" in tumor biology.

Dr. Noma's excellence has been recognized through **merit-based awards and honors**, which were conferred in recognition of her outstanding research performance and academic rigor. These distinctions, coupled with her recruitment to perform a **critical research role at the H. Lee Moffitt Cancer Center**, an **NCI-designated Comprehensive Cancer Center**, provide compelling evidence of her elite status. Moffitt's decision to recruit Dr. Noma to advance its mission in melanoma research constitutes a "critical role" determination by one of the most distinguished oncology institutions in the United States.

Finally, the fact that USCIS has previously approved Dr. Noma's **O-1A nonimmigrant petition** provides persuasive evidence of her sustained status at the top of her field. While the EB-1A standard is distinct, the continuity of her high-level achievements, from her doctoral work in Brazil to her high-level research in the U.S., demonstrates that she has not only reached the top of her field but has maintained that position through continuous, impactful contribution.

Under the *Kazarian* framework, the evidence demonstrates that Dr. Noma is an individual of extraordinary ability who has:

- Produced clinically validated discoveries (PRDX2/HDAC8) that have materially advanced melanoma biology;
- Achieved an accelerating citation impact (31% growth) reflecting global reliance on her work;

- Been recognized through merit-based awards;
- Served as an authoritative judge of the work of other scientists in her field;
- Exhibited her research at the world's most prestigious melanoma congresses (SMR); and
- Been recruited for a critical role by a premier NCI-designated Cancer Center (Moffitt).

Taken together, these accomplishments establish that Dr. Noma is among the small percentage of scientists who have risen to the very top of the field of oncology and tumor biology. Her work has moved the needle of scientific progress, and her continued presence in the field ensures the advancement of critical cancer research. Accordingly, Dr. Noma qualifies for classification as an Alien of Extraordinary Ability.

Conclusion and Request for Favorable Adjudication

In conclusion, Dr. Noma's high-level achievements demonstrate that she meets and exceeds the criteria required for the EB-1A visa classification. Her work has left a significant and lasting impact on the oncology community, as evidenced by her numerous accolades, strong citation metrics, and the widespread recognition her research has received from independent experts. Dr. Noma stands out as a leading researcher in her specialized area and a vital asset to the American scientific infrastructure.

Applying the preponderance of the evidence standard, the totality of the record contains extensive, credible, and objective documentation demonstrating that Dr. Noma satisfies **at least six** of the ten regulatory criteria. More importantly, these achievements place her among the small percentage of scientists who have risen to the very top of the field. The evidence establishes sustained national and international recognition over time, repeated selection by distinguished organizations, and a consistent pattern of high-level performance in cutting-edge cancer research.

Granting Dr. Noma eligibility for this immigrant classification will enable her to continue making valuable contributions to the United States as a researcher of sustained elite status. We kindly request that you consider her application favorably and grant her the opportunity to further her outstanding career in the United States.

If you have any questions, please do not hesitate to contact us.

Sincerely,

Otavio Haverroth Silva
California Bar # 343486

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Exhibit G. Evidence that the Petitioner has performed in a critical role for an organization of distinguished reputation

G.1. Postdoctoral Fellow Offer Letter, H. Lee Moffitt Cancer Center and Research Institute (April 28, 2025) 639-641

G.2. Letter Confirming International Scientific Award Recognition and Critical Role – Dr. Keiran Smalley, Director, Melanoma and Skin Cancer Center of Excellence, Moffitt Cancer Center (November 6, 2025) 642-667

G.3. Institutional Profile and Economic Impact Documentation, "About Moffitt," confirming NCI Comprehensive Cancer Center designation and $2.4 billion economic impact 668-675

G.4. National Ranking Reports, Newsweek "America's Best Cancer Hospitals" (2023 and 2024), identifying Moffitt as #1 in Florida and the Southeast and Top 10 in the Nation 676-683

G.5. Institutional Awards and Recognition Records, confirming Moffitt as a Forbes "America's Best Large Employer" and Becker’s "150 Top Places to Work in Healthcare" 684

G.6. Departmental Overview, "Tumor Microenvironment and Metastasis Department," detailing the specialized research program and elite clinician-scientist faculty 685-691

G.7. Strategic Partnership Announcements, documenting high-profile oncology collaborations between Moffitt Cancer Center, AstraZeneca, and CMIC Group 692-698

G.8. Institutional Fellowship Standards, "Postdoctoral Training" and "Become a Moffitt Postdoc," identifying fellows as "outstanding scientific leaders" within the NCI-designated research environment.	699-706

Exhibit H. Final Merits Determination

Previously Approved Dr. Noma’s O-1A Nonimmigrant Petition	707-708

Exhibit I. Personal Documents

Birth Certificate and Corresponding Translation	709-711
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U.S. Visa Page	713-714
I-94	715-716
Marriage certificate and Corresponding Translation	717-719
Derivative’s Birth Certificate	720-722
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Derivative’ s I-94	725-726

Exhibit A. Evidence on Dr. Noma

To the Authorities of the United States,

Subject: Petition Regarding Professional Career and Contributions

Dear Sir or Madam,

My name is Isabella Harumi Yonehara Noma, and this petition reflects my deep commitment to professional excellence, research, and education. Through this letter, I present a detailed account of my academic background and my contributions over 10 years of experience, as well as my commitment to professional and academic development.

With a diverse educational background and extensive experience across several areas of biomedical research, I believe I am fully prepared to make a meaningful contribution to my field of expertise in the United States.

Academic and Professional Background

My academic background includes a bachelor's degree in Biomedical Science, as well as a specialization in Biomedical Engineering and an MBA in Data Analytics. I also completed a Master's degree in Health Sciences and a Doctorate in Science with an emphasis on pathophysiology and toxicology. Postdoctoral research conducted in collaboration with the University of São Paulo (USP) and the Moffitt Cancer Center, USA.

I have worked in academia for 10 years, with experience in several biomedical fields, including molecular biology, cell biology, cancer pathophysiology, genetics, and epigenetics. Throughout my career, I participated in outreach projects aimed at providing information about genetic diseases to the public in a simple and accessible way. I participated in teaching projects during my undergraduate studies in microbiology and immunology, in addition to providing tutoring sessions for undergraduate students. Presentation of posters and research papers during academic week events and other conferences. During my master's degree, I had the opportunity to participate in the organization of symposia related to pharmacy (VI and VII International Meeting of Biosciences and Physiopathology and the Symposium on Biosciences Applied to Pharmacy), in addition to publishing articles resulting from my master's research and other collaborative publications. During my doctoral studies, due to the pandemic, I began my research and meetings remotely, with participation in various online conferences and lectures. After the pandemic, it was possible to begin

the studies, resulting in publications arising from my dissertation, as well as from national and international collaborations. Participation in international conferences, including receiving travel awards that enabled my in-person attendance and presentation of research results, I also received an honorable mention for the presentation of a research project, as well as the opportunity to deliver a lecture in a lecture series by invitation of the Department of Graduate Studies in Pharmacy at the University of São Paulo. In summary, I have published 13 articles in indexed journals, presented 10 extended abstracts at conferences, presented 13 short abstracts at conferences, delivered 28 research presentations, attended 39 events as a participant, and contributed to the organization of 3 events.

I have experience in drafting academic articles, presenting research, conducting scientific research, performing *in vitro and in vivo* biological experiments, problem-solving, statistical analysis, teamwork, and scientific writing.

Contributions and Potential for Professional Development in the United States of America

Based on my professional experience, diverse educational background, and contributions over the years, I believe I can add value to the academic and professional community in the United States. My work is aligned with the mission of promoting improvements in melanoma treatments and providing important new insights into how it can be prevented and treated. My research in this field leads national efforts to improve the treatment of melanoma patients, supporting new strategies and public health information focused on prevention, early detection, and innovative treatment approaches for melanoma and other skin cancers, saving the lives of many Americans facing this terrible disease.

I intend to develop projects that address important biological questions, foster international collaborations, and support the training of future professionals. With my experience and academic and professional output, I am confident that my contribution will bring significant impacts to the community, such as new studies in the field of cancer, collaborations with other countries, and advancements in research and technology in the field.

Final Considerations

This petition reflects my commitment to professional excellence, research, and development. I stand ready to contribute in a substantial and lasting manner to the United States by promoting advances in the fields in which I work and strengthening professional and academic ties.

Thank you for your attention to this petition, and I remain available to provide any clarification that may be necessary.

Sincerely,

Isabella H. Y. Noma

Date:11/14/2025

Isabella Harumi Yonehara Noma – Postdoctoral Researcher.

I, André Vinícius Inacio Penna Mello, telephone number 415 425-2508, mailing address P.O. Box 90487, San Diego, CA 92169, certify that the professional translation of this document from Portuguese to English has been performed by myself, a qualified translator fluent in both languages, and that the following is an accurate and complete translation of the document.

Date: April 23, 2026.

Às Autoridades dos Estados Unidos,

Assunto: Petição sobre Trajetória Profissional e Contribuições

Prezados Senhores,

Meu nome é Isabella Harumi Yonehara Noma, e esta petição reflete meu profundo comprometimento com a excelência profissional, pesquisa e formação. Por meio desta carta, apresento um detalhamento da minha trajetória acadêmica e minhas contribuições ao longo de 10 anos de experiência, bem como meu compromisso com o desenvolvimento profissional e acadêmico.

Com uma formação diversificada e uma vasta experiência em diversas áreas de atuação na pesquisa biomédica, acredito estar plenamente preparado para contribuir de forma significativa para a minha área de atuação nos Estados Unidos.

Trajetória Acadêmica a e Profissional

Minha formação acadêmica inclui graduação em Biomedicina, além de especialização em Engenharia biomédica e MBA em análises de dados. Também concluí meu Mestrado em Ciências da Saúde e Doutorado em Ciências com ênfase em fisiopatologia e toxicologia. Pós-doutorado em pesquisa colaboração com a USP e o Moffitt Cancer Center, USA.

Atuo na academia há 10 anos, com experiência em diversas áreas biomédicas desde biologia molecular, biologia celular, fisiopatologia do câncer, genética e epigenética. Durante minha trajetória, participei de projetos de extensão voltados para a trazer informações de doenças genéticas de uma forma simples para sociedade. Participei de projetos de ensino durante a graduação em microbiologia e imunologia, além de ministrar monitorias para alunos da graduação. Apresentação de posters e trabalhos durante a semana acadêmica e outros congressos. Durante meu mestrado tive a oportunidade de participar como na organização de simpósios aplicados a farmácia (VI e VII International Meeting of Biosciences and Physiopathology, Simpósio de Biociências Aplicadas à Farmácia), além de publicação de artigos do mestrado e outros em colaboração. Durante o doutorado, devido a pandemia iniciei meus estudos e reuniões no modo remoto, com diversas participações em congressos e palestras on-line. Após a pandemia, foi possível iniciar os estudos resultando em publicações decorrentes da tese, assim como de

colaboração nacionais e internacionais. Participações de congressos internacionais com a obtenção de prêmios viagens que possibilitaram a participação presencial com apresentação de resultados, também obtive uma menção honrosa decorrente da apresentação de um trabalho, e apresentação de uma palestra em um ciclo de palestras a convite do departamento de pós-graduação em farmácia da USP. Em resumo foram 13 artigos publicados em revistas indexadas, 10 resumos expandidos apresentados em congressos, 13 resumos simples apresentados em congressos, 28 apresentações de trabalhos, 39 participações como ouvinte em eventos e 3 participações em organização de eventos.

Tenho experiência na elaboração de artigos acadêmicos, apresentação de trabalhos, pesquisa científica, experimentos biológicos *in vitro* e *in vivo*, resolução de problemas, análises estatísticas, trabalho em equipe, e escrita científica.

Contribuições e Potencial para o Desenvolvimento Profissional nos Estados Unidos da América

Com base em minha experiência profissional, formação diversificada e contribuições ao longo dos anos, acredito que posso agregar valor à comunidade acadêmica e profissional nos Estados Unidos. Minha atuação está alinhada com a missão de promover o aprimoramento dos tratamentos para o melanoma oferecem novos insights importantes sobre como ele pode ser prevenido e tratado. Minha pesquisa nessa área lidera os esforços nacionais para aprimorar o tratamento de pacientes com melanoma, fundamentando novas estratégias e informações para a saúde pública com foco na prevenção, detecção precoce e abordagens inovadoras de tratamento para melanoma e outros cânceres de pele, salvando a vida de muitos americanos que enfrentam essa terrível doença.

Pretendo desenvolver projetos que abordem questões biológicas importantes, fomentar colaborações internacionais e oferecer suporte à formação de futuros profissionais. Com minha experiência e produção acadêmica e profissional, estou confiante de que minha contribuição trará impactos significativos à comunidade, como novos estudos na área do câncer, colaborações com outros países, aprimoramento de estudos e tecnologia na área.

Considerações Finais

Esta petição reflete meu comprometimento com a excelência profissional, pesquisa e desenvolvimento. Coloco-me à disposição para contribuir de maneira substancial e duradoura com os Estados Unidos, promovendo avanços nas áreas em que atuo e fortalecendo laços profissionais e acadêmicos.

Agradeço pela atenção dedicada a esta petição e coloco-me à disposição para quaisquer esclarecimentos necessários.

Atenciosamente,

Isabella H. Y. Noma

Data:14/11/2025

Isabella Harumi Yonehara Noma – Pós doutora.

Isabella Harumi Yonehara Noma

URL to access this CV: http://lattes.cnpq.br/6549549395123599
Lattes ID: **6549549395123599**
Last updated on 07/31/2025

Postdoctoral Researcher at the H. Lee Moffitt Cancer Center. Doctor of Science from the School of Pharmaceutical Sciences (FCF) (CAPES score: 7) at the University of São Paulo (USP), with a focus on metastatic melanoma. We investigated the complexity of the microenvironment in the melanoma invasion process using three-dimensional reconstructed skin models at the Skin Pathophysiology Laboratory-USP. Bachelor's degree in Biomedicine from the State University of Maringá (2017), with undergraduate research projects in the areas of Basic Microbiology and Clinical Immunology (UEM). Master's degree in Health Sciences, working at the Clinical Cytology and Sexually Transmitted Diseases Laboratory, with a focus on the detection of HPV and other associated bacteria using molecular biology techniques. **(Text provided by the author)**

Personal Information

Name

Isabella Harumi Yonehara Noma

Name in bibliographic citations

NOMA, I. H. Y.;NOMA, ISABELLA;YONEHARA NOMA, ISABELLA HARUMI;HARUMI YONEHARA NOMA, ISABELLA;NOMA, ISABELLA HARUMI YONEHARA

Lattes iD

http://lattes.cnpq.br/6549549395123599

Orcid iD

? https://orcid.org/0000-0002-7413-1316

Country of Nationality

Brazil

Academic Background/Degree

2020 - 2023

Ph.D. in Pharmacy (Pathophysiology and Toxicology).
University of São Paulo, USP, Brazil.
Title: Peroxiredoxin 2 in the modulation of the invasive phenotype in NRAS-mutant melanoma, Year of completion: 2023.
Advisor: Silvya Stuchi Maria-Engler.
Fellow of: National Council for Scientific and Technological Development, CNPq, Brazil.

2018 - 2020

Master's degree in Health Sciences.
State University of Maringá, UEM, Brazil.
Title: Prevalence of Ureaplasma and

Mycoplasma in women of childbearing age from Maringá, Year of Completion: 2020.
Advisor: Marcia Edilaine Lopes Consolaro.
Fellow of: Coordination for the Improvement of Higher Education Personnel, CAPES, Brazil.
Areas of activity: Scientific research and development.

2023 - 2025

MBA in Data Science and Analytics, Distance Learning. (Course Load: 425h).
University of São Paulo, Luiz de Queiroz College of Agriculture, USP ESALQ, Brazil.
Title: Clustering of Cutaneous and Acral Melanoma: Impact of genetic alterations on immune response.
Advisor: William Barbosa.

2019 - 2020

Specialization in Biomedical Engineering with an emphasis in Clinical Engineering. (Course Load: 360h).
Estácio de Sá University, UNESA, Brazil.
Title: GEOSPATIAL ANALYSIS OF DIAGNOSTIC IMAGING EQUIPMENT ASSOCIATED WITH SOCIODEMOGRAPHIC CHARACTERISTICS IN BRAZIL.

2014 - 2017

Bachelor's degree in Biomedicine.
State University of Maringá, UEM, Brazil.
Title: Evaluation of the nociceptive response of mice infected with Leishmania (Leishmania) amazonensis and its relationship with nitric oxide.
Advisor: Sandra Mara Alessi Aristides.
Scholarship holder of: Coordination for the Improvement of Higher Education Personnel, CAPES, Brazil.

2011 - 2013

High School. (Secondary education).
Santa Cruz School, CSC, Brazil.

Postdoctoral Research

2023 - 2024

Postdoctoral Research.
H. Lee Moffitt Cancer Center and Research Institute, HLMCCRI, United States.
Fellow of: National Council for Scientific and Technological Development, CNPq, Brazil.
Major area: Health Sciences

Complementary Education

2020 - 2020

University extension course in DES-UEM Distance Courses – Applied Biostatistics. (Course load: 34h).
State University of Maringá, UEM, Brazil.

2020 - 2020

Health in Data: How to Work with the DataSUS Systems. (Course load: 2h).
State University of Maringá, UEM, Brazil.

2020 - 2020

Introduction to Machine Learning. (Course load: 2h).
State University of Maringá, UEM, Brazil.

2020 - 2020

French Language A2 - Paraná fala Francês (Paraná Speaks Ffrench – Language program)- Module 2 Online. (Couser load: 30h).
State University of Maringá, UEM, Brazil.

2020 - 2020

1st International Digital Congress on 3D Biofabrication and Bioprinting. (Course load: 8h).
Araraquara University, UNIARA, Brazil.

2020 - 2020

1st Basic Oncology Course of LAOMA.
(Course load: 10h).
Araraquara University, UNIARA, Brazil.

2020 - 2020

Understanding Cancer Metastasis. (Course load: 4h).
Johns Hopkins University, JHU, United States.

2020 - 2020

Introduction to the Biology of Cancer. (Course load: 8h).
Johns Hopkins University, JHU, United States.

2020 - 2020

Cell Culture and Applications in the Study of Chronic and Infectious Diseases. (Course load: 15h).
Federal University of Paraná, UFPR, Brazil.

2020 - 2020

XI Board Review in Medical Oncology: Update, Discussion, and Innovation and II. (Course load: 40h).
Einstein's Teaching and Research Institute, IIEPAE, Brazil.

2020 - 2020

Systematic Review: Essential Elements for Reliable Evidence. (Carga horária: 2h). State University of Maringá, UEM, Brazil.

2020 - 2020

Oral Communication and Academic Activities-Online Module. (Course load: 35h).
University of São Paulo, USP, Brazil.

2019 - 2019

University extension course in the 1st Vacation Course in Immunology and Immunogenetics. (Course load:

40h).
State University of Maringá, UEM, Brazil.

2019 - 2019

University extension course in UEM at Expoingá. (Course load: 5h).
State University of Maringá, UEM, Brazil.

2019 - 2019

University extension course in Active Learning. (Carga horária: 4h).
State University of Maringá, UEM, Brazil.

2019 - 2019

University extension course in Paraná fala francês A1- level 2. (Course load: 60h).
State University of Maringá, UEM, Brazil.

2019 - 2019

Conversation in French laguage. (Course load: 8h).
State University of Maringá, UEM, Brazil.

2019 - 2019

Discovering Phages. (Course load: 15h).
State University of Maringá, UEM, Brazil.

2019 - 2019

Extracellular Vesicles Involved in Chronic and Infectious Diseases. (Course load: 4h).
State University of Maringá, UEM, Brazil.

2019 - 2019

Primers: who are they and how to outline them?.
State University of Londrina, UEL, Brazil.

2018 - 2018

University extension course in Paraná fala Francês. (Course load: 60h).
State University of Maringá, UEM, Brazil.

2018 - 2018

University extension course in Paraná fala Inglês (Paraná Speaks Englsih). (Course load: 30h).
State University of Maringá, UEM, Brazil.

2016 - 2016

Antibiotic as Promotors of Antibiotic Resistance. (Course load: 2h).
Graduate Program in Biosciences and Pathophysiology, PBF, Brazil.

2014 - 2016

Japanese language. (Course load: 300h). State University of Maringá, UEM, Brazil.

2015 - 2015

In Situ Hepatic Perfusion. (Course load: 4h).
State University of Maringá, UEM, Brazil.

2015 - 2015

Study and Guidance for the Japanese Language Proficiency Examination N.05. (Course load: 12h).
State University of Maringá, UEM, Brazil.

2014 - 2014

Assisted Reproduction. (Course load: 4h).
State University of Londrina, UEL, Brazil.

Professional Experience

H. Lee Moffitt Cancer Center and Research Institute, HLMCCRI, United States.

Institutional Affiliation

2025 - Current

Employment Type: Employee under the Brazilian Labor Code (CLT), Position: Postdoctoral Researcher, Workload: 40, Work Schedule: Exclusive dedication.

Institutional Affiliation

2023 - 2024

Employment Type: Fellow, Position: Postdoctoral Researcher, Workload: 40

State University of Maringá, UEM, Brazil.

Institutional Affiliation

2018 - 2020

Employment Type: Fellow, Position: Graduate Student, Workload: 40, Work Schedule: Exclusive dedication.

Other information

Master's Degree in the Health Sciences Program - Fellow

Institutional Affiliation

2017 - 2017

Employment Type: Intern, Position: Mandatory Internship in Immunogenetics, Workload: 40

Institutional Affiliation

2017 - 2017

Employment Type: Intern, Position: Mandatory Internship in Clinical Analysis, Workload: 40

Institutional Affiliation

2014 - 2017

Employment Type: Teaching Project, Position: Laboratory Practice, Workload: 4

Institutional Affiliation

2015 - 2016

Employment Type: Fellow, Position: Microbiology Teaching Assistant, Workload: 20

University of São Paulo, USP, Brazil.

Institutional Affiliation

2022 - 2022

Employment Type: Fellow, Position: Teaching Intern, Workload: 6

Research projects

2023 - 2024

The role of HDAC8 in UVR-driven melanoma initiation

Description: Exposure to ultraviolet radiation (UVR) is the major risk factor for melanoma development and leads to complex phenotypic responses in melanocytes, including dendrite formation, enhanced melanogenesis, and cell cycle arrest. Previous studies identified the type I histone deacetylase,HDAC8, as an epigenetic regulator of melanoma cells induced following exposure to UVR,hypoxia, and BRAF inhibitor therapy. Our studies suggest that HDAC8 expression is upregulated in melanocytes that experience high levels of UVR-induced damage. No strategies yet exist to prevent the accumulation of sun-damaged melanocytes in the skin, representing a significant barrier to effective melanoma prevention. In this new proposal, we aimed to investigate whether UVR-induced HDAC8 activation allows some melanocytes to evade cell death, enabling cells with pathogenic mutations to survive and potentially develop into melanoma. We utilized a unique mouse melanoma model driven by mutant BRAF and heterozygous PTEN loss, in which HDAC8expression can be conditionally regulated in skin melanocytes, to elucidate the

role of epigenetic reprogramming in UVR-induced melanoma initiation. Additionally, we employed siRNA mediated overexpression of HDAC8 in cell lines. Our findings demonstrated that HDAC8overexpression following UV irradiation increases the survival of UVR-damaged melanocytes and melanoma cells, mediated by the downregulation of p53 and acetylated p53. This research provides a novel perspective on how UVR-induced epigenetic reprogramming targeting HDAC8contributes to the persistence of melanocytes and melanoma cells harboring pathogenic mutations, allowing them to grow and evade apoptosis..
Status: Completed; Type: Research.

Participants: Isabella Harumi Yonehara Noma - Participant / Silvya Stuchi Maria-Engler - Coordinator / SMALLEY, KEIRAN S. M. - Participant.

2023 - Current

METASTATIC POTENTIAL OF PEROXIREDOXIN 2 IN NRAS-MUTANT MELANOMAS

Description: Description: Cutaneous melanoma is a curable neoplasm when diagnosed at early stages, but it is associated with a worse disease course in advanced stages. Melanoma lesions in sun-exposed sites, associated with a high mutational burden and aberrant epigenetic regulation, contribute to the development and progression of the disease. This is the type of skin cancer with the highest metastatic potential, which leads to its high mortality rate. The main mutations occur in the MAPK pathway: 50% of patients have alterations in BRAF, 15-20% in NRAS, and the remainder in NF1 or are triple wild-type. Targeted therapy revolutionized the treatment of melanoma with the use of BRAF and MEK inhibitors; however, intrinsic and acquired resistance leads to treatment failure. In this context, NRAS mutations characterize an even more aggressive tumor with no specific therapeutic options, associating this mutation with a poorer prognosis. Intratumoral heterogeneity occurs as a consequence of the phenotypic plasticity of the cells, which assume and transition through different transcriptional states controlled by MITF levels, making the cells more proliferative or invasive. This event is associated with metastasis and therapy failure. Therefore, alternative pathways should be explored as potential options for the treatment of patients with NRAS mutations. Given that one of the factors limiting the efficacy of targeted therapy is resistance to oxidative stress, the control of redox homeostasis is an interesting starting point. Hydrogen peroxide plays a central role in redox signaling and is involved in all hallmarks of cancer, from proliferation to metabolism, angiogenesis, and immune system evasion. In addition, hydrogen peroxide is capable of altering MITF levels. In this context, molecules that regulate its activity are of substantial interest. Peroxiredoxins (PRDXs) are the main hydrogen peroxide-consuming enzymes in cells due to their high expression and catalytic capacity. Several studies report the role of antioxidant enzymes in redox homeostasis and tumorigenesis. In addition to the exceptional role of PRDXs in removing the oxidant from the environment, when they become oxidized and

inactive, they allow hydrogen peroxide to react directly or indirectly with other targets, thereby regulating the cell's redox signaling. Due to their central role in controlling hydrogen peroxide, PRDXs are directly involved in different types of cancer. In fact, the PRDX2 isoform is epigenetically silenced in some cancers, and our group recently demonstrated that it is also downregulated in patients with melanoma metastasis, reinforcing its involvement in the invasion process. In this context, it has been shown that PRDX2 is capable of inducing the stability of adherens junctions in BRAF-mutant melanomas. However, there are still no studies relating PRDXs to NRAS mutation; thus, we observed that NRAS-mutant melanoma cell lines showed different proliferative and invasive profiles, corresponding to differences in PRDX2 expression. Thus, our proposal is to investigate the role of PRDX2 in the phenotypic plasticity of NRAS-mutant melanomas. To this end, the silencing of this protein will be studied through gene editing (shRNA) and by a pharmacological PRDX inhibitor (Conoidin A), in addition to the effect of its restoration through the use of gliotoxin, a PRDX mimetic. Functional parameters such as proliferation, colony-forming ability, migration, and invasion in 3D skin models will be evaluated. Such observations reinforce our hypothesis that PRDX2 may be a novel target in the search for more specific therapies for NRAS-mutant melanomas.
Status: In progress; Type: Research.
Students involved: Doctoral students: (3) .

Participants: Isabella Harumi Yonehara Noma - Coordinator / Silvya Stuchi Maria-Engler - Participant / Larissa Anastácio da Costa Carvalho - Participant.

2016 - 2017

Variability of β-lhamases present in Gram-negative bacilli isolated during the hospitalization of patients in a Teaching Hospital

Project certified by Coordinator Maria Cristina Bronharo Tognim on 11/11/2019.

Description: Considering the importance of Gram-negative bacteria (GNB) in hospitals, especially in intensive care units, due to their antimicrobial resistance, and also considering that patients may be sources or reservoirs of these microorganisms, this project aims to assess the presence of different GNB that may colonize or infect patients during their hospital stay. As methodology, we will use the selection of patients who were registered in an Excel database from the project "Epidemiological Surveillance of Multidrug-Resistant Bacteria at the University Hospital of Maringá". Patients will be selected by the initials of their names, and only patients who present microbiological data on GNB isolates from colonization and infection will be included. All microorganisms will be retrieved from the freezer collection, and resistance to carbapenems will be confirmed by the agar dilution method. For all isolates, tests will be performed to detect β-lactam resistance

markers by PCR for ESBLs (bla CTX, bla SHV e bla TEM), as well as for oxacillinase-type carbapenemases and metallo-β-lactamases. The isolates will be molecularly characterized using the ERIC technique. For some cases, the use of antimicrobials in the patients' medical records will be evaluated. The aim is to use the findings to better understand the dynamics of the microorganism and its control.
Status: Completed; Type: Research.
Students involved: Undergraduate: (2) / Doctoral: (1).

Participants: Isabella Harumi Yonehara Noma - Participant / Alessandra Barrochelli da Silva Ecker - Participant / Déborah de Castro Moreira - participant / Maria Cristina Bronharo Tognim - Coordinator.

2016 - 2016

RELATIONSHIP BETWEEN NOCICEPTION AND NITRIC OXIDE PRODUCTION IN ANIMALS INFECTED WITH LEISHMANIA (LEISHMANIA) AMAZONENSIS

Project certified by Coordinator Thais Gomes Verzignassi Silveira on 03/02/2021.

Description: American tegumentary leishmaniasis, considered a neglected disease, is endemic in many countries, including Brazil. The disease is characterized by ulcers and may heal spontaneously or present severe and mutilating metastases in the mucosa. The lesions generally progress to ulcerations in the absence of pain, suggesting that the infection interferes with pain perception. In this project, we evaluated the pain threshold using increasing doses of the parasite with an electronic aesthesiometer, as well as paw edema in lesions induced by parasite inoculation, measured weekly with the aid of a plethysmometer and a caliper. The results showed an increase in edema as the parasite concentrations increased, as well as an increase in pain intensity. We also evaluated nitric oxide production in the paw lesions of animals inoculated with the parasite; however, the preliminary results were negative. In conclusion, we found that edema formation is accompanied by increased pain; however, nitric oxide production does not appear to be related to pain or edema.
Status: Completed; Type: Research.
Students involved: Undergraduate: (2) / Academic master's: (1).

Participants: Isabella Harumi Yonehara Noma - Participant / Paula Karoline Sfordi da Silva - Participant / Valéria Medrado Pereira - Participant / Sandra Mara Alessi Aristides - Participant / Thais Gomes Verzignassi Silveira - Coordinator.

Outreach projects

2016 - 2017

Dr. Genetics: Online Informational Content to Assist with Human Genetic Testing

Description: The dissemination of information through the internet has become increasingly rapid and accessible to the population as a whole. At the beginning of its use, access to it was, for the most part, limited to technology enthusiasts and professionals in the field; however, over the years, both access and the content available have come to be largely produced by laypeople on the subject. Brazilians represent the largest share worldwide in terms of average time spent browsing, enabling a major exchange of information, including in the field of health. Although very useful for access to information, many specialists emphasize that much of the information made available is inadequate or incomplete. In Brazil, there is no regulation or certification of websites containing health information. Therefore, it becomes essential to make information available to the general public, information provided by universities and grounded in scientific knowledge, which can thus help strengthen physician-patient trust and reduce the anxiety and discomfort resulting from illnesses. The objective of this project is to develop a website with information about genetic tests, including cytogenetic and molecular tests, in the various areas of genetics, including genetic counseling and oncology, among others. In addition to supplementary information, such as interpretation, where they can be performed, and their costs both through the Unified Health System and through health insurance. The website will be promoted through visits and posters at health clinics in Maringá, Maringá Municipal Hospital, and Maringá University Hospital. The information made available will be based on books, indexed articles, and supporting laboratories and hospitals. Students in the fields of Biomedicine and Medicine participating in the project will gain knowledge about genetic tests and will have the opportunity to transmit this knowledge to the general population, since the demand for such tests is increasing, whether for diagnostic assistance, prognosis, or even the establishment of therapeutic approaches, taking into account the promising development of new targeted molecular drugs.
Status: Completed; Type: Outreach.
Students Involved: Undergraduate: (7) .

Participants: Isabella Harumi Yonehara Noma - Participant / Tamires Leite Valverde - Participant / Veronica Elisa Pimenta Vicentini - Participant / Luiz Felipe MSB Blanco - Participant / Lucas B Gremaschi - Participant / Patrícia Alexandra da Silva Saavedra Romão - Participant / Natalia Mestre Braz - Participant / Eliane Papa Ambrosio Albuquerque - Coordinator.

2016 - 2016

Student integration in the execution of volunteer activities, aiming at greater dissemination and awareness among the Maringá community about little-known diseases that manifest in childhood, progressive muscular dystrophies with emphasis on Muscular Dystrophy.

Project certified by Coordinator Maria Ida Bonini Ravanelli Speziali on 19/10/2022.

Description: It is believed that the vast majority of society is unaware of the occurrence of some diseases that affect children. Muscular dystrophies fit perfectly within the group of little-known diseases. Although they are considered rare, their incidence strongly contradicts this premise. Through this work, the aim is, through the integration of students from the various areas of the State University of Maringá, to carry out activities that promote greater knowledge within society about diseases that affect children. In this way, the first year of this project, which intends to address one disease per year, will direct its activities toward muscular dystrophies, thus allowing society to mobilize by participating in events committed to improving the quality of life of patients affected by Duchenne muscular dystrophy, chosen as the slogan of this project because it is the most frequent as well as the most lethal among all muscular dystrophies.
Status: Completed; Type: Outreach.
Students Involved: Undergraduate: (13) .

Participants: Isabella Harumi Yonehara Noma - Participant / Paula Karoline Sfordi da Silva - Participant / Tamires Leite Valverde - Participant / Patrícia Alexandra da Silva Saavedra Romão - Participant / Natalia Mestre Braz - Participant / Lais de Sena Marques - Participant / Maria Ida Bonini Ravanelli Speziali - Coordinator / Fabiana Galvão da Motta Lima - Participant / Francyhellen Franco Montanha - Participant / Gabriela Fernanda Martinez - Participant / Maria Naomi Yamanaka - Participant / Sara Fabricio dos Santos - Participant / Aarão Fabrício dos Santos - Participant / Leonardo de Oliveira Figueiredo - Participant.

Areas of Expertise

1.

Major Area: Health Sciences / Area: Pharmacy / Subarea: Clinical Cytology.

2.

Major Area: Health Sciences / Area: Pharmacy / Subarea: Pathophysiology and Laboratory Diagnosis.

Languages

English

Understands Well, Speaks Fairly, Reads Well, Writes Fairly.

Awards and Honors

2022

Honorable Mention for the study entitled "Targeting Peroxiredoxin-2 as a potential treatment for NRAS-mutated metastatic melanoma", University of São Paulo.

2022

SMR PARTICIPATION AWARD, Society for Melanoma Research.

Publications

Bibliographic Production

Citations

Google Scholar

Total number of studies: 19 Total number of citations: 74 Date: 02/10/2025

https://scholar.google.com/citations?user=gtlvO4YAAAAJ&hl=pt-BR

Full Articles Published in Journals

Sort by

Chronological Order

1.

NORONHA,, NATÁLIA YUMI ; RODRIGUES, GUILHERME DA SILVA ; WATANABE, LÍGIA MORIGUCHI ; **NOMA, ISABELLA HARUMI YONEHARA** ; CAZIER, JEAN-BAPTISTE ; SAE-LEE, CHANACHAI ; CHITTA, PITAKSIN ; PEREIRA, VANESSA APARECIDA BATISTA ; PINHEL, MARCELA AUGUSTA DE SOUZA ; DIANI, LUISA MARIA ; BARBOSA, FERNANDO ; PLÖSCH, TORSTEN ; NONINO, CARLA BARBOSA . DNA methylation signatures of arsenic exposure and obesity. Environmental Epigenetics JCR , v. -, p. -, 2025.

2.

DA SILVA RODRIGUES, GUILHERME ; NORONHA, NATÁLIA YUMI ; **NOMA, ISABELLA HARUMI YONEHARA** ; DE LIMA, JOÃO GABRIEL RIBEIRO ; DA SILVA SOBRINHO, ANDRESSA CRYSTINE ; DE SOUZA PINHEL, MARCELA AUGUSTA ; DE ALMEIDA, MARIANA LUCIANO ; WATANABE, LÍGIA MORIGUCHI ; NONINO, CARLA BARBOSA ; JÚNIOR, CARLOS ROBERTO BUENO . 14-Week exercise training modifies the DNA methylation levels at gene sites in non-Alzheimer's disease women aged 50 to 70 years. EXPERIMENTAL GERONTOLOGY JCR , v. 186, p. 112362, 2024. **Citations:** WEB OF SCIENCE™ 2 | SCOPUS 2

3.

NORONHA, NATÁLIA YUMI ; **NOMA, ISABELLA HARUMI YONEHARA** ; FERNANDES FERREIRA, RAFAEL ; RODRIGUES, GUILHERME DA SILVA ; MARTINS, LUZANIA DOS SANTOS ; WATANABE, LÍGIA MORIGUCHI ; PINHEL, MARCELA AUGUSTA DE SOUZA ; MELLO SCHINEIDER, ISABELLE ; DIANI, LUISA MARIA ; CARLOS, DANIELA ; NONINO, CARLA BARBOSA . Association between the relative abundance of phyla actinobacteria, vitamin C consumption,

and DNA methylation of genes linked to immune response pathways. FRONTIERS IN NUTRITION JCR , v. 11, p. -, 2024. **Citations:** WEB OF SCIENCE ™ 2 | SCOPUS 1

4.

NOMA, ISABELLA HARUMI YONEHARA; CARVALHO, LARISSA ANASTACIO DA COSTA ; CAMARENA, DENISSE ESTHER MALLAUPOMA ; SILVA, RENAIRA OLIVEIRA ; MORAES JUNIOR, MANOEL OLIVEIRA DE ; DE SOUZA, SOPHIA TAVARES ; NEWTON-BISHOP, JULIA ; NSENGIMANA, JEREMIE ; MARIA-ENGLER, SILVYA STUCHI . Peroxiredoxin-2 represses NRAS-mutated melanoma cells invasion by modulating EMT markers. BIOMEDICINE & PHARMACOTHERAPY JCR , v. 177, p. 116953, 2024. **Citations:** WEB OF SCIENCE ™ 2

5.

CARVALHO, LARISSA ANASTACIO DA COSTA ; **NOMA, ISABELLA HARUMI YONEHARA** ; UEHARA, ADRIANA HIROMI ; SIENA, ADAMO DAVI DIOGENES ; OSAKI, LUCIANA HARUMI ; MORI, MATEUS PRATES ; PINTO, NADJA CRISTHINA DE SOUZA ; FREITAS, VANESSA MORAIS ; JUNIOR, WILSON ARAUJO SILVA ; SMALLEY, KEIRAN S. M. ; MARIA-ENGLER, SILVYA STUCHI . Modeling Melanoma Heterogeneity In Vitro: Redox, Resistance and Pigmentation Profiles. ANTIOXIDANTS JCR , v. 13, p. 555, 2024. **Citations:** WEB OF SCIENCE ™ 2 | SCOPUS 2

6.

FRANCO, YLANA ADAMI ; DE MORAES, MANOEL OLIVEIRA ; CARVALHO, LARISSA A. C. ; DOHLE, WOLFGANG ; DA SILVA, RENAIRA OLIVEIRA ; **NOMA, ISABELLA HARUMI YONEHARA** ; LIMA, KELI ; POTTER, BARRY V. L. ; MACHADO-NETO, JOÃO A. ; MARIA-ENGLER, SILVYA STUCHI . 2-Methoxyestradiol-3,17-O,O-bis-sulfamate (STX140) Inhibits Proliferation and Invasion via Senescence Pathway Induction in Human BRAFi-Resistant Melanoma Cells. INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES JCR , v. 24, p. 11314, 2023. **Citations:** WEB OF SCIENCE ™ 1 | SCOPUS 1

7.

DA SILVA RODRIGUES, GUILHERME ; **NOMA, ISABELLA HARUMI YONEHARA** ; NORONHA, NATALIA YUMI ; WATANABE, LIGIA MORIGUCHI ; DA SILVA SOBRINHO, ANDRESSA CRYSTINE ; DE LIMA, JOÃO GABRIEL RIBEIRO ; SAE-LEE, CHANACHAI ; BENJAMIM, CICERO JONAS RODRIGUES ; NONINO, CARLA BARBOSA ; BUENO, CARLOS ROBERTO . Eight Weeks of Physical Training Decreases 2 Years of DNA Methylation Age of Sedentary Women. RESEARCH QUARTERLY FOR EXERCISE AND SPORT JCR , v. -, p. 1-11, 2023. **Citations:** WEB OF SCIENCE ™ 2 | SCOPUS 2

8.

WATANABE, LÍGIA MORIGUCHI ; PEREIRA, VANESSA APARECIDA BATISTA ; NORONHA, NATALIA YUMI ; DE SOUZA PINHEL, MARCELA AUGUSTA ; WOLF, LETICIA SANTANA ; DE OLIVEIRA, CRISTIANA CORTES ; PLAÇA, JESSICA RODRIGUES ; **NOMA, ISABELLA HARUMI YONEHARA** ; DA SILVA RODRIGUES, GUILHERME ; DE SOUZA, VANESSA CRISTINA OLIVEIRA ; JUNIOR, FERNANDO BARBOSA ; NONINO, CARLA BARBOSA . The influence of serum selenium in differential epigenetic and transcriptional regulation of CPT1B gene in women with obesity. JOURNAL OF TRACE ELEMENTS IN MEDICINE AND BIOLOGY JCR , v. -, p. 127376, 2023. **Citations:** WEB OF SCIENCE ™ 3 | SCOPUS 2

9.

YUMI NORONHA, NATÁLIA ; DA SILVA RODRIGUES, GUILHERME ; **HARUMI YONEHARA NOMA, ISABELLA** ; FERNANDA CUNHA BRANDAO, CAMILA ; PEREIRA RODRIGUES, KARINE ; COLELLO BRUNO, ALEXANDRE ; SAE-LEE, CHANACHAI ; MORIGUCHI WATANABE, LIGIA ; AUGUSTA DE SOUZA PINHEL, MARCELA ; MELLO SCHINEIDER, ISABELLE ; LUCIANO DE ALMEIDA, MARIANA ; BARBOSA JUNIOR, FERNANDO ; ARAUJO MORAIS, DÉBORAH ; TAVARES DE SOUSA JUNIOR, WELLINGTON ; PLÖSCH, TORSTEN ; ROBERTO BUENO JUNIOR, CARLOS ; BARBOSA NONINO, CARLA . 14-weeks combined exercise epigenetically modulated 118 genes of menopausal women with prediabetes. Frontiers in Endocrinology JCR, v. 13, p. 895489, 2022. **Citations:** WEB OF SCIENCE ℠ 14 | SCOPUS 11

10.

DOMINGUES, KARLA LARISSA TRASSI GANAZA ; TANOSHI, CÍNTHIA AKEMI ; **NOMA, ISABELLA HARUMI YONEHARA** ; CARVALHO, MARIA DALVA DE BARROS ; PELLOSO, SANDRA MARISA ; CONSOLARO, MARCIA EDILAINE LOPES ; ARISTIDES, SANDRA MARA ALESSI ; DEMARCHI, IZABEL GALHARDO ; PEDROSO, RAISSA BOCCHI ; LONARDONI, MARIA VALDRINEZ CAMPANA . Economic impact of hospitalizations due to leishmaniasis in southern Brazil. RESEARCH, SOCIETY AND DEVELOPMENT, v. 11, p. e39211831139, 2022.

11.

NORONHA, NATÁLIA YUMI ; DA SILVA RODRIGUES, GUILHERME ; DE SOUZA PINHEL, MARCELA AUGUSTA ; CAZIER, JEAN-BAPTISTE ; WATANABE, LIGIA MORIGUCHI ; MENEZES, ALBERT NOBRE ; BUENO, CARLOS ROBERTO ; NICOLETTI, CAROLINA FERREIRA ; DE OLIVEIRA, BRUNO AFFONSO PARENTI ; SCHINEIDER, ISABELLE MELLO ; **YONEHARA NOMA, ISABELLA HARUMI** ; DIAS ALCARÁS, IGOR CAETANO ; BARBOSA, FERNANDO ; BARBOSA NONINO, CARLA . Sample Preparation to Bioinformatics Analysis of DNA Methylation: Association Strategy for Obesity and Related Trait Studies. Jove-Journal of Visualized Experiments JCR, v. 183, p. e62598, 2022. **Citations:**
WEB OF SCIENCE ℠ 8 | SCOPUS 7

12.

NOMA, ISABELLA; SHINOBU-MESQUITA, CRISTIANE ; SUEHIRO, TAMY ; MORELLI, FABRICIO ; DE SOUZA, MARIA ; DAMKE, EDILSON ; DA SILVA, VÂNIA ; CONSOLARO, MARCIA . Association of Righ-Risk Human Papillomavirus and Ureaplasma parvum Co-Infections with Increased Risk of Low-Grade Squamous Intraepithelial Cervical Lesions. Asian Pacific Journal of Cancer Prevention, v. 22, p. 1239-1246, 2021. **Citations:** SCOPUS 8

13.

SANTOS E SILVA, JUAN CARLO ; VASCONCELOS, AMANDA PEREIRA ; **YONEHARA NOMA, ISABELLA HARUMI** ; NORONHA, NATÁLIA YUMI ; AQUINO, RODRIGO ; GIDDALURU, JEEVAN ; DURÃO, LUIZ ; COSTA-MARTINS, ANDRÉ GUILHERME ; SCHUCH, VIVIANE ; MORAES-VIEIRA, PEDRO M. ; NAKAYA, HELDER I. . Gene Signatures of Autopsy Lungs from Obese Patients with COVID-19. CLINICAL NUTRITION ESPEN JCR , v. 43, p. 986, 2021. **Citations:**
WEB OF SCIENCE ℠
8 | SCOPUS 8

1.

TANOSHI, C. A. ; **NOMA, I. H. Y.** ; GANAZA, K. L. T. ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. ; ARISTIDES, S. M. A. . HOSPITAL MORBIDITY EXPENDITURES DUE TO VISCERAL LEISHMANIASIS IN A REGION OF SOUTHERN BRAZIL. In: 5th International Congress of the CCS, 3rd International Congress on Obesity Treatment and 7th PCS Meeting. State University of Maringá, 2018, Maringá. Proceedings of the 5th International Congress of the CCS, 3rd International Congress on Obesity Treatment and 7th PCS Meeting, 2018. p. 5.

2.

GANAZA, K. L. T. ; TANOSHI, C. A. ; **NOMA, I. H. Y.** ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; CONSOLARO, M. E. L. ; ARISTIDES, S. M. A. ; DEMARCHI, I. G. ; LONARDONI, M. V. C. . ANALYSIS OF HOSPITAL MORBIDITY DUE TO CUTANEOUS LEISHMANIASIS IN THE STATE OF PARANÁ, BRAZIL, 2008-2018. In: 5th International Congress of the CCS, 3rd International Congress on Obesity Treatment and 7th PCS Meeting. State University of Maringá, 2018, Maringá. Proceedings of the 5th International Congress of the CCS, 3rd International Congress on Obesity Treatment and 7th PCS Meeting, 2018.

3.

NOMA, I. H. Y.; GANAZA, K. L. T. ; TANOSHI, C. A. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; ARISTIDES, S. M. A. ; DEMARCHI, I. G. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. . MUCOCUTANEOUS LEISHMANIASIS IN PARANÁ: EVALUATION OF HOSPITAL MORBIDITY FROM 2008 TO 2018. In: 5th International Congress of the CCS, 3rd International Congress on Obesity Treatment and 7th PCS Meeting. State University of Maringá, 2018, Maringá. Proceedings of the 5th International Congress of the CCS, 3rd International Congress on Obesity Treatment and 7th PCS Meeting, 2018. p. 6.

4.

TANOSHI, C. A. ; GANAZA, K. L. T. ; **NOMA, I. H. Y.** ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; CONSOLARO, M. E. L. ; LONARDONI, M. V. C. ; ARISTIDES, S. M. A. . The Prevalence of a Neglected Disease: Epidemiology and Morbidity of Leishmaniases in Paraná. In: V International Meeting of Biosciences and Physiopathology and 9th Symposium on Biosciences Applied to Pharmacy., 2018, Maringá. Proceedings of the 9th Academic Week of Biomedicine, 2018.

5.

NOMA, I. H. Y.; ECKER, A. B. S. ; MOREIRA, D. C. ; SILVA, L. C. ; PEREIRA, P. M. ; SZCZEREPA, M. M. A. ; TOGNIM, M. C. B. . VARIABILITY OF β-LACTAMASES PRESENT IN GRAM-NEGATIVE BACILLI ISOLATED DURING PATIENTS' STAY IN A TEACHING HOSPITAL. In: 26th Annual Scientific Initiation Meeting and 6th Annual Junior Scientific Initiation Meeting, 2017, Maringá. Proceedings of the XXVI EAIC VI EAIC Jr., 2017. p. 67.

6.

ALBUQUERQUE, E. P. A. ; MONTANHA, F. F. ; **NOMA, I. H. Y.** ; GREMASCHI, L. B. ; BLANCO, L. F. M. ; BRAZ, N. M. ; ROMAO, P. A. S.

S. ; VALVERDE, T. L. ; VICENTINI, V. E. P. . Dr. Genetics: Information in the Service of the Community. In: UEM Outreach and Culture Forum: University Outreach, Commitment and Social Transformation, 2016, Maringá. Proceedings of the 14th UEM Outreach and Culture Forum: University Outreach: Commitment and Social Transformation, 2016. v. 23. p. 13-16.

7.

NOMA, I. H. Y.; PEREIRA, V. M. ; SANUKI, M. S. ; BORGHI, S. M. ; VERRI JUNIOR, W. A. ; SILVA, P. K. S. ; ARISTIDES, S. M. A. . RELATIONSHIP BETWEEN NOCICEPTION AND NITRIC OXIDE PRODUCTION IN ANIMALS INFECTED WITH LEISHMANIA (LEISHMANIA) AMAZONENSIS. In: 25th Annual Scientific Initiation Meeting and 5th Annual Junior Scientific Initiation Meeting, 2016, Maringá. Proceedings of the XXV EAIC V EAIC Jr., 2016. p. 62.

8.

NOMA, I. H. Y.; WATANABE, A. S. M. ; SILVA, M. R. T. ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . Evaluation of the Pathogenicity and Virulence Factors of Candida albicans in the Experimental Candidemia Model. In: 3rd Academic Week of Biomedicine ? UEM, 2015, Maringá. PROCEEDINGS OF THE THIRD ACADEMIC WEEK OF BIOMEDICINE , 2015.

9.

WATANABE, A. S. M. ; SILVA, M. R. T. ; **NOMA, I. H. Y.** ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . EVALUATION OF THE VIRULENCE FACTORS OF CANDIDA TROPICALIS AFTER EXPERIMENTAL CANDIDEMIA. In: 3rd Academic Week of Biomedicine ? UEM, 2015, Maringá. PROCEEDINGS OF THE THIRD ACADEMIC WEEK OF BIOMEDICINE , 2015.

10.

SILVA, M. R. T. ; WATANABE, A. S. M. ; **NOMA, I. H. Y.** ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . EVALUATION OF THE PATHOGENICITY PROFILE OF CANDIDA ALBICANS AFTER SUBSEQUENT CONTACTS WITH THE HOST. In: 3rd Academic Week of Biomedicine ? UEM, 2015, Maringá. PROCEEDINGS OF THE THIRD ACADEMIC WEEK OF BIOMEDICINE , 2015.

Abstracts Published in Conference Proceedings

1.

RODRIGUES, R. F. ; SILVA, L. L. ; TANOSHI, C. A. ; **NOMA, I. H. Y.** ; SILVA, G. E. C. . ANALYSIS OF THE EPIDEMIOLOGICAL PROFILE OF ACCIDENTS INVOLVING VENOMOUS ANIMALS IN THE STATE OF PARANA: DURING THE PERIOD FROM 2015 TO 2019. In: VII International Meeting of Biosciences and Physiopathology and 11th Symposium on Biosciences Applied to Pharmacy, 2020, Maringá. Proceedings of the VII International Meeting of Biosciences and Physiopathology and 11th Symposium on Biosciences Applied to Pharmacy. Maringá: Isabella Harumi Yonehara Noma, 2020. v. 1.

2.

NOMA, I. H. Y.; TANOSHI, C. A. ; SANTOS, D. S. ; RODRIGUES, R. F. ; SILVA, L. L. ; CONSOLARO, M. E. L. ; SOUZA, E. M. ; PELLOSO, S. M.

; PEDROSO, R. B. . THE PREVALENCE OF SYPHILIS IN PRENATAL CARE WOMEN IN PARANA STATE, BRAZIL. In: III INTERNATIONAL SYMPOSIUM OF EXPERIMENTAL PATHOLOGY, 2019, Londrina. Proceedings of the III International Symposium os Experimental Pathology. Londrina : UEL, 2019: Eduardo José de Almeida Araújo ...[et al.], 2019. p. 48.

3.

TANOSHI, C. A. ; **NOMA, I. H. Y.** ; RODRIGUES, R. F. ; SILVA, L. L. ; ROSSONI, D. F. ; PEDROSO, R. B. ; ARISTIDES, S. M. A. . CHALLENGES TO HUMAN LEISHMANIASIS: DISCUSSION OF THE DISEASE BASED ON ITS BEHAVIOR. In: III INTERNATIONAL SYMPOSIUM OF EXPERIMENTAL PATHOLOGY, 2019, Londrina. Proceedings of the
III International Symposium os Experimental Pathology, 2019. p. 40.

4.

NOMA, I. H. Y.; SUEHIRO, T. T. ; MORELLI, F. ; SOUZA, M. V. F. ; MESQUITA, C. S. S. ; SILVA, V. R. S. ; CONSOLARO, M. E. L. . Co-infections between Human Papillomavirus (HPV) and genital mycoplasmas in asymptomatic women. In: VI International Meeting of Biosciences and Physiopathology ? 10th Symposium on Biosciences Applied to Pharmacy, 2019, Ivatuba. Proceedings of the VI International Meeting of Biosciences & Physiopathology. Maringá, 2019. p. 227.

5.

NOMA, I. H. Y.; GANAZA, K. L. T. ; TANOSHI, C. A. ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; ARISTIDES, S. M. A. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. . PUBLIC EXPENDITURES ON HOSPITAL MORBIDITY DUE TO LEISHMANIASIS IN PARANA, BRAZIL, BRAZIL. In: V International Meeting of Biosciences and Physiopathology e 9th Symposium on Biosciences Applied to Pharmacy., 2018, Maringá. Proceedings of the de Simpósio do Programa de Biociências e Fisiopatologia- UEM, 2018. v. 1. p. 175.

6.

NOMA, I. H. Y.; ECKER, A. B. S. ; SZCZEREPA, M. M. A. ; TOGNIM, M. C. B. . Dissemination of enterobacteria among patients during hospitalization in the intensive care unit of a teaching hospital in southern Brazill. In: IV International Meeting of Biosciences and Physiopathology, 2017, Maringá. 7th Symposium on Biosciences Applied to Pharmacy, 2017.

7.

MOREIRA, D. C. ; ECKER, A. B. S. ; **NOMA, I. H. Y.** ; FEDRIGO, N. H. ; FABRI, F. V. ; COSTA, B. B. ; ZERBINATI, H. T. ; MENEZES, A. V. ; FERNANDES, A. S. ; TOGNIM, M. C. B. . SURVEILLANCE OF CARBAPENEM-RESISTANT ENTEROBACTERIACEAE IN PATIENTS DURING HOSPITALIZATION IN AN ADULT INTENSIVE CARE UNIT. In: 29th BRAZILIAN CONGRESS OF MICROBIOLOGY - CBM, 2017, Foz do Iguaçu. 29th Brazilian Congress of Microbiology 2017, 2017.

8.

SILVA, L. C. ; PEREIRA, P. M. ; DURANTE, P. C. ; MATTOS, M. S. F. ; ZERBINATI, H. T. ; **NOMA, I. H. Y.** ; FERNANDES, A. S. ; CARDOSO, C. L. ; TOGNIM, M. C. B. ; NISHIYAMA, S. A. B. . PRELIMINARY EVALUATION OF THE FREQUENCY OF ACINETOBACTER BAUMANNI ISOLATE AT THE BUCAL AND NASAL CAVITY FROM UNIVERSITY STUDENTS. In: 29th Brazilian Congress of Microbiology, 2017, Foz do Iguaçu. 29th BRAZILIAN CONGRESS OF MICROBIOLOGY 2017, 2017.

9.

FEDRIGO, N. H. ; MATTA, P. E. G. ; PEREIRA, P. M. ; **NOMA, I. H. Y.** ; TOGNIM, M. C. B. . Activity of fosfomycin against Acinetobacter baumannii and performance of susceptibility testing methods. In: 7th Symposium on Biosciences Applied to Pharmacy and III International Meeting of Biosciences and Physiopathology, 2016, Maringá. Proceedings of the 7th Symposium on Biosciences Applied to Pharmacy, 2016.

10.

NOMA, I. H. Y.; PEREIRA, V. M. ; SILVA, P. K. S. ; SANUKI, M. S. ; SILVEIRA, T. G. V. ; ARISTIDES, S. M. A. . RELATIONSHIP BETWEEN NOCICEPTION AND NITRIC OXIDE PRODUCTION IN ANIMALS INFECTED WITH Leishmania (Leishmania) amazonensis. In: 4th Academic Week of Biomedicine - UEM, 2016, Maringá. Proceedings of the 4th Week of Biomedicine, 2016.

11.

MATTA, P. E. G. ; ECKER, A. B. S. ; ZARPELLON, M. N. ; MOREIRA, D. C. ; **NOMA, I. H. Y.** ; TAMURA, N. K. ; GARCIA, L. B. ; TOGNIM, M. C. B. . PREVALENCE OF RESISTANCE TO CARBAPENEMS IN THE MAIN GRAM-NEGATIVE BACILLI ISOLATED IN A TEACHING HOSPITAL. In: 14th Brazilian Congress on Infection Control and Hospital Epidemiology, 2014, Curitiba. Journal of Infection Control, 2014. v. 3. p. 139.

12.

VIANA, G. F. ; ZAGO, M. C. B. ; SAALFELD, S. M. S. ; **NOMA, I. H. Y.** ; MATTA, P. E. G. ; MITSUGUI, C. S. ; ABREU FILHO, B. A. ; TOGNIM, M. C. B. . CHANGES IN THE MODE OF DISSEMINATION OF Acinetobacter baumannii ISOLATES IN A UNIVERSITY HOSPITAL. In: 14th Brazilian Congress on Infection Control and Hospital Epidemiology, 2014, Curitiba. Journal of Infection Control, 2014. v. 3. p. 148.

13.

ZAGO, M. C. B. ; VIANA, G. F. ; SAALFELD, S. M. S. ; **NOMA, I. H. Y.** ; FARIAS, A. P. M. ; HELBEL, C. ; TOGNIM, M. C. B. . DISSEMINATION OF Acinetobacter baumannii IN A TERTIARY HOSPITAL IN THE NORTHERN REGION OF PARANA. In: 14th BRAZILIAN CONGRESS ON INFECTION CONTROL AND HOSPITAL EPIDEMIOLOGY, 2014, Curitiba. DISSEMINATION OF Acinetobacter baumannii IN A TERTIARY HOSPITAL IN THE NORTHERN REGION OF PARANA, 2014. v. 3. p. 135.

Paper Presentations

1.

NOMA, I. H. Y.; CRUZ, E. ; DUTRA, A. C. ; NEGRI, M. F. N. . Geospatial Analysis of Diagnostic Imaging Equipment in Brazil. 2020. (Paper Presentation/Conference or Lecture).

2.

CRUZ, E. ; **NOMA, I. H. Y.** ; DUTRA, A. C. ; NEGRI, M. F. N. . Life-Sustaining Equipment: A Demographic Geospace Analysis in National Territory. 2020. (Paper Presentation/Conference or Lecture).

3.

RODRIGUES, R. F. ; SILVA, L. L. ; TANOSHI, C. A. ; **NOMA, I. H. Y.** ; SILVA, G. E. C. . ANALYSIS OF THE EPIDEMIOLOGICAL PROFILE OF SCORPIONISM IN THE SOUTHERN STATES OF BRAZIL: A STUDY REGARDING THE PERIOD FROM 2015 TO 2019. 2020. (Paper Presentation/Other).

4.

NOMA, I. H. Y.; SUEHIRO, T. T. ; MORELLI, F. ; SOUZA, M. V. F. ; MESQUITA, C. S. S. ; SILVA, V. R. S. ; CONSOLARO, M. E. L. . Co-infections between Human Papillomavirus (HPV) and genital mycoplasmas in asymptomatic women. (Paper Presentation/Symposium).

5.

TANOSHI, C. A. ; **NOMA, I. H. Y.** ; RODRIGUES, R. F. ; SILVA, L. L. ; PEDROSO, R. B. ; ARISTIDES, S. M. A. . Challenges to human leishmaniasis: discussion of the disease based on its behavior. 2019. (Paper Presentation/Symposium).

6.

NOMA, I. H. Y.; TANOSHI, C. A. ; RODRIGUES, R. F. ; SILVA, L. L. ; CONSOLARO, M. E. L. ; PEDROSO, R. B. . The prevalence of syphilis in prenatal care women in Paraná State, Brazil. 2019. (Paper Presentation/Symposium).

7.

NOMA, I. H. Y.; GANAZA, K. L. T. ; TANOSHI, C. A. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; ARISTIDES, S. M. A. ; DEMARCHI, I. G. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. . Mucocutaneous leishmaniasis in Paraná, evaluation of hospital morbidity from 2008 to 2018.. 2018. (Paper Presentation/Congress).

8.

GANAZA, K. L. T. ; TANOSHI, C. A. ; **NOMA, I. H. Y.** ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; CONSOLARO, M. E. L. ; ARISTIDES, S. M. A. ; DEMARCHI, I. G. ; LONARDONI, M. V. C. . Analysis of hospital morbidity due to cutaneous leishmaniasis in the state of Paraná, Brazil, 2008-2018. 2018. (Paper Presentation/Congress).

9.

TANOSHI, C. A. ; **NOMA, I. H. Y.** ; GANAZA, K. L. T. ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. ; ARISTIDES, S. M. A. . HOSPITAL MORBIDITY EXPENDITURES DUE TO VISCERAL LEISHMANIASIS IN A REGION OF SOUTHERN BRAZIL.. 2018. (Paper Presentation/Congress).

10.

TANOSHI, C. A. ; GANAZA, K. L. T. ; **NOMA, I. H. Y.** ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; CONSOLARO, M. E. L. ; LONARDONI, M. V. C. ; ARISTIDES, S. M. A. . The prevalence of a neglected infectious disease: epidemiology and morbidity of leishmaniases in Paraná. 2018. 2018. (Paper Presentation/Symposium).

11.

NOMA, I. H. Y.; GANAZA, K. L. T. ; TANOSHI, C. A. ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; ARISTIDES, S. M. A. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. . PUBLIC EXPENDITURES ON HOSPITAL MORBIDITY DUE TO LEISHMANIASIS IN PARANÁ, BRAZIL. 2018. (Paper Presentation/Symposium).

12.

ECKER, A. B. S. ; MOREIRA, D. C. ; **NOMA, I. H. Y.** ; VIANA, G. F. ; MITSUGUI, C. S. ; PEREIRA, P. M. ; TOGNIM, M. C. B. . CLONAL VARIABILITY AND INVESTIGATION OF BETA-LACTAMASE GENES IN ACINETOBACTER BAUMANNII ISOLATES OBTAINED DURING PATIENTS' STAY IN THE INTENSIVE CARE UNIT.
2017. (Paper Presentation/Congress).

13.

PEREIRA, P. M. ; MATTA, P. E. G. ; **NOMA, I. H. Y.** ; SILVA, L. C. ; FARIAS, A. P. M. ; ZARPELLON, M. N. ; TOGNIM, M. C. B. . ACTIVITY OF FOSFOMYCIN AGAINST ACINETOBACTER BAUMANNII ISOLATES: EVALUATION OF DIFFERENT METHODOLOGIES. 2017.
(Paper Presentation/Congress).

14.

NOMA, I. H. Y.; ECKER, A. B. S. ; MOREIRA, D. C. ; SILVA, L. C. ; PEREIRA, P. M. ; SZCZEREPA, M. M. A. ; TOGNIM, M. C. B. . VARIABILITY OF β-LACTAMASES PRESENT IN GRAM-NEGATIVE BACILLI ISOLATED DURING PATIENTS' STAY IN A TEACHING HOSPITAL. 2017. (Paper Presentation/Other).

15.

NOMA, I. H. Y.; ECKER, A. B. S. ; SZCZEREPA, M. M. A. ; TOGNIM, M. C. B. . Dissemination of enterobacteria among patients during hospitalization in the intensive care unit of a teaching hospital in southern Brazill.. 2017. (Paper Presentation/Symposium).

16.

SILVA, L. C. ; PEREIRA, P. M. ; DURANTE, P. C. ; MATTOS, M. S. F. ; ZERBINATI, H. T. ; **NOMA, I. H. Y.** ; FERNANDES, A. S. ; CARDOSO, C. L. ; TOGNIM, M. C. B. ; NISHIYAMA, S. A. B. . PRELIMINARY EVALUATION OF THE FREQUENCY OF ACINETOBACTER BAUMANNI ISOLATE AT THE BUCAL AND NASAL CAVITY FROM UNIVERSITY STUDENTS. 2017. (Paper Presentation/Congress).

17.

MOREIRA, D. C. ; ECKER, A. B. S. ; **NOMA, I. H. Y.** ; FEDRIGO, N. H. ; FABRI, F. V. ; COSTA, B. B. ; ZERBINATI, H. T. ; MENEZES, A. V. ; FERNANDES, A. S. ; TOGNIM, M. C. B. . SURVEILLANCE OF CARBAPENEM-RESISTANT ENTEROBACTERIACEAE IN PATIENTS DURING HOSPITALIZATION IN AN ADULT INTENSIVE CARE UNIT.. 2017. (Paper Presentation/Congress).

18.

ROMAO, P. A. S. S. ; ALBUQUERQUE, E. P. A. ; MONTANHA, F. F. ; **NOMA, I. H. Y.** ; GREMASCHI, L. B. ; BLANCO, L. F. M. ; BRAZ, N. M. ; VALVERDE, T. L. ; VICENTINI, V. E. P. . Dr. Genetics: Online informational content to support genetic testing. 2016. (Paper Presentation/Other).

19.

NOMA, I. H. Y.; PEREIRA, V. M. ; SANUKI, M. S. ; BORGHI, S. M. ; VERRI JUNIOR, W. A. ; SILVA, P. K. S. ; ARISTIDES, S. M. A. . RELATIONSHIP BETWEEN NOCICEPTION AND NITRIC OXIDE PRODUCTION IN ANIMALS INFECTED WITH LEISHMANIA (LEISHMANIA) AMAZONENSIS. 2016. (Paper Presentation/Other).

20.

FEDRIGO, N. H. ; MATTA, P. E. G. ; PEREIRA, V. M. ; **NOMA, I. H. Y.** ; TOGNIM, M. C. B. . Activity of fosfomycin against Acinetobacter baumannii and performance of susceptibility testing methods. 2016. (Paper Presentation/Symposium).

21.

NOMA, I. H. Y.; WATANABE, A. S. M. ; SILVA, M. R. T. ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . Evaluation of the pathogenicity and virulence factors of Candida albicans in the experimental candidemia model. 2015. (Paper Presentation/Other).

22.

LODI, F. G. ; ZAGO, M. C. B. ; MATTA, P. E. G. ; **NOMA, I. H. Y.** ; TAMURA, N. K. ; AOKI, E. E. ; TOGNIM, M. C. B. . Temporal evolution of the minimum inhibitory concentration for daptomycin in gram-positive cocci. 2015. (Paper Presentation/Congress).

23.

FEDRIGO, N. H. ; TREVIZOLI, T. M. B. ; **NOMA, I. H. Y.** ; MITSUGUI, C. S. ; AOKI, E. E. ; TAMANINI, J. B. ; TOGNIM, M. C. B. . Temporal analysis of resistance to carbapenems in non-fermenting gram-negative bacilli. 2015. (Paper Presentation/Congress).

24.

WATANABE, A. S. M. ; SILVA, M. R. T. ; **NOMA, I. H. Y.** ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . EVALUATION OF THE VIRULENCE FACTORS OF CANDIDA TROPICALIS AFTER EXPERIMENTAL CANDIDEMIA. 2015.(Paper Presentation/Other).

25.

SILVA, M. R. T. ; WATANABE, A. S. M. ; **NOMA, I. H. Y.** ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . EVALUATION OF THE PATHOGENICITY PROFILE OF CANDIDA ALBICANS AFTER SUBSEQUENT CONTACTS WITH THE HOST. 2015. (Paper Presentation/Other).

26.

MATTA, P. E. G. ; ECKER, A. B. S. ; ZARPELLON, M. N. ; MOREIRA, D. C. ; **NOMA, I. H. Y.** ; TAMURA, N. K. ; GARCIA, L. B. ; TOGNIM, M. C. B. . PREVALENCE OF RESISTANCE TO CARBAPENEMS IN THE MAIN GRAM-NEGATIVE BACILLI ISOLATED IN A TEACHING HOSPITAL. 2014. (Paper Presentation/Congress).

27.

VIANA, G. F. ; ZAGO, M. C. B. ; SAALFELD, S. M. S. ; **NOMA, I. H. Y.** ; MATTA, P. E. G. ; MITSUGUI, C. S. ; ABREU FILHO, B. A. ; TOGNIM, M. C. B. . CHANGES IN THE MODE OF DISSEMINATION OF Acinetobacter baumannii ISOLATES IN A UNIVERSITY HOSPITAL. 2014. (Paper Presentation/Congress).

28.

ZAGO, M. C. B. ; VIANA, G. F. ; SAALFELD, S. M. S. ; **NOMA, I. H. Y.** ; FARIAS, A. P. M. ; HELBEL, C. ; TOGNIM, M. C. B. DISSEMINATION OF Acinetobacter baumannii IN A TERTIARY HOSPITAL IN THE NORTHERN REGION OF PARANA. 2014. (Paper Presentation/Congress).

Technical Production

Social media, websites and blogs

1.

NOMA, I. H. Y.. IS IT POSSIBLE TO RECREATE AGED SKIN IN VITRO?. 2020; Topic: Scientific Outreach of the Skin Biology and Melanoma Laboratory. (Social media).

Participation in events, congresses, exhibitions, and fairs

1.

19th International Congress of the Society for Melanoma Research. PRDX2 as a possible phenotype switcher in NRAS-mutated melanomas. 2022. (Congress).

2.

Symposium in Commemoration of the 50th Anniversary of the Graduate Program in Pharmacy and Pathophysiology and Toxicologia.Targeting Peroxiredoxin 2 as a potential treatment for NRAS mutated metastatic melanoma. 2022. (Symposium).

3.

XXII Brazilian Congress of Toxicology. Gliotoxin and its potential cytotoxicity against NRAS mutated-melanoma. 2022. (Congress).

4.

The importance of the study of in vitro dermal irritation/corrosion in toxicological evaluation. 2021. (Other).

5.

Host-Pathogen Interaction Meeting. 2021. (Meeting).

6.

1st Symposium on Challenges in Molecular Oncology. 2021. (Symposium).

7.

Brazilian Congress in Biomedical Engineering (CBEB2020). GEOSPATIAL ANALYSIS OF DIAGNOSTIC IMAGING EQUIPMENT IN BRAZIL. 2020. (Congress).

8.

FisioTox Lecture Series. 2020. (Seminar).

9.

Unifesp Virtual Academic Congress 2020 - Science and University: Transformations for Society. 2020. (Congress).

10.

2nd Meeting on Genetics, Hemotherapy, and Hematological Pathologies. 2020. (Meeting).

11.

Increasing CRISPR editing efficiency with novel guide RNA methods. 2020. (Seminar).

12.

HPV Infection - Infecto in Series. 2020. (Seminar).

13.

Diagnostic and Therapeutic Perspectives Using Technology CRISPR. 2020. (Other).

14.

ONCOLOGY PILLS: Principles of hormone therapy in oncological treatment. 2020. (Seminar).

15.

ONCOLOGY PILLS: Principles of Oncological Treatment and Mechanisms of Chemotherapy. 2020. (Seminar).

16.

ONCOLOGY PILLS: Targeted Therapy and Immunotherapy in Modern Oncology. 2020. (Seminar).

17.

ONCOLOGY PILLS - Clinical cases in oncology: application of basic concepts. 2020. (Seminar).

18.

VII International Meeting of Biosciences and Physiopathology and 11th Symposium on Biosciences Applied to Pharmacy. 2020. (Symposium).

19.

Varilux-UEM French Film Festival 2019. 2019. (Exposition).

20.

III INTERNATIONAL SYMPOSIUM OF EXPERIMENTAL PATHOLOGY.THE PREVALENCE OF SYPHILIS IN PRENATAL CARE WOMEN IN PARANA STATE, BRAZIL. 2019. (Symposium).

21.

VI International Meeting of Biosciences and Physiopathology ? 10th Symposium on Biosciences Applied to Pharmacy.Co-infections between Human Papillomavirus (HPV) and genital mycoplasmas in asymptomatic women. 2019. (Meeting).

22.

8th Annual Meeting on Technological Initiation and Innovation. Session Coordinator. 2018. (Meeting).

23.

2nd Brazil-United States Workshop on Cervical Cancer. 2018. (Other).

24.

SUS and Brazilian Public Health: current scenario and perspectives. 2018. (Other).

25.

Shall We Talk About Rare Diseases?. 2018. (Other).

26.

5th International Congress of the CCS, 3rd International Congress on Obesity Treatment and 7th PCS Meeting. State University of Maringá. Mucocutaneous leishmaniasis in Paraná, evaluation of hospital morbidity from 2008 to 2018. 2018. (Congress).

27.

V International Meeting of Biosciences and Physiopathology e 9th Symposium on Biosciences Applied to Pharmacy..PUBLIC EXPENDITURES ON HOSPITAL MORBIDITY DUE TO LEISHMANIASIS IN PARANA, BRAZIL, BRAZIL.
2018. (Symposium).

28.

26th Annual Scientific Initiation Meeting and 6th Annual Junior Scientific Initiation Meeting. VARIABILITY OF β-LACTAMASES PRESENT IN GRAM-NEGATIVE BACILLI ISOLATED DURING PATIENTS' STAY IN A TEACHING HOSPITAL. 2017.
(Meeting).

29.

1st Workshop on Medical Genetics. 2017. (Other).

30.

25th Annual Scientific Initiation Meeting and 5th Annual Junior Scientific Initiation Meeting. RELATIONSHIP BETWEEN NOCICEPTION AND NITRIC OXIDE PRODUCTION IN ANIMALS INFECTED WITH LEISHMANIA (LEISHMANIA) AMAZONENSIS. 2016. (Meeting).

31.

1st Regional Symposium on Integrative and Exercise Physiology. 2016. (Symposium).

32.

4th Academic Week of Biomedicine - UEM. Relationship between nociception and nitric oxide production in animals infected with Leishmania (Leishmania) amazonensis. 2016. (Other).

33.

PROSED. Socio-educational Project of the 3rd Academic Week of Biomedicine - UEM. 2016. (Oficina).

34.

Health Conexion. 2015. (Other).

35.

3rd Academic Week of Biomedicine ? UEM. Evaluation of the Pathogenicity and Virulence Factors of Candida albicans in the Experimental Candidemia Model. 2015. (Other).

36.

4th Paraná Congress of Biomedical Sciences and 1st Pro-Health Workshop. 2014. (Congress).

37.

3rd Film and Scientific Outreach Exhibition in Biosciences. 2014. (Other).

38.

2nd Academic Week of Biomedicine - UEM. 2014. (Other).

39.

1st Student Discussion Forum. Alternative Energy Sources (Biodiesel and Biogas). 2011. (Other).

Organization of events, congresses, exhibitions, and fairs

1.

NOMA, ISABELLA HARUMI YONEHARA. 2nd FisioTox Lecture Series of the Graduate Program in Pharmacy (Pathophysiology and Toxicology). 2021. (Other).

2.

NOMA, I. H. Y.. VII International Meeting of Biosciences and Physiopathology and 11th Symposium on Biosciences Applied to Pharmacy. 2020. (Other).

3.

NOMA, I. H. Y.. VI International Meeting of Biosciences and Physiopathology ? 10th Symposium on Biosciences Applied to Pharmacy. 2019. (Other).

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I, André Vinícius Inacio Penna Mello, telephone number 415 425-2508, mailing address P.O. Box 90487, San Diego, CA 92169, certify that the professional translation of this document from Portuguese to English has been performed by myself, a qualified translator fluent in both languages, and that the following is an accurate and complete translation of the document.

Date: April 24, 2026.

Isabella Harumi Yonehara Noma

Endereço para acessar este CV: http://lattes.cnpq.br/6549549395123599
ID Lattes: **6549549395123599**
Última atualização do currículo em 31/07/2025

Pós-doutoranda pelo H. Lee Moffitt Cancer Center. Doutora em Ciências pela Faculdade de Ciências Farmacêuticas (FCF) (Capes-7) da USP com foco em melanomas metastáticos. Exploramos a complexidade do microambiente no processo de invasão do melanoma por meio de modelos tridimensionais de pele reconstruída no Laboratório de Fisiopatologia da pele-USP. Graduada em Biomedicina pela Universidade Estadual de Maringá (2017), com projetos de iniciação científica na área de Microbiologia Básica e Imunologia Clínica (UEM). Mestre pelo programa de Ciências da Saúde, atuando no Laboratório de Citologia Clínica e Doenças Sexualmente Transmissíveis com foco em detecção de HPV e outras bactérias associadas pela técnica de biologia molecular. **(Texto informado pelo autor)**

Identificação

Nome

Isabella Harumi Yonehara Noma

Nome em citações bibliográficas

NOMA, I. H. Y.;NOMA, ISABELLA;YONEHARA NOMA, ISABELLA HARUMI;HARUMI YONEHARA NOMA, ISABELLA;NOMA, ISABELLA HARUMI YONEHARA

Lattes iD

http://lattes.cnpq.br/6549549395123599

Orcid iD

? https://orcid.org/0000-0002-7413-1316

País de Nacionalidade

Brasil

Formação acadêmica/titulação

2020 - 2023

Doutorado em Farmácia (Fisiopatologia e Toxicologia).
Universidade de São Paulo, USP, Brasil.
Título: Peroxirredoxina 2 na modulação do fenótipo invasivo em melanoma com mutação em NRAS, Ano de obtenção: 2023.
Orientador: Silvya Stuchi Maria-Engler.
Bolsista do(a): Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq, Brasil.

2018 - 2020

Mestrado em Ciências da Saúde.
Universidade Estadual de Maringá, UEM, Brasil.
Título: Prevalência de Ureaplasmas e

Micoplasmas em mulheres Maringaenses em de idade fértil, Ano de Obtenção: 2020.
Orientador: Marcia Edilaine Lopes Consolaro.
Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES, Brasil.
Setores de atividade: Pesquisa e desenvolvimento científico.

2023 - 2025

Especialização em MBA em Data Science e Analytics EaD. (Carga Horária: 425h).
Universidade de São Paulo, Escola Superior de Agricultura Luiz de Queiroz, USP ESALQ, Brasil.
Título: Clusterização de melanoma cutâneo e acral: Impacto das alterações genéticas na resposta imune.
Orientador: William Barbosa.

2019 - 2020

Especialização em Engenharia Biomédica com ênfase em engenharia clínica. (Carga Horária: 360h).
Universidade Estácio de Sá, UNESA, Brasil.
Título: ANÁLISE GEOESPACIAL DE EQUIPAMENTOS DE DIAGNÓSTICO POR IMAGEM ASSOCIADO ÀS CARACTERÍSTICAS SOCIODEMOGRÁFICAS NO BRASIL.

2014 - 2017

Graduação em Biomedicina.
Universidade Estadual de Maringá, UEM, Brasil.
Título: Avaliação da resposta nociceptiva de camundongos infectados por Leishmania (Leishmania) amazonensis e a sua relação com o óxido nítrico.
Orientador: Sandra Mara Alessi Aristides.
Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES, Brasil.

2011 - 2013

Ensino Médio (2º grau).
Colégio Santa Cruz, CSC, Brasil.

Pós-doutorado

2023 - 2024

Pós-Doutorado.
H. Lee Moffitt Cancer Center and Research Institute, HLMCCRI, Estados Unidos.
Bolsista do(a): Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq, Brasil.
Grande área: Ciências da Saúde

Formação Complementar

2020 - 2020

Extensão universitária em Cusos a distância DES-UEM - Bioestatística Aplicada. (Carga horária: 34h).
Universidade Estadual de Maringá, UEM, Brasil.

2020 - 2020

Saúde em dados: Como trabalhar com os sistemas do DataSUS. (Carga horária: 2h).
Universidade Estadual de Maringá, UEM, Brasil.

2020 - 2020

Introdução à Machine Learning. (Carga horária: 2h).
Universidade Estadual de Maringá, UEM, Brasil.

2020 - 2020

Língua Francesa A2- Paraná fala Francês-Modulo 2 Online. (Carga horária: 30h).
Universidade Estadual de Maringá, UEM, Brasil.

2020 - 2020

1st International Digital Congress on 3D Biofabrication and Bioprinting. (Carga horária: 8h).
Universidade de Araraquara, UNIARA, Brasil.

2020 - 2020

I CURSO DE ONCOLOGIA BÁSICA DA LAOMA. (Carga horária: 10h).
Universidade de Araraquara, UNIARA, Brasil.

2020 - 2020

Understanding Cancer Metastasis. (Carga horária: 4h).
Johns Hopkins University, JHU, Estados Unidos.

2020 - 2020

Introduction to the Biology of Cancer. (Carga horária: 8h).
Johns Hopkins University, JHU, Estados Unidos.

2020 - 2020

Cultivo celular e aplicações no estudo de doenças crônicas e infecciosas. (Carga horária: 15h).
Universidade Federal do Paraná, UFPR, Brasil.

2020 - 2020

XI Board Review in Medical Oncology: Atualização, Discussão e Inovação e II. (Carga horária: 40h).
Instituto Israelita de Ensino e Pesquisa Albert Einstein, IIEPAE, Brasil.

2020 - 2020

Revisão sistemática ? Elementos essenciais para evidências confiáveis. (Carga horária: 2h).
Universidade Estadual de Maringá, UEM, Brasil.

2020 - 2020

Oral Communication and Academic Activities-Modalidade Online. (Carga horária: 35h).
Universidade de São Paulo, USP, Brasil.

2019 - 2019

Extensão universitária em I Curso de Férias em Imunologia e Imunogenética. (Carga horária:

40h).
Universidade Estadual de Maringá, UEM, Brasil.

2019 - 2019

Extensão universitária em UEM na Expoingá. (Carga horária: 5h).
Universidade Estadual de Maringá, UEM, Brasil.

2019 - 2019

Extensão universitária em Active Learning. (Carga horária: 4h).
Universidade Estadual de Maringá, UEM, Brasil.

2019 - 2019

Extensão universitária em Paraná fala francês A1- nível 2. (Carga horária: 60h).
Universidade Estadual de Maringá, UEM, Brasil.

2019 - 2019

Conversation en langue française. (Carga horária: 8h).
Universidade Estadual de Maringá, UEM, Brasil.

2019 - 2019

Descobrindo fagos. (Carga horária: 15h).
Universidade Estadual de Maringá, UEM, Brasil.

2019 - 2019

Vesículas extracelulares envolvidas em doenças crônicas e infecciosas. (Carga horária: 4h).
Universidade Estadual de Maringá, UEM, Brasil.

2019 - 2019

Primers: who are they and how to outline them?.
Universidade Estadual de Londrina, UEL, Brasil.

2018 - 2018

Extensão universitária em Paraná fala Francês. (Carga horária: 60h).
Universidade Estadual de Maringá, UEM, Brasil.

2018 - 2018

Extensão universitária em Paraná fala Inglês. (Carga horária: 30h).
Universidade Estadual de Maringá, UEM, Brasil.

2016 - 2016

Antibiotic as Promotors of Antibiotic Resistence.. (Carga horária: 2h).
Programa de Pós-Graduação em Biociências e Fisiopatologia, PBF, Brasil.

2014 - 2016

Língua japonesa. (Carga horária: 300h).
Universidade Estadual de Maringá, UEM, Brasil.

2015 - 2015

Perfusão Hepática in situ.. (Carga horária: 4h).
Universidade Estadual de Maringá, UEM, Brasil.

2015 - 2015

Estudos e Orientações para Exame de Proeficiência em Língua Japonesa N.05. (Carga horária: 12h).
Universidade Estadual de Maringá, UEM, Brasil.

2014 - 2014

Reprodução Assistida.. (Carga horária: 4h).
Universidade Estadual de Londrina, UEL, Brasil.

Atuação Profissional

H. Lee Moffitt Cancer Center and Research Institute, HLMCCRI, Estados Unidos.

Vínculo institucional

2025 - Atual

Vínculo: Celetista, Enquadramento Funcional: Pós doutorando, Carga horária: 40, Regime: Dedicação exclusiva.

Vínculo institucional

2023 - 2024

Vínculo: Bolsista, Enquadramento Funcional: Pós doutorando, Carga horária: 40

Universidade Estadual de Maringá, UEM, Brasil.

Vínculo institucional

2018 - 2020

Vínculo: Bolsista, Enquadramento Funcional: Estudante de pós-graduação, Carga horária: 40, Regime: Dedicação exclusiva.

Outras informações

Mestrado no Programa de Ciências da Saúde - Bolsista

Vínculo institucional

2017 - 2017

Vínculo: Estagiario, Enquadramento Funcional: Estágio obrigatório em imunogenética, Carga horária: 40

Vínculo institucional

2017 - 2017

Vínculo: Estagiario, Enquadramento Funcional: Estágio obrigatório em análises clínicas, Carga horária: 40

Vínculo institucional

2014 - 2017

Vínculo: Projeto Ensino, Enquadramento Funcional: Práticas em laboratório, Carga horária: 4

Vínculo institucional

2015 - 2016

Vínculo: Bolsista, Enquadramento Funcional: Monitor de microbiologia, Carga horária: 20

Universidade de São Paulo, USP, Brasil.

Vínculo institucional

2022 - 2022

Vínculo: Bolsista, Enquadramento Funcional: Estagiário em Docência, Carga horária: 6

Projetos de pesquisa

2023 - 2024

The role of HDAC8 in UVR-driven melanoma initiation

Descrição: Exposure to ultraviolet radiation (UVR) is the major risk factor for melanoma development andleads to complex phenotypic responses in melanocytes, including dendrite formation, enhancedmelanogenesis, and cell cycle arrest. Previous studies identified the type I histone deacetylase,HDAC8, as an epigenetic regulator of melanoma cells induced following exposure to UVR,hypoxia, and BRAF inhibitor therapy. Our studies suggest that HDAC8 expression is upregulatedin melanocytes that experience high levels of UVR-induced damage. No strategies yet exist toprevent the accumulation of sun-damaged melanocytes in the skin, representing a significantbarrier to effective melanoma prevention. In this new proposal, we aimed to investigate whetherUVR-induced HDAC8 activation allows some melanocytes to evade cell death, enabling cells withpathogenic mutations to survive and potentially develop into melanoma. We utilized a uniquemouse melanoma model driven by mutant BRAF and heterozygous PTEN loss, in which HDAC8expression can be conditionally regulated in skin melanocytes, to elucidate the

role of epigeneticreprogramming in UVR-induced melanoma initiation. Additionally, we employed siRNAmediatedoverexpression of HDAC8 in cell lines. Our findings demonstrated that HDAC8overexpression following UV irradiation increases the survival of UVR-damaged melanocytes andmelanoma cells, mediated by the downregulation of p53 and acetylated p53. This research providesa novel perspective on how UVR-induced epigenetic reprogramming targeting HDAC8contributes to the persistence of melanocytes and melanoma cells harboring pathogenic mutations,allowing them to grow and evade apoptosis..
Situação: Concluído; Natureza: Pesquisa.

Integrantes: Isabella Harumi Yonehara Noma - Integrante / Silvya Stuchi Maria-Engler - Coordenador / SMALLEY, KEIRAN S. M. - Integrante.

2023 - Atual

POTENCIAL METASTÁTICO DA PEROXIRREDOXINA 2 EM MELANOMAS NRAS-MUTADOS

Descrição: Descrição: O melanoma cutâneo é uma neoplasia curável quando diagnosticada em estágios iniciais, mas está associada a uma pior evolução na doença avançada. Lesões de melanoma em locais expostos ao sol e associadas a uma alta carga mutacional e regulação epigenética aberrante, contribuem para o desenvolvimento e progressão da doença. Este é o tipo de câncer de pele com maior potencial metastático, o que leva a sua alta taxa de mortalidade. As principais mutações ocorrem na via das MAPKs: 50% dos pacientes apresentam alterações em BRAF, 15-20% em NRAS e os demais em NF1 ou triplo selvagem. A terapia alvo-dirigida revolucionou o tratamento do melanoma com o uso de inibidores de BRAF e MEK, porém a resistência intrínseca e adquirida acarreta na falha do tratamento. Nesse sentido, mutações em NRAS caracterizam um tumor ainda mais agressivo e sem opções terapêuticas específicas, relacionando esta mutação a um pior prognóstico. A heterogeneidade intratumoral ocorre como consequência da plasticidade fenotípica das células, que assumem e transitam por diferentes estados transcricionais controlados pelos níveis de MITF, tornando as células mais proliferativas ou invasivas. Tal evento está relacionado à metástase e falha da terapia. Dessa forma, vias alternativas devem ser exploradas como potenciais opções para o tratamento de pacientes com mutações em NRAS. Tendo em vista que um dos fatores que limitam a eficácia da terapia alvo-dirigida é a resistência ao estresse oxidativo, o controle da homeostase redox é um interessante ponto de partida. O peróxido de hidrogênio desempenha um papel central na sinalização redox, estando envolvido em todos os hallmarks do câncer, desde proliferação a metabolismo, angiogênese e escape do sistema imune. Além disso, o peróxido de hidrogênio é capaz de alterar os níveis de MITF. Nesse contexto, moléculas que controlem sua ação apresentam substancial interesse. As peroxiredoxinas (PRDXs) são as principais enzimas consumidoras de peróxido de hidrogênio nas células devido a sua alta expressão e capacidade catalítica. Diversos estudos relatam a ação de enzimas antioxidantes na homeostase redox e na tumorigênese. Além do papel excepcional das PRDXs removendo o oxidante do meio, ao se tornarem oxidadas e inativas, elas permitem

que o peróxido de hidrogênio reaja direta ou indiretamente com outros alvos, regulando assim a sinalização redox da célula. Devido a sua função central no controle do peróxido de hidrogênio, as PRDXs estão envolvidas diretamente em diferentes tipos de câncer. De fato, a isoforma PRDX2 está epigeneticamente silenciada em alguns canceres e nosso grupo demonstrou recentemente que ela também está diminuída em pacientes com metástase de melanoma, reforçando seu envolvimento com o processo de invasão. Nesse sentido, foi demonstrado que a PRDX2 é capaz de induzir a estabilidade de junções aderentes em melanomas com mutação em BRAF. Porém ainda não existem estudos que relacionem PRDXs a mutação em NRAS, sendo assim, observamos que linhagens de melanomas com mutação em NRAS mostraram diferentes perfis proliferativos e invasivos, correspondente à diferença de expressão de PRDX2. Assim, nossa proposta é investigar o papel da PRDX2 na plasticidade fenotípica de melanomas com mutação em NRAS. Para isso, será estudado o silenciamento dessa proteína por edição gênica (shRNA) e por um inibidor farmacológico de PRDXs (Conoidina A), além do efeito da sua restauração pelo uso da gliotoxina, um mimético de PRDX. Serão avaliados os parâmetros funcionais como proliferação, capacidade de formação de colônias, migração e invasão em modelos de pele 3D. Tais observações reforçam nossa hipótese de que a PRDX2 pode ser um novo alvo na busca de medicamentos mais específicos para melanomas com mutação em NRAS..
Situação: Em andamento; Natureza: Pesquisa.
Alunos envolvidos: Doutorado: (3) .

Integrantes: Isabella Harumi Yonehara Noma - Coordenador / Silvya Stuchi Maria-Engler - Integrante / Larissa Anastácio da Costa Carvalho - Integrante.

2016 - 2017

Variabilidade de B-lactamase presentes em bacilos Gram negativos isolados durante a permanência de pacientes em Hospital Ensino

Projeto certificado pelo(a) coordenador(a) Maria Cristina Bronharo Tognim em 11/11/2019.

Descrição: Considerando a importância das bactérias Gram Negativas (BGN) nos hospitais, principalmente em UTI, devido à sua resistência antimicrobiana e ainda considerando que os pacientes podem ser fontes ou reservatórios destes microrganismos, este projeto tem como objetivo avaliar a presença dos diferentes BGN que podem colonizar ou infectar os pacientes durante sua permanência hospitalar. Como metodologia utilizaremos a seleção de pacientes que estavam cadastrados em banco de dados do Excel provenientes do projeto ?Vigilância Epidemiológica de Bactérias Multirresistentes no Hospital Universitário de Maringá?. Os pacientes serão selecionados pelas iniciais dos nomes, onde só serão incluídos pacientes que apresentarem dados microbiológicos de isolados em colonização e infecção por BGN. Todos os microrganismos serão recuperados da coleção do freezer, será confirmada a resistência aos carbapenêmicos pelo método de ágar diluição. E todos os isolados serão

realizados testes para detecção de marcadores de resistência aos β lactâmicos por PCR para ESBL (bla CTX, bla SHV e bla TEM), para carbapenêmicos do tipo Oxacilinases e Metalo β lactamases. Os isolados serão caracterizados molecularmente pela técnica de ERIC. Para alguns casos serão avaliados o uso de antimicrobianos nos prontuários dos pacientes. Pretende-se com os achados compreender melhor a dinâmica do microrganismo e seu controle..
Situação: Concluído; Natureza: Pesquisa.
Alunos envolvidos: Graduação: (2) / Doutorado: (1) .

Integrantes: Isabella Harumi Yonehara Noma - Integrante / Alessandra Barrochelli da Silva Ecker - Integrante / Déborah de Castro Moreira - Integrante / Maria Cristina Bronharo Tognim - Coordenador.

2016 - 2016

RELAÇÃO DA NOCICEPÇÃO COM A PRODUÇÃO DE ÓXIDO NÍTRICO EM ANIMAIS INFECTADOS COM LEISHMANIA (LEISHMANIA) AMAZONENSIS

Projeto certificado pelo(a) coordenador(a) Thais Gomes Verzignassi Silveira em 02/03/2021.

Descrição: A leishmaniose tegumentar americana, considerada negligenciada é endêmica em muitos países, inclusive no Brasil. A doença é caracterizada por úlceras e pode ser curada espontaneamente ou apresentar metástases graves e multilantes na mucosa. As lesões geralmente progridem para ulcerações na ausência de dor, sugerindo que a infecção interfere com a percepção de dor. Nesse projeto avaliamos o limiar de dor com doses crescentes do parasito utilizando o anestesiômetro eletrônico e o edema de pata nas lesões induzidas pela inoculação do parasito medidos semanalmente com ajuda de um pletismômetro e com um paquímetro, os resultados mostraram um aumento no edema conforme o aumento das concentrações do parasito e também aumento na intensidade da dor. Avaliamos também a produção de óxido nítrico nas lesões da pata dos animais inoculada com o parasito, porém os resultados preliminares se mostraram negativos. Como conclusão avaliamos que a formação de edema é acompanhada pelo aumento de dor, porém a produção de óxido nítrico não estaria relacionada com a dor e o edema..
Situação: Concluído; Natureza: Pesquisa.
Alunos envolvidos: Graduação: (2) / Mestrado acadêmico: (1) .

Integrantes: Isabella Harumi Yonehara Noma - Integrante / Paula Karoline Sfordi da Silva - Integrante / Valéria Medrado Pereira - Integrante / Sandra Mara Alessi Aristides - Integrante / Thais Gomes Verzignassi Silveira - Coordenador.

Projetos de extensão

2016 - 2017

Dr genética: conteúdo informacional online no auxílio a exames genéticos humanos

Descrição: A dissipação de informação por meio da internet tem se tornado cada vez mais rápida e acessível à população como um todo. No inicio da sua utilização seu acesso era feito em sua grande maioria por amantes de tecnologias e profissionais da área, contudo, com o passar dos anos tanto o acesso como o conteudo disponiveis são feito em grande parte por leigos no assunto. Os brasileiros representam a maior fatia a nivel mundial no quesito tempo médio gasto navegando, possibilitando grande troca de informações incluido na área da saúde . Apesar de muito ùtil no acesso à informação muitos especialistas salientam que muita dessa informação disponibilizada são inadequadas ou incompletas, . No Brasil não há regulamentação ou certificação dos sites com informações sobre saúde.. Desta forma, torna-se essencial a disponibilidade de informação ao público em geral, informação essa oferecida por universidades, embasada em conhecimentos científicos, podendo assim auxiliar na confiança médico-paciente e na diminuição da ansiedade e desconforto decorrentes das enfermidades. O objetivo deste projeto é desenvolver um site com informações sobre exames genéticos, sejam citogenéticos e moleculares, nas diversas áreas da genética, incluindo aconselhamento genético e oncologia, entre outros. Além de informações adicionais, como interpretação, locais em que podem ser feitos e seus custos tanto através do Sistema Unico de Saúde como por Plano de saúde. A divulgação do site será feita através de visitas e cartazes nos postos de saúde em Maringá, Hospital Municipal de Maringá, Hospital Universitário de Maringá. As informações disponibilizadas terão como fonte livros, artigos indexados, laboratórios e hospitais de apoio. Os alunos das áreas de Biomedicina e Medicina participantes do projeto terão acesso ao conhecimento sobre exames geneticos e terão a oportunidade de transmitir esse conhecimento a população em geral, uma vez que a demanda de exames é crescente, sejam de auxílio diagnóstico, prognóstico ou ainda no estabelecimento de condutas terapêuticas, levando-se em conta o desenvolvimento promissor de novas drogas alvo-moleculares.
Situação: Concluído; Natureza: Extensão.
Alunos envolvidos: Graduação: (7) .

Integrantes: Isabella Harumi Yonehara Noma - Integrante / Tamires Leite Valverde - Integrante / Veronica Elisa Pimenta Vicentini - Integrante / Luiz Felipe MSB Blanco - Integrante / Lucas B Gremaschi - Integrante / Patrícia Alexandra da Silva Saavedra Romão - Integrante / Natalia Mestre Braz - Integrante / Eliane Papa Ambrosio Albuquerque - Coordenador.

2016 - 2016

Integração estudantil na execução de ações voluntárias, visando uma maior divulgação e conscientização da comunidade maringaense sobre doenças pouco conhecidas que se manifestam na infância, distrofias musculares progressivas com ênfase na Dist. Muscular

Projeto certificado pelo(a) coordenador(a) Maria Ida Bor[illegible] Ravanelli Speziali em

19/10/2022.

Descrição: Acredita-se que a sociedade em sua grande maioria, desconhece a ocorrência de algumas doenças que acometem crianças. As distrofias musculares se encaixam perfeitamente no grupo de doenças desconhecidas. Apesar de consideradas como raras a incidência das mesmas contraria fortemente essa premissa. Com este trabalho pretende-se através da integração de alunos das diversas áreas da Universidade Estadual de Maringá a realização de ações que promovam junto da sociedade um maior conhecimento sobre doenças que acometem crianças. Desta maneira, o primeiro ano de realização deste projeto, que pretende abordar uma doença por ano, estará direcionando suas ações para as distrofias musculares, permitindo assim que a sociedade se mobilize, participando de eventos que se comprometem com a melhoria da qualidade de vida de pacientes acometidos com distrofia muscular de Duchenne escolhida como slogan deste projeto por ser a distrofia mais frequente bem como a mais letal dentre todas as distrofias..
Situação: Concluído; Natureza: Extensão.
Alunos envolvidos: Graduação: (13) .

Integrantes: Isabella Harumi Yonehara Noma - Integrante / Paula Karoline Sfordi da Silva - Integrante / Tamires Leite Valverde - Integrante / Patrícia Alexandra da Silva Saavedra Romão - Integrante / Natalia Mestre Braz - Integrante / Lais de Sena Marques - Integrante / Maria Ida Bonini Ravanelli Speziali - Coordenador / Fabiana Galvão da Motta Lima - Integrante / Francyhellen Franco Montanha - Integrante / Gabriela Fernanda Martinez - Integrante / Maria Naomi Yamanaka - Integrante / Sara Fabricio dos Santos - Integrante / Aarão Fabrício dos Santos - Integrante / Leonardo de Oliveira Figueiredo - Integrante.

Áreas de atuação

1.

Grande área: Ciências da Saúde / Área: Farmácia / Subárea: Citologia Clínica.

2.

Grande área: Ciências da Saúde / Área: Farmácia / Subárea: Fisiopatologia e diagnóstico laboratorial.

Idiomas

Inglês

Compreende Bem, Fala Razoavelmente, Lê Bem, Escreve Razoavelmente.

Prêmios e títulos

2022

Menção honrosa do trabalho intitulado ? Targeting Peroxiredoxin-2 as a potential treatment for NRAS-mutated metastatic melanoma?, Universidade de São Paulo.

2022

SMR PARTICIPATION AWARD, Society for Melanoma Research.

Produções

Produção bibliográfica

Citações

Google Scholar

Total de trabalhos: 19 Total de citações: 74 Data: 10/02/2025

https://scholar.google.com/citations?user=gtlvO4YAAAAJ&hl=pt-BR

Artigos completos publicados em periódicos

Ordenar por

Ordem Cronológica

1.

NORONHA, NATÁLIA YUMI ; RODRIGUES, GUILHERME DA SILVA ; WATANABE, LÍGIA MORIGUCHI ; **NOMA, ISABELLA HARUMI YONEHARA** ; CAZIER, JEAN-BAPTISTE ; SAE-LEE, CHANACHAI ; CHITTA, PITAKSIN ; PEREIRA, VANESSA APARECIDA BATISTA ; PINHEL, MARCELA AUGUSTA DE SOUZA ; DIANI, LUISA MARIA ; BARBOSA, FERNANDO ; PLÖSCH, TORSTEN ; NONINO, CARLA BARBOSA . DNA methylation signatures of arsenic exposure and obesity. Environmental Epigenetics JCR, v. -, p. -, 2025.

2.

DA SILVA RODRIGUES, GUILHERME ; NORONHA, NATÁLIA YUMI ; **NOMA, ISABELLA HARUMI YONEHARA** ; DE LIMA, JOÃO GABRIEL RIBEIRO ; DA SILVA SOBRINHO, ANDRESSA CRYSTINE ; DE SOUZA PINHEL, MARCELA AUGUSTA ; DE ALMEIDA, MARIANA LUCIANO ; WATANABE, LÍGIA MORIGUCHI ; NONINO, CARLA BARBOSA ; JÚNIOR, CARLOS ROBERTO BUENO . 14-Week exercise training modifies the DNA methylation levels at gene sites in non-Alzheimer's disease women aged 50 to 70 years. EXPERIMENTAL GERONTOLOGY JCR, v. 186, p. 112362, 2024. **Citações:** WEB OF SCIENCE 2 | SCOPUS 2

3.

NORONHA, NATÁLIA YUMI ; **NOMA, ISABELLA HARUMI YONEHARA** ; FERNANDES FERREIRA, RAFAEL ; RODRIGUES, GUILHERME DA SILVA ; MARTINS, LUZANIA DOS SANTOS ; WATANABE, LÍGIA MORIGUCHI ; PINHEL, MARCELA AUGUSTA DE SOUZA ; MELLO SCHINEIDER, ISABELLE ; DIANI, LUISA MARIA ; CARLOS, DANIELA ; NONINO, CARLA BARBOSA . Association between the relative abundance of phyla a[illegible]obacteria, vitamin C consumption,

and DNA methylation of genes linked to immune response pathways. FRONTIERS IN NUTRITION JCR, v. 11, p. -, 2024. **Citações:** WEB OF SCIENCE ™ 2 | SCOPUS 1

4.

NOMA, ISABELLA HARUMI YONEHARA; CARVALHO, LARISSA ANASTACIO DA COSTA ; CAMARENA, DENISSE ESTHER MALLAUPOMA ; SILVA, RENAIRA OLIVEIRA ; MORAES JUNIOR, MANOEL OLIVEIRA DE ; DE SOUZA, SOPHIA TAVARES ; NEWTON-BISHOP, JULIA ; NSENGIMANA, JEREMIE ; MARIA-ENGLER, SILVYA STUCHI . Peroxiredoxin-2 represses NRAS-mutated melanoma cells invasion by modulating EMT markers. BIOMEDICINE & PHARMACOTHERAPY JCR, v. 177, p. 116953, 2024. **Citações:** WEB OF SCIENCE ™ 2

5.

CARVALHO, LARISSA ANASTACIO DA COSTA ; **NOMA, ISABELLA HARUMI YONEHARA** ; UEHARA, ADRIANA HIROMI ; SIENA, ADAMO DAVI DIOGENES ; OSAKI, LUCIANA HARUMI ; MORI, MATEUS PRATES ; PINTO, NADJA CRISTHINA DE SOUZA ; FREITAS, VANESSA MORAIS ; JUNIOR, WILSON ARAUJO SILVA ; SMALLEY, KEIRAN S. M. ; MARIA-ENGLER, SILVYA STUCHI . Modeling Melanoma Heterogeneity In Vitro: Redox, Resistance and Pigmentation Profiles. ANTIOXIDANTS JCR, v. 13, p. 555, 2024. **Citações:** WEB OF SCIENCE ™ 2 | SCOPUS 2

6.

FRANCO, YLANA ADAMI ; DE MORAES, MANOEL OLIVEIRA ; CARVALHO, LARISSA A. C. ; DOHLE, WOLFGANG ; DA SILVA, RENAIRA OLIVEIRA ; **NOMA, ISABELLA HARUMI YONEHARA** ; LIMA, KELI ; POTTER, BARRY V. L. ; MACHADO-NETO, JOÃO A. ; MARIA-ENGLER, SILVYA STUCHI . 2-Methoxyestradiol-3,17-O,O-bis-sulfamate (STX140) Inhibits Proliferation and Invasion via Senescence Pathway Induction in Human BRAFi-Resistant Melanoma Cells. INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES JCR, v. 24, p. 11314, 2023. **Citações:** WEB OF SCIENCE ™ 1 | SCOPUS 1

7.

DA SILVA RODRIGUES, GUILHERME ; **NOMA, ISABELLA HARUMI YONEHARA** ; NORONHA, NATÁLIA YUMI ; WATANABE, LÍGIA MORIGUCHI ; DA SILVA SOBRINHO, ANDRESSA CRYSTINE ; DE LIMA, JOÃO GABRIEL RIBEIRO ; SAE-LEE, CHANACHAI ; BENJAMIM, CICERO JONAS RODRIGUES ; NONINO, CARLA BARBOSA ; BUENO, CARLOS ROBERTO . Eight Weeks of Physical Training Decreases 2 Years of DNA Methylation Age of Sedentary Women. RESEARCH QUARTERLY FOR EXERCISE AND SPORT JCR, v. -, p. 1-11, 2023. **Citações:** WEB OF SCIENCE ™ 2 | SCOPUS 2

8.

WATANABE, LÍGIA MORIGUCHI ; PEREIRA, VANESSA APARECIDA BATISTA ; NORONHA, NATALIA YUMI ; DE SOUZA PINHEL, MARCELA AUGUSTA ; WOLF, LETICIA SANTANA ; DE OLIVEIRA, CRISTIANA CORTES ; PLAÇA, JESSICA RODRIGUES ; **NOMA, ISABELLA HARUMI YONEHARA** ; DA SILVA RODRIGUES, GUILHERME ; DE SOUZA, VANESSA CRISTINA OLIVEIRA ; JUNIOR, FERNANDO BARBOSA ; NONINO, CARLA BARBOSA . The influence of serum selenium in differential epigenetic and transcriptional regulation of CPT1B gene in women with obesity. JOURNAL OF TRACE ELEMENTS IN MEDICINE AND BIOLOGY JCR, v. -, p. 127376, 2023. **Citações:** WEB OF SCIENCE ™ 3 | SCOPUS 2

9.

YUMI NORONHA, NATÁLIA ; DA SILVA RODRIGUES, GUILHERME ; **HARUMI YONEHARA NOMA, ISABELLA** ; FERNANDA CUNHA BRANDAO, CAMILA ; PEREIRA RODRIGUES, KARINE ; COLELLO BRUNO, ALEXANDRE ; SAE-LEE, CHANACHAI ; MORIGUCHI WATANABE, LIGIA ; AUGUSTA DE SOUZA PINHEL, MARCELA ; MELLO SCHINEIDER, ISABELLE ; LUCIANO DE ALMEIDA, MARIANA ; BARBOSA JUNIOR, FERNANDO ; ARAUJO MORAIS, DEBORAH ; TAVARES DE SOUSA JUNIOR, WELLINGTON ; PLÖSCH, TORSTEN ; ROBERTO BUENO JUNIOR, CARLOS ; BARBOSA NONINO, CARLA . 14-weeks combined exercise epigenetically modulated 118 genes of menopausal women with prediabetes. Frontiers in Endocrinology JCR, v. 13, p. 895489, 2022. **Citações:** WEB OF SCIENCE ℠ 14 | SCOPUS 11

10.

DOMINGUES, KARLA LARISSA TRASSI GANAZA ; TANOSHI, CÍNTHIA AKEMI ; **NOMA, ISABELLA HARUMI YONEHARA** ; CARVALHO, MARIA DALVA DE BARROS ; PELLOSO, SANDRA MARISA ; CONSOLARO, MARCIA EDILAINE LOPES ; ARISTIDES, SANDRA MARA ALESSI ; DEMARCHI, IZABEL GALHARDO ; PEDROSO, RAISSA BOCCHI ; LONARDONI, MARIA VALDRINEZ CAMPANA . Economic impact of hospitalizations due to leishmaniasis in southern Brazil. RESEARCH, SOCIETY AND DEVELOPMENT, v. 11, p. e39211831139, 2022.

11.

NORONHA, NATÁLIA YUMI ; DA SILVA RODRIGUES, GUILHERME ; DE SOUZA PINHEL, MARCELA AUGUSTA ; CAZIER, JEAN-BAPTISTE ; WATANABE, LIGIA MORIGUCHI ; MENEZES, ALBERT NOBRE ; BUENO, CARLOS ROBERTO ; NICOLETTI, CAROLINA FERREIRA ; DE OLIVEIRA, BRUNO AFFONSO PARENTI ; SCHINEIDER, ISABELLE MELLO ; **YONEHARA NOMA, ISABELLA HARUMI** ; DIAS ALCARAS, IGOR CAETANO ; BARBOSA, FERNANDO ; BARBOSA NONINO, CARLA . Sample Preparation to Bioinformatics Analysis of DNA Methylation: Association Strategy for Obesity and Related Trait Studies. Jove-Journal of Visualized Experiments JCR, v. 183, p. e62598, 2022. **Citações:** WEB OF SCIENCE ℠ 8 | SCOPUS 7

12.

NOMA, ISABELLA; SHINOBU-MESQUITA, CRISTIANE ; SUEHIRO, TAMY ; MORELLI, FABRICIO ; DE SOUZA, MARIA ; DAMKE, EDILSON ; DA SILVA, VÂNIA ; CONSOLARO, MARCIA . Association of Righ-Risk Human Papillomavirus and Ureaplasma parvum Co-Infections with Increased Risk of Low-Grade Squamous Intraepithelial Cervical Lesions. Asian Pacific Journal of Cancer Prevention, v. 22, p. 1239-1246, 2021. **Citações:** SCOPUS 8

13.

SANTOS E SILVA, JUAN CARLO ; VASCONCELOS, AMANDA PEREIRA ; **YONEHARA NOMA, ISABELLA HARUMI** ; NORONHA, NATÁLIA YUMI ; AQUINO, RODRIGO ; GIDDALURU, JEEVAN ; DURÃO, LUIZ ; COSTA-MARTINS, ANDRÉ GUILHERME ; SCHUCH, VIVIANE ; MORAES-VIEIRA, PEDRO M. ; NAKAYA, HELDER I. . Gene Signatures of Autopsy Lungs from Obese Patients with COVID-19. CLINICAL NUTRITION ESPEN JCR, v. 43, p. 986, 2021. **Citações:** WEB OF SCIENCE ℠ 8 | SCOPUS 8

Resumos expandidos publicados em anais de congressos

1.

TANOSHI, C. A. ; **NOMA, I. H. Y.** ; GANAZA, K. L. T. ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. ; ARISTIDES, S. M. A. . DESPESAS EM MORBIDADE HOSPITALAR DEVIDO A LEISHMANIOSE VISCERAL EM UMA REGIÃO DO SUL DO BRASIL. In: V Congresso Internacional do CCS, III Congresso Internacional de Tratamento da Obesidade e VII Encontro do PCS. Universidade Estadual de Maringá, 2018, Maringá. Anais V Congresso Internacional do CCS, III Congresso Internacional de Tratamento da Obesidade e VII Encontro do PCS, 2018. p. 5.

2.

GANAZA, K. L. T. ; TANOSHI, C. A. ; **NOMA, I. H. Y.** ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; CONSOLARO, M. E. L. ; ARISTIDES, S. M. A. ; DEMARCHI, I. G. ; LONARDONI, M. V. C. . ANÁLISE DA MORBIDADE HOSPITALAR POR LEISHMANIOSE CUTÂNEA NO ESTADO DO PARANÁ, BRASIL, 2008-2018. In: V Congresso Internacional do CCS, III Congresso Internacional de Tratamento da Obesidade e VII Encontro do PCS. Universidade Estadual de Maringá, 2018, Maringá. Anais V Congresso Internacional do CCS, III Congresso Internacional de Tratamento da Obesidade e VII Encontro do PCS, 2018.

3.

NOMA, I. H. Y.; GANAZA, K. L. T. ; TANOSHI, C. A. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; ARISTIDES, S. M. A. ; DEMARCHI, I. G. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. . LEISHMANIOSE MUCOCUTÂNEA NO PARANÁ, AVALIAÇÃO DA MORBIDADE HOSPITALAR DE 2008 A 2018.. In: V Congresso Internacional do CCS, III Congresso Internacional de Tratamento da Obesidade e VII Encontro do PCS. Universidade Estadual de Maringá, 2018, Maringá. Anais V Congresso Internacional do CCS, III Congresso Internacional de Tratamento da Obesidade e VII Encontro do PCS, 2018. p. 6.

4.

TANOSHI, C. A. ; GANAZA, K. L. T. ; **NOMA, I. H. Y.** ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; CONSOLARO, M. E. L. ; LONARDONI, M. V. C. ; ARISTIDES, S. M. A. . A prevalência de uma doença negligenciada: epidemiologia e morbidade das leishmanioses no Parana. In: V International Meeting of Biosciences and Physiopathology e IX Simpósio de Biociências Aplicadas à Farmácia., 2018, Maringá. Anais IX Semana Acadêmica de Biomedicina, 2018.

5.

NOMA, I. H. Y.; ECKER, A. B. S. ; MOREIRA, D. C. ; SILVA, L. C. ; PEREIRA, P. M. ; SZCZEREPA, M. M. A. ; TOGNIM, M. C. B. . VARIABILIDADE DE β-LACTAMASES PRESENTES EM BACILOS GRAM NEGATIVOS ISOLADOS DURANTE A PERMANÊNCIA DE PACIENTES EM HOSPITAL DE ENSINO. In: 26.o Encontro Anual de Iniciação Científica e 6.o Encontro Anual de Iniciação Científica Júnior, 2017, Maringá. Anais XXVI EAIC VI EAIC Jr., 2017. p. 67.

6.

ALBUQUERQUE, E. P. A. ; MONTANHA, F. F. ; **NOMA, I. H. Y.** ; GREMASCHI, L. B. ; BLANCO, L. F. M. ; BRAZ, N. M. ; ROMAO, P. A. S.

S. ; VALVERDE, T. L. ; VICENTINI, V. E. P. . Dr. Genética: informação a serviço da comunidade. In: Fórum de Extensão e Cultura da UEM Extensão Universitária: compromisso e transformação social, 2016, Maringá. Anais do 14o Fórum de Extensão e Cultura da UEM : Extensão Universitária : compromisso e transformação social, 2016. v. 23. p. 13-16.

7.

NOMA, I. H. Y.; PEREIRA, V. M. ; SANUKI, M. S. ; BORGHI, S. M. ; VERRI JUNIOR, W. A. ; SILVA, P. K. S. ; ARISTIDES, S. M. A. . RELAÇÃO DA NOCICEPÇÃO COM A PRODUÇÃO DE ÓXIDO NÍTRICO EM ANIMAIS INFECTADOS COM LEISHMANIA (LEISHMANIA) AMAZONENSIS. In: 25.o Encontro Anual de Iniciação Científica e 5.o Encontro Anual de Iniciação Científica Júnior, 2016, Maringá. Anais XXV EAIC V EAIC Jr., 2016. p. 62.

8.

NOMA, I. H. Y.; WATANABE, A. S. M. ; SILVA, M. R. T. ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . Avaliação da patogenicidade e fatores de virulência de Candida albicans no modelo experimental de Candidemia. In: III Semana Acadêmica de Biomedicina ? UEM, 2015, Maringá. ANAIS DA TERCEIRA SEMANA ACADÊMICA DE BIOMEDICINA, 2015.

9.

WATANABE, A. S. M. ; SILVA, M. R. T. ; **NOMA, I. H. Y.** ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . AVALIAÇÃO DOS FATORES DE VIRULÊNCIA DE CANDIDA TROPICALIS APÓS CANDIDEMIA EXPERIMENTAL. In: III Semana Acadêmica de Biomedicina ? UEM, 2015, Maringá. ANAIS DA TERCEIRA SEMANA ACADÊMICA DE BIOMEDICINA, 2015.

10.

SILVA, M. R. T. ; WATANABE, A. S. M. ; **NOMA, I. H. Y.** ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . AVALIAÇÃO DO PERFIL DE PATOGENICIDADE DE CANDIDA ALBICANS APÓS CONTATOS SUBSEQUENTES COM O HOSPEDEIRO. In: III Semana Acadêmica de Biomedicina ? UEM, 2015, Maringá. ANAIS DA TERCEIRA SEMANA ACADÊMICA DE BIOMEDICINA, 2015.

Resumos publicados em anais de congressos

1.

RODRIGUES, R. F. ; SILVA, L. L. ; TANOSHI, C. A. ; **NOMA, I. H. Y.** ; SILVA, G. E. C. . ANÁLISE DO PERFIL EPIDEMIOLÓGICO DE ACIDENTES POR ANIMAIS PEÇONHENTOS NO ESTADO DO PARANÁ: NO PERÍODO DE 2015 A 2019. In: VII International Meeting of Biosciences and Physiopathology e XI Simpósio de Biociências Aplicadas à Farmácia, 2020, Maringá. Anais do VII International Meeting of Biosciences and Physiopathology e XI Simpósio de Biociências Aplicadas à Farmácia. Maringá: Isabella Harumi Yonehara Noma, 2020. v. 1.

2.

NOMA, I. H. Y.; TANOSHI, C. A. ; SANTOS, D. S. ; RODRIGUES, R. F. ; SILVA, L. L. ; CONSOLARO, M. E. L. ; SOUZA, E. M. ; PELLOSO, S. M.

; PEDROSO, R. B. . THE PREVALENCE OF SYPHILIS IN PRENATAL CARE WOMEN IN PARANA STATE, BRAZIL. In: III INTERNATIONAL SYMPOSIUM OF EXPERIMENTAL PATHOLOGY, 2019, Londrina. Annais III International Symposium os Experimental Pathology. Londrina : UEL, 2019: Eduardo José de Almeida Araújo ...[et al.], 2019. p. 48.

3.

TANOSHI, C. A. ; **NOMA, I. H. Y.** ; RODRIGUES, R. F. ; SILVA, L. L. ; ROSSONI, D. F. ; PEDROSO, R. B. ; ARISTIDES, S. M. A. . CHALLENGES TO HUMAN LEISHMANIASIS: DISCUSSION OF THE DISEASE BASED ON ITS BEHAVIOR. In: III INTERNATIONAL SYMPOSIUM OF EXPERIMENTAL PATHOLOGY, 2019, Londrina. Annais III International Symposium os Experimental Pathology, 2019. p. 40.

4.

NOMA, I. H. Y.; SUEHIRO, T. T. ; MORELLI, F. ; SOUZA, M. V. F. ; MESQUITA, C. S. S. ; SILVA, V. R. S. ; CONSOLARO, M. E. L. . Co-infecções entre Papillomavirus Humano (HPV) e micoplasmas genitais em mulheres assintomáticas. In: VI International Meeting of Biosciences and Physiopathology ? X Simpósio de Biociências Aplicadas à Farmácia, 2019, Ivatuba. Annais VI International Meeting of Biosciences & Physiopathology. Maringá, 2019. p. 227.

5.

NOMA, I. H. Y.; GANAZA, K. L. T. ; TANOSHI, C. A. ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; ARISTIDES, S. M. A. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. . GASTOS PÚBLICOS NA MORBIDADE HOSPITALAR POR LEISHMANIOSE NO PARANÁ, BRASIL. In: V International Meeting of Biosciences and Physiopathology e IX Simpósio de Biociências Aplicadas à Farmácia., 2018, Maringá. Anais de Simpósio do Programa de Biociências e Fisiopatologia- UEM, 2018. v. 1. p. 175.

6.

NOMA, I. H. Y.; ECKER, A. B. S. ; SZCZEREPA, M. M. A. ; TOGNIM, M. C. B. . Disseminação de enterobactérias em pacientes durante a internação em unidade de tratamento intensivo de hospital escola do sul do Brasil. In: IV International Meeting of Biosciences and Physiopathology, 2017, Maringá. VII Simpósio de Biociências Aplicadas à Farmácia, 2017.

7.

MOREIRA, D. C. ; ECKER, A. B. S. ; **NOMA, I. H. Y.** ; FEDRIGO, N. H. ; FABRI, F. V. ; COSTA, B. B. ; ZERBINATI, H. T. ; MENEZES, A. V. ; FERNANDES, A. S. ; TOGNIM, M. C. B. . SURVEILLANCE OF CARBAPENEM-RESISTANT ENTEROBACTERIACEAE IN PATIENTS DURING HOSPITALIZATION IN AN ADULT INTENSIVE CARE UNIT. In: 29º CONGRESSO BRASILEIRO DE MICROBIOLOGIA - CBM, 2017, Foz do Iguaçu. 29º Congresso Brasileiro de Microbiologia 2017, 2017.

8.

SILVA, L. C. ; PEREIRA, P. M. ; DURANTE, P. C. ; MATTOS, M. S. F. ; ZERBINATI, H. T. ; **NOMA, I. H. Y.** ; FERNANDES, A. S. ; CARDOSO, C. L. ; TOGNIM, M. C. B. ; NISHIYAMA, S. A. B. . PRELIMINARY EVALUATION OF THE FREQUENCY OF ACINETOBACTER BAUMANNI ISOLATE AT THE BUCAL AND NASAL CAVITY FROM UNIVERSITY STUDENTS. In: 29° Congresso Brasileiro de Microbiologia, 2017, Foz do Iguaçu. 29º Congresso Brasileiro de Microbiologia 2017, 2017.

9.

FEDRIGO, N. H. ; MATTA, P. E. G. ; PEREIRA, P. M. ; **NOMA, I. H. Y.** ; TOGNIM, M. C. B. . Atividade da fosfomicina contra Acinetobacter baumannii e desempenho de métodos de avaliação de sensibilidade. In: VII Simpósio de Biociências Aplicadas à Farmácia e III International Meeting of Biosciences and Physiopathology, 2016, Maringá. Anais Simpósio VII Simpósio de Biociências Aplicadas à Farmácia, 2016.

10.

NOMA, I. H. Y.; PEREIRA, V. M. ; SILVA, P. K. S. ; SANUKI, M. S. ; SILVEIRA, T. G. V. ; ARISTIDES, S. M. A. . RELAÇÃO DA NOCICEPÇÃO COM A PRODUÇÃO DE ÓXIDO NÍTRICO EM ANIMAIS INFECTADOS COM Leishmania (Leishmania) amazonensis. In: IV Semana Acadêmica de Biomedicina - UEM, 2016, Maringá. Anais da IV Semana de Biomedicina, 2016.

11.

MATTA, P. E. G. ; ECKER, A. B. S. ; ZARPELLON, M. N. ; MOREIRA, D. C. ; **NOMA, I. H. Y.** ; TAMURA, N. K. ; GARCIA, L. B. ; TOGNIM, M. C. B. . PREVALÊNCIA DE RESISTÊNCIA AOS CARBAPENÊMICOS NOS PRINCIPAIS BACILOS GRAM-NEGATIVOS ISOLADO SEM UM HOSPITAL DE ENSINO. In: XIV Congresso Brasileiro de Controle de Infecção e Epidemiologia Hospitalar, 2014, Curitiba. Journal of Infection Control, 2014. v. 3. p. 139.

12.

VIANA, G. F. ; ZAGO, M. C. B. ; SAALFELD, S. M. S. ; **NOMA, I. H. Y.** ; MATTA, P. E. G. ; MITSUGUI, C. S. ; ABREU FILHO, B. A. ; TOGNIM, M. C. B. . ALTERAÇÃO NO MODO DE DISSEMINAÇÃO DE ISOLADOS DE Acinetobacter baumannii EM UM HOSPITAL UNIVERSITÁRIO. In: XIV Congresso Brasileiro de Controle de Infecção e Epidemiologia Hospitalar, 2014, Curitiba. Journal of Infection Control, 2014. v. 3. p. 148.

13.

ZAGO, M. C. B. ; VIANA, G. F. ; SAALFELD, S. M. S. ; **NOMA, I. H. Y.** ; FARIAS, A. P. M. ; HELBEL, C. ; TOGNIM, M. C. B. . DISSEMINAÇÃO DE Acinetobacter baumannii EM UM HOSPITAL TERCIÁRIO NA REGIÃO NORTE DO PARANA. In: XIV CONGRESSO BRASILEIRO DE CONTROLE DE INFECÇÃO E EPIDEMIOLOGIA HOSPITALAR, 2014, Curitiba. DISSEMINAÇÃO DE Acinetobacter baumannii EM UM HOSPITAL TERCIÁRIO NA REGIÃO NORTE DO PARANÁ, 2014. v. 3. p. 135.

Apresentações de Trabalho

1.

NOMA, I. H. Y.; CRUZ, E. ; DUTRA, A. C. ; NEGRI, M. F. N. . Geospatial Analysis of Diagnostic Imaging Equipment in Brazil. 2020. (Apresentação de Trabalho/Conferência ou palestra).

2.

CRUZ, E. ; **NOMA, I. H. Y.** ; DUTRA, A. C. ; NEGRI, M. F. N. . Life-Sustaining Equipment: A Demographic Geospace Analysis in National Territory. 2020. (Apresentação de Trabalho/Conferência ou palestra).

3.

RODRIGUES, R. F. ; SILVA, L. L. ; TANOSHI, C. A. ; **NOMA, I. H. Y.** ; SILVA, G. E. C. . ANÁLISE DO PERFIL EPIDEMIOLÓGICO DE ESCORPIONISMO NOS ESTADOS DO SUL DO BRASIL: UM ESTUDO REFERENTE AO PERÍODODE 2015 A 2019. 2020. (Apresentação de Trabalho/Outra).

4.

NOMA, I. H. Y.; SUEHIRO, T. T. ; MORELLI, F. ; SOUZA, M. V. F. ; MESQUITA, C. S. S. ; SILVA, V. R. S. ; CONSOLARO, M. E. L. . Co-infecções entre Papillomavirus Humano (HPV) e micoplasmas genitais em mulheres assintomáticas. 2019. (Apresentação de Trabalho/Simpósio).

5.

TANOSHI, C. A. ; **NOMA, I. H. Y.** ; RODRIGUES, R. F. ; SILVA, L. L. ; PEDROSO, R. B. ; ARISTIDES, S. M. A. . Challenges to human leishmaniasis: discussion of the disease based on its behavior. 2019. (Apresentação de Trabalho/Simpósio).

6.

NOMA, I. H. Y.; TANOSHI, C. A. ; RODRIGUES, R. F. ; SILVA, L. L. ; CONSOLARO, M. E. L. ; PEDROSO, R. B. . The prevalence of syphilis in prenatal care women in Paraná State, Brazil. 2019. (Apresentação de Trabalho/Simpósio).

7.

NOMA, I. H. Y.; GANAZA, K. L. T. ; TANOSHI, C. A. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; ARISTIDES, S. M. A. ; DEMARCHI, I. G. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. . Leishmaniose mucocutânea no Paraná, avaliação da morbidade hospitalar de 2008 a 2018.. 2018. (Apresentação de Trabalho/Congresso).

8.

GANAZA, K. L. T. ; TANOSHI, C. A. ; **NOMA, I. H. Y.** ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; CONSOLARO, M. E. L. ; ARISTIDES, S. M. A. ; DEMARCHI, I. G. ; LONARDONI, M. V. C. . Análise da morbidade hospitalar por leishmaniose cutânea no estado do Paraná, Brasil, 2008-2018. 2018. (Apresentação de Trabalho/Congresso).

9.

TANOSHI, C. A. ; **NOMA, I. H. Y.** ; GANAZA, K. L. T. ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. ; ARISTIDES, S. M. A. . Despesas em morbidade hospitalar devido a leishmaniose visceral em uma região do sul do Brasil.. 2018. (Apresentação de Trabalho/Congresso).

10.

TANOSHI, C. A. ; GANAZA, K. L. T. ; **NOMA, I. H. Y.** ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; CONSOLARO, M. E. L. ; LONARDONI, M. V. C. ; ARISTIDES, S. M. A. . A prevalência de uma doença infecciosa negligenciada: epidemiologia e morbidade das leishmanioses no Paraná. 2018. 2018. (Apresentação de Trabalho/Simpósio).

11.

NOMA, I. H. Y.; GANAZA, K. L. T. ; TANOSHI, C. A. ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; ARISTIDES, S. M. A. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. . GASTOS PÚBLICOS NA MORBIDADE HOSPITALAR POR LEISHMANIOSE NO PARANA, BRASIL. 2018. (Apresentação de Trabalho/Simpósio).

12.

ECKER, A. B. S. ; MOREIRA, D. C. ; **NOMA, I. H. Y.** ; VIANA, G. F. ; MITSUGUI, C. S. ; PEREIRA, P. M. ; TOGNIM, M. C. B. . VARIABILIDADE CLONAL E PESQUISA DE GENES DE BETA LACTAMASES DE ACINETOBACTER BAUMANNII ISOLADOS DURANTE A PERMANÊNCIA DE PACIENTES EM UNIDADE DE TERAPIA INTENSIVA. 2017. (Apresentação de Trabalho/Congresso).

13.

PEREIRA, P. M. ; MATTA, P. E. G. ; **NOMA, I. H. Y.** ; SILVA, L. C. ; FARIAS, A. P. M. ; ZARPELLON, M. N. ; TOGNIM, M. C. B. . ATIVIDADE DE FOSFOMICINA CONTRA ISOLADOS DE ACINETOBACTER BAUMANNII: AVALIAÇÃO DE DIFERENTES METODOLOGIAS. 2017. (Apresentação de Trabalho/Congresso).

14.

NOMA, I. H. Y.; ECKER, A. B. S. ; MOREIRA, D. C. ; SILVA, L. C. ; PEREIRA, P. M. ; SZCZEREPA, M. M. A. ; TOGNIM, M. C. B. . VARIABILIDADE DE β-LACTAMASES PRESENTES EM BACILOS GRAM NEGATIVOS ISOLADOS DURANTE A PERMANÊNCIA DE PACIENTES EM HOSPITAL DE ENSINO. 2017. (Apresentação de Trabalho/Outra).

15.

NOMA, I. H. Y.; ECKER, A. B. S. ; SZCZEREPA, M. M. A. ; TOGNIM, M. C. B. . Disseminação de enterobactérias em pacientes durante a internação em unidade de tratamento intensivo de hospital escola do sul do Brasil.. 2017. (Apresentação de Trabalho/Simpósio).

16.

SILVA, L. C. ; PEREIRA, P. M. ; DURANTE, P. C. ; MATTOS, M. S. F. ; ZERBINATI, H. T. ; **NOMA, I. H. Y.** ; FERNANDES, A. S. ; CARDOSO, C. L. ; TOGNIM, M. C. B. ; NISHIYAMA, S. A. B. . PRELIMINARY EVALUATION OF THE FREQUENCY OF ACINETOBACTER BAUMANNI ISOLATE AT THE BUCAL AND NASAL CAVITY FROM UNIVERSITY STUDENTS. 2017. (Apresentação de Trabalho/Congresso).

17.

MOREIRA, D. C. ; ECKER, A. B. S. ; **NOMA, I. H. Y.** ; FEDRIGO, N. H. ; FABRI, F. V. ; COSTA, B. B. ; ZERBINATI, H. T. ; MENEZES, A. V. ; FERNANDES, A. S. ; TOGNIM, M. C. B. . SURVEILLANCE OF CARBAPENEM-RESISTANT ENTEROBACTERIACEAE IN PATIENTS DURING HOSPITALIZATION IN AN ADULT INTENSIVE CARE UNIT.. 2017. (Apresentação de Trabalho/Congresso).

18.

ROMAO, P. A. S. S. ; ALBUQUERQUE, E. P. A. ; MONTANHA, F. F. ; **NOMA, I. H. Y.** ; GREMASCHI, L. B. ; BLANCO, L. F. M. ; BRAZ, N. M. ; VALVERDE, T. L. ; VICENTINI, V. E. P. . Dr. Genética: Conteúdo informacional online de apoio a exames genéticos. 2016. (Apresentação de Trabalho/Outra).

19.

NOMA, I. H. Y.; PEREIRA, V. M. ; SANUKI, M. S. ; BORGHI, S. M. ; VERRI JUNIOR, W. A. ; SILVA, P. K. S. ; ARISTIDES, S. M. A. . RELAÇÃO DA NOCICEPÇÃO COM A PRODUÇÃO DE ÓXIDO NÍTRICO EM ANIMAIS INFECTADOS COM LEISHMANIA (LEISHMANIA) AMAZONENSIS. 2016. (Apresentação de Trabalho/Outra).

20.

FEDRIGO, N. H. ; MATTA, P. E. G. ; PEREIRA, V. M. ; **NOMA, I. H. Y.** ; TOGNIM, M. C. B. . Atividade da fosfomicina contra Acinetobacter baumannii e desempenho de métodos de avaliação de sensibilidade. 2016. (Apresentação de Trabalho/Simpósio).

21.

NOMA, I. H. Y.; WATANABE, A. S. M. ; SILVA, M. R. T. ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . Avaliação da patogenicidade e fatores de virulência de Candida albicans no modelo experimental de Candidemia. 2015. (Apresentação de Trabalho/Outra).

22.

LODI, F. G. ; ZAGO, M. C. B. ; MATTA, P. E. G. ; **NOMA, I. H. Y.** ; TAMURA, N. K. ; AOKI, E. E. ; TOGNIM, M. C. B. . Evolução temporal da concentração inibitória minima para daptomicina em cocos gram-positivos. 2015. (Apresentação de Trabalho/Congresso).

23.

FEDRIGO, N. H. ; TREVIZOLI, T. M. B. ; **NOMA, I. H. Y.** ; MITSUGUI, C. S. ; AOKI, E. E. ; TAMANINI, J. B. ; TOGNIM, M. C. B. . Análise temporal da resistência aos carbapenêmicos em bacilos gram-negativos não fermentadores. 2015. (Apresentação de Trabalho/Congresso).

24.

WATANABE, A. S. M. ; SILVA, M. R. T. ; **NOMA, I. H. Y.** ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . AVALIAÇÃO DOS FATORES DE VIRULÊNCIA DE CANDIDA TROPICALIS APÓS CANDIDEMIA EXPERIMENTAL. 2015. (Apresentação de Trabalho/Outra).

25.

SILVA, M. R. T. ; WATANABE, A. S. M. ; **NOMA, I. H. Y.** ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . AVALIAÇÃO DO PERFIL DE PATOGENICIDADE DE CANDIDA ALBICANS APÓS CONTATOS SUBSEQUENTES COM O HOSPEDEIRO. 2015. (Apresentação de Trabalho/Outra).

26.

MATTA, P. E. G. ; ECKER, A. B. S. ; ZARPELLON, M. N. ; MOREIRA, D. C. ; **NOMA, I. H. Y.** ; TAMURA, N. K. ; GARCIA, L. B. ; TOGNIM, M. C. B. . PREVALÊNCIA DE RESISTÊNCIA AOS CARBAPENÊMICOS NOS PRINCIPAIS BACILOS GRAM-NEGATIVOS ISOLADO SEM UM HOSPITAL DE ENSINO.. 2014. (Apresentação de Trabalho/Congresso).

27.

VIANA, G. F. ; ZAGO, M. C. B. ; SAALFELD, S. M. S. ; **NOMA, I. H. Y.** ; MATTA, P. E. G. ; MITSUGUI, C. S. ; ABREU FILHO, B. A. ; TOGNIM, M. C. B. . ALTERAÇÃO NO MODO DE DISSEMINAÇÃO DE ISOLADOS DE Acinetobacter baumannii EM UM HOSPITAL UNIVERSITÁRIO. 2014. (Apresentação de Trabalho/Congresso).

28.

ZAGO, M. C. B. ; VIANA, G. F. ; SAALFELD, S. M. S. ; **NOMA, I. H. Y.** ; FARIAS, A. P. M. ; HELBEL, C. ; TOGNIM, M. C. B. . DISSEMINAÇÃO DE Acinetobacter baumannii EM UM HOSPITAL TERCIÁRIO NA REGIÃO NORTE DO PARANÁ. 2014. (Apresentação de Trabalho/Congresso).

Produção técnica

Redes sociais, websites e blogs

1.

NOMA, I. H. Y.. É POSSÍVEL RECRIAR PELE ENVELHECIDA IN VITRO?. 2020; Tema: Divulgação científica do Laboratório de Biologia da Pele e Melanoma. (Rede social).

Participação em eventos, congressos, exposições e feiras

1. 19th International Congress of the Society for Melanoma Research. PRDX2 as a possible phenotype switcher in NRAS-mutated melanomas. 2022. (Congresso).

2. Simpósio em comemoração dos 50 anos do Programa de Pós Graduação em Farmácia Fisiopatologia e Toxicologia.Targeting Peroxiredoxin 2 as a potential treatment for NRAS mutated metastatic melanoma. 2022. (Simpósio).

3. XXII Brazilian Congress of Toxicology. Gliotoxin and its potential cytotoxicity against NRAS mutated-melanoma. 2022. (Congresso).

4. A importância do estudo de Irritação/Corrosão Dérmica in vitro na avaliação toxicológica. 2021. (Outra).

5. Host-Pathogen Interaction Meeting. 2021. (Encontro).

6. I Simpósio Desafios em Oncologia Molecular. 2021. (Simpósio).

7. Brazilian Congress in Biomedical Engineering (CBEB2020). GEOSPATIAL ANALYSIS OF DIAGNOSTIC IMAGING EQUIPMENT IN BRAZIL. 2020. (Congresso).

8. Ciclo de Palestras FisioTox. 2020. (Seminário).

9. Congresso Acadêmico Unifesp Virtual 2020 - Ciência e Universidade: Transformações para a Sociedade. 2020. (Congresso).

10.

II Encontro De Genética, Hemoterapia e Patologias Hematológicas. 2020. (Encontro).

11.

Increasing CRISPR editing efficiency with novel guide RNA methods. 2020. (Seminário).

12.

Infecção por HPV- Infecto em Série. 2020. (Seminário).

13.

Perspectivas Diagnósticas e Terapêuticas utilizando a Tecnologia CRISPR. 2020. (Outra).

14.

PÍLULAS DE ONCOLOGIA: Princípios de hormonioterapia no tratamento oncológico. 2020. (Seminário).

15.

PÍLULAS DE ONCOLOGIA: Princípios do Tratamento Oncológico e Mecanismos da Quimioterapia. 2020. (Seminário).

16.

PÍLULAS DE ONCOLOGIA: Terapia-alvo e Imunoterapia na Oncologia Moderna. 2020. (Seminário).

17.

PÍLULAS DE ONCOLOGIA - Casos clínicos em oncologia: aplicação de conceitos básicos. 2020. (Seminário).

18.

VII International Meeting of Biosciences and Physiopathology e XI Simpósio de Biociências Aplicadas à Farmácia. 2020. (Simpósio).

19.

Festival Varilux-UEM de Cinema Francês 2019. 2019. (Exposição).

20.

III INTERNATIONAL SYMPOSIUM OF EXPERIMENTAL PATHOLOGY.THE PREVALENCE OF SYPHILIS IN PRENATAL CARE WOMEN IN PARANA STATE, BRAZIL. 2019. (Simpósio).

21.

VI International Meeting of Biosciences and Physiopathology ? X Simpósio de Biociências Aplicadas à Farmácia.Co-infecções entre Papillomavirus Humano (HPV) e micoplasmas genitais em mulheres assintomáticas. 2019. (Encontro).

22.

8.o Encontro Anual de Iniciação Tecnológica e Inovação.Coordenador(a) de sessão. 2018. (Encontro).

23.

II Workshop Brasil-Estados Unidos em câncer cervical. 2018. (Outra).

24.

O SUS e a Saúde Pública brasileira: cenário atual e perspectivas. 2018. (Outra).

25.

Vamos falar sobre Doenças Raras?. 2018. (Outra).

26.

V Congresso Internacional do CCS, III Congresso Internacional de Tratamento da Obesidade e VII Encontro do PCS. Universidade Estadual de Maringá. Leishmaniose mucocutânea no Paraná, avaliação da morbidade hospitalar de 2008 a 2018. 2018. (Congresso).

27.

V International Meeting of Biosciences and Physiopathology e IX Simpósio de Biociências Aplicadas à Farmácia..GASTOS PUBLICOS NA MORBIDADE HOSPITALAR POR LEISHMANIOSE NO PARANA, BRASIL. 2018. (Simpósio).

28.

26.o Encontro Anual de Iniciação Científica e 6.o Encontro Anual de Iniciação Científica Júnior.VARIABILIDADE DE ?-LACTAMASES PRESENTES EM BACILOS GRAM NEGATIVOS ISOLADOS DURANTE A PERMANÊNCIA DE PACIENTES EM HOSPITAL DE ENSINO. 2017. (Encontro).

29.

I Workshop de genética médica. 2017. (Outra).

30.

25.o Encontro Anual de Iniciação Científica e 5.o Encontro Anual de Iniciação Científica Júnior.RELAÇÃO DA NOCICEPÇÃO COM A PRODUÇÃO DE ÓXIDO NÍTRICO EM ANIMAIS INFECTADOS COM LEISHMANIA (LEISHMANIA) AMAZONENSIS. 2016. (Encontro).

31.

I Simpósio Regional de Fisiologia Integrativa e do Exercício. 2016. (Simpósio).

32.

IV Semana Acadêmica de Biomedicina - UEM.Relação da nocicepção com a produção de óxido nítrico em animais infectados com Leishmania (Leishmania) amazonenses. 2016. (Outra).

33.

PROSED.Projeto Socioeducacional da III Semana Acadêmica de Biomedicina - UEM. 2016. (Oficina).

34.

Conexão Saúde. 2015. (Outra).

35.

III Semana Acadêmica de Biomedicina ? UEM.Avaliação da patogenicidade e fatores de virulência de Candida albicans no modelo experimental de Candidemia. 2015. (Outra).

36.

4º Congresso Paranaense de Ciências Biomédicas e 1ª Oficina Pró-saúde. 2014. (Congresso).

37.

III Mostra de Cinema e Divulgação Cientifica em Biociências. 2014. (Outra).

38.

II Semana Acadêmica de Biomedicina - UEM. 2014. (Outra).

39.

I Fórum de Discussão Estudantil.Fontes alternativas de energia (biodiesel e biogás). 2011. (Outra).

Organização de eventos, congressos, exposições e feiras

1.

NOMA, ISABELLA HARUMI YONEHARA. II Ciclo de Palestra da FisioTox, do Programa de Pós-Graduação em Farmácia (Fisiopatologia e Toxicologia). 2021. (Outro).

2.

NOMA, I. H. Y.. VII International Meeting of Biosciences and Physiopathology e XI Simpósio de Biociências Aplicadas à Farmácia. 2020. (Outro).

3.

NOMA, I. H. Y.. VI International Meeting of Biosciences and Physiopathology ? X Simpósio de Biociências Aplicadas à Farmácia. 2019. (Outro).

Página gerada pelo Sistema Currículo Lattes em 05/08/2025 às 19:15:48

Somente os dados identificados como públicos pelo autor são apresentados na consulta do seu Currículo Lattes.
Configuração de privacidade na Plataforma Lattes

State University of Maringá

RECOGNIZED BY FEDERAL DECREE NO. 77,583 OF 05/11/1976, PUBLISHED IN THE FEDERAL OFFICIAL GAZETTE OF 05/12/1976
MARINGÁ – PARANÁ

The President of the State University of Maringá
in the exercise of his duties and in view of the completion of the Biomedical Sciences Program
in the academic year of 2017, hereby confers the degree of

Bachelor of Biomedical Sciences

to

Isabella Harumi Yonehara Noma

Brazilian, born in the State of Paraná, on September 3, 1996
R.G. No. 10.873.717-4 - PR

and, considering the Graduation Ceremony held on March 9, 2018,
issues this diploma, granting her all the rights and privileges pertaining thereto.

Maringá, March 9, 2018.

-----// signature //-----
Prof. Dr. Mauro Luciano Baesso
President

-----// signature //-----
Prof. Dr. Ronaldo Augusto de Lara Gonçalves
Director of Academic Affairs

-----// signature //-----
Prof. Dr. Juliana Curi Martinichen Herrero
Program Coordinator

-----// signature //-----
Isabella Harumi Yonehara Noma
Graduate

Undergraduate Program in BIOMEDICINE, recognized by State Decree No. 506 of 02/24/2015, published in the State Official Gazette on 02/25/2015.

STATE UNIVERSITY OF MARINGÁ
PRESIDENT'S OFFICE – VICE PRESIDENT'S OFFICE FOR EDUCATION
Directorate of Academic Affairs
Division of Diploma Registration

ADDENDUM

The holder of this diploma completed the following qualification(s): BACHELOR'S DEGREE.

This addendum is hereby issued so that the holder may enjoy all the prerogatives provided by law.

Maringá, March 9, 2018.

-----// signature //-----
Vilson Franciscon Jacob
Head of the Division of Diploma Registration

By delegation of authority, in accordance with Ordinance No. 1338-GRE/UEM, of 11/03/2006.

STATE UNIVERSITY OF MARINGÁ
PRESIDENT'S OFFICE – VICE PRESIDENT'S OFFICE FOR EDUCATION
Directorate of Academic Affairs
Division of Diploma Registration

REGISTERED DIPLOMA under

No.: **2326/2018** Book: **RG-202**

Page: **083** Process: **2326/2018**

In accordance with the provisions of Article 48, §1, of Law No. 9,394, of 12/20/1996.

The addendum(s) noted herein are recorded on this date in the registration log.

Maringá, March 9, 2018.

-----// signature //-----
Vilson Franciscon Jacob
Head of the Division of Diploma Registration

By delegation of authority, in accordance with Ordinance No. 1338-GRE/UEM, of 11/03/2006.

90681

I, Raphael Michels Fantinato de Moura, telephone number 415 425-2508, mailing address P.O. Box 90487, San Diego, CA 92169, certify that the professional translation of this document from Portuguese to English has been performed by myself, a qualified translator fluent in both languages, and that the following is an accurate and complete translation of the document.

Raphael Michels Fantinato de Moura **Date: October 27, 2025.**

Universidade Estadual de Maringá

RECONHECIDA PELO DECRETO FEDERAL N.º 77.583, DE 11/05/76, PUBLICADO NO DIÁRIO OFICIAL DA UNIÃO DE 12/05/76
MARINGÁ - PARANÁ

O Reitor da Universidade Estadual de Maringá,
no uso de suas atribuições e tendo em vista a conclusão do Curso de Biomedicina
no ano letivo de 2017, confere o título de

Bacharela em Biomedicina

a

Isabella Harumi Yonehara Noma

brasileira, natural do Estado do Paraná, nascida no dia 03 de setembro de 1996
R.G. n.º 10.873.717-4 - PR

e considerando o termo de Colação de Grau lavrado no dia 09 de março de 2018, outorga o presente diploma, a fim de que possa gozar de todos os direitos e prerrogativas legais.

Maringá, 09 de março de 2018.

Prof. Dr. Mauro Luciano Baesso
Reitor

Prof. Dr. Ronaldo Augusto de Lara Gonçalves
Diretor de Assuntos Acadêmicos

Profa. Dra. Juliana Curi Martinichen Herrero
Coordenadora do Curso

Isabella H. Y. Noma
Isabella Harumi Yonehara Noma
Diplomada

Curso de Graduação em BIOMEDICINA, reconhecido pelo Decreto Estadual nº 506 de 24/02/2015, publicado no D.O.E. de 25/02/2015.

UNIVERSIDADE ESTADUAL DE MARINGÁ
REITORIA - PRÓ-REITORIA DE ENSINO
Diretoria de Assuntos Acadêmicos
Divisão de Registro de Diplomas

APOSTILA

A portadora do presente Diploma cursou a(s) habilitação(ões): BACHARELADO.

Para que possa gozar de todas as prerrogativas previstas em Lei, procedeu-se à presente apostila.

Maringá , 09 de março de 2018.

Vilson Franciscon Jacob
Chefe da Divisão de Registro de Diplomas
Por delegação de competência, de acordo com a Portaria nº 1338-GRE/UEM, de 03/11/2006.

UNIVERSIDADE ESTADUAL DE MARINGÁ
REITORIA - PRÓ-REITORIA DE ENSINO
Diretoria de Assuntos Acadêmicos
Divisão de Registro de Diplomas

DIPLOMA REGISTRADO sob

Nº: **2326/2018** Livro: **RG-202**

Fls: **083** Processo: **2326/2018**

de acordo com o disposto no artigo 48, §1º, da Lei nº 9.394, de 20/12/1996.

Apostila(s) anotada(s), nesta data, no termo de registro.

Maringá , 09 de março de 2018.

Vilson Franciscon Jacob
Chefe da Divisão de Registro de Diplomas
Por delegação de competência, de acordo com a Portaria nº 1338-GRE/UEM, de 03/11/2006.

90681

ESTACIO DE SÁ UNIVERSITY

GRADUATE STUDIES DEPARTMENT
CERTIFICATE

We hereby certify that ISABELLA HARUMI YONEHARA NOMA

has completed the course in SPECIALIZATION IN BIOMEDICAL ENGINEERING WITH EMPHASIS IN CLINICAL ENGINEERING

held from January 1, 2019, to June 30, 2020,

with a total workload of 360 hours.

Rio de Janeiro - RJ, December 1, 2020

----- // signature //-----
President

----- // signature //-----
Vice President

ESTÁCIO DE SÁ UNIVERSITY

Accredited by Ordinance No. 592, of 11/29/1988, published in the Federal Official Gazette on 11/30/1988. Reaccredited by MEC Ordinance No. 1095, of 08/31/2012, published in the Federal Official Gazette on 09/04/2012. Accredited by MEC Ordinance No. 442, of 05/11/2009, published in the Federal Official Gazette on 05/12/2009. The program was carried out in compliance with the provisions of Resolution No. 1 of the Higher Education Chamber of the National Education Council, published in the Federal Official Gazette on 04/09/2018.

Field of Knowledge: MEDICAL ENGINEERING

President: FLÁVIO MURILO OLIVEIRA DE GOUVÊA
Vice President: RAFAEL MARIO IORIO FILHO

ADDENDUM

Program conducted under a technical-pedagogical cooperation agreement between Estácio de Sá University and Harvard Business Publishing, an affiliate publisher of Harvard Business School.

Certificate registered under No. 52402
on 12/01/2020.

-----// signature //-----

S.R.D. Secretary:

PAGE 116222

Academic Transcript
LATO SENSU GRADUATE STUDIES

Student: 2019.02.10659-8- ISABELLA HARUMI YONEHARA NOMA

Program: 2897 - SPECIALIZATION IN BIOMEDICAL ENGINEERING WITH EMPHASIS IN CLINICAL ENGINEERING

Campus: GRADUATE STUDIES UNIT **Program Total Hours:** 360

Period: 01/01/2019 a 06/30/2020 **Total Hours Completed:** 360

Courses and Faculty	Final Grade	Hours
CAREER GUIDANCE WITH COACHING NELSON ROQUE SCHNEIDER – MASTER'S	**10**	**18**
CONSULTING CLAUDIA MARCIA PEREIRA LOUREIRO – MASTER'S	**9**	**18**
CORPORATE GOVERNANCE AND BUSINESS EXCELLENCE RICARDO BARBOSA DA SILVEIRA – MASTER'S	**10**	**18**
RESEARCH METHODS AND TECHNIQUES ERIKA CONCEICAO GELENSKE CUNHA – MASTER'S	**10**	**36**
INTRODUCTION TO BIOMEDICAL ENGINEERING AND CLINICAL ENGINEERING GISELE TEIXEIRA SALEIRO – MASTER'S	**10**	**18**
BASICS OF HUMAN PHYSIOLOGY GISELE TEIXEIRA SALEIRO – MASTER'S	**9.8**	**36**
MEDICAL-HOSPITAL INSTRUMENTATION I MIRNA MIGUEL PASSOS - PHD	**10**	**36**
CLINICAL ENGINEERING COMPETENCIES I GISELE TEIXEIRA SALEIRO – MASTER'S	**10**	**36**
MEDICAL-HOSPITAL INSTRUMENTATION II MIRNA MIGUEL PASSOS - PHD	**10**	**36**
FUNDAMENTALS OF BASIC ELECTRICITY AND HOSPITAL ELECTRICAL INSTALLATIONS MARCIO JORGE GOMES VICENTE - SPECIALIZATION	**10**	**18**
MEDICAL-HOSPITAL INSTRUMENTATION III MIRNA MIGUEL PASSOS - PHD	**10**	**36**

Courses and Faculty	Final Grade	Hours
CLINICAL ENGINEERING COMPETENCIES II MARCIO JORGE GOMES VICENTE - SPECIALIZATION	**9.9**	**36**
MAINTENANCE ENGINEERING CARLOS ALBERTO MARTINS FERREIRA - PHD	**10**	**18**
FINAL THESIS FABIO MACEDO SIMAS - PHD	**10**	**-**

Type of Evaluation: The assessment of academic performance will be expressed in grades, on a scale from zero to ten. To be approved, the student must achieve a final grade of no less than seven in each course and a minimum attendance of 75% (seventy-five percent) in the Program under formal evaluation.

Note: The program was completed in compliance with the provisions of Resolution No. 1/2018 of the Higher Education Chamber of the National Education Council, published in the Federal Official Gazette on 04/09/2018. Accredited by Ordinance No. 592 of 11/29/1988, published in the Federal Official Gazette on 11/30/1988. Reaccredited by MEC Ordinance No. 1095 of 08/31/2012, published in the Federal Official Gazette on 09/04/2012. Accredited by MEC Ordinance No. 442 of 05/11/2009, published in the Federal Official Gazette on 05/12/2009.

Final Thesis Topic: GEOSPATIAL ANALYSIS OF IMAGING DIAGNOSTIC EQUIPMENT ASSOCIATED WITH SOCIO-DEMOGRAPHIC CHARACTERISTICS IN BRAZIL

-----// signature //-----

Rio de Janeiro – RJ, November 30, 2020.

Roberta Martins Ramos

General Student Secretary

I, Raphael Michels Fantinato de Moura, telephone number 415 425-2508, mailing address P.O. Box 90487, San Diego, CA 92169, certify that the professional translation of this document from Portuguese to English has been performed by myself, a qualified translator fluent in both languages, and that the following is an accurate and complete translation of the document.

Raphael Michels Fantinato de Moura. **Date: October 27, 2025.**

UNIVERSIDADE ESTÁCIO DE SÁ

CENTRO DE PÓS-GRADUAÇÃO
CERTIFICADO

Certificamos que ISABELLA HARUMI YONEHARA NOMA

concluiu o Curso de ESPECIALIZAÇÃO EM ENGENHARIA BIOMÉDICA COM ÊNFASE EM ENGENHARIA CLÍNICA

realizado no período de 01 *de* Janeiro *de* 2019 *a* 30 *de* Junho *de* 2020

com carga horária total de 360 *horas.*

Rio de Janeiro - RJ, 01 *de* Dezembro *de* 2020

Reitor(a)

Estácio

Vice-Reitor(a)

UNIVERSIDADE ESTÁCIO DE SÁ

Credenciada pela Portaria Nº 592, de 29/11/1988, publicada no D.O.U. de 30/11/1988 e Recredenciada pela Portaria MEC Nº 1095, de 31/08/2012, publicada no D.O.U. de 04/09/2012. Credenciada pela Portaria MEC Nº 442, de 11/05/2009, publicada no D.O.U. de 12/05/2009. O curso foi realizado em cumprimento ao disposto na Resolução nº 1 da Câmara de Educação Superior do Conselho Nacional de Educação, Publicada no D.O.U em 09/04/2018.

Área de Conhecimento : ENGENHARIA MEDICA

Reitor(a): FLÁVIO MURILO OLIVEIRA DE GOUVÊA

Vice-Reitor(a): RAFAEL MARIO IORIO FILHO

APOSTILA

Curso objeto de convênio de cooperação técnico-pedagógica entre a Universidade Estácio de Sá e Harvard *Business Publishing*, editora afiliada da Harvard *Business School*.

Certificado registrado sob o n° 52402

em 01/12/2020.

Secretário(a) da S.R.D

PG 116222

Estácio

Histórico Escolar
PÓS – GRADUAÇÃO LATO SENSU

Aluno: 2019.02.10659-8 - ISABELLA HARUMI YONEHARA NOMA

Curso: 2897 - ESPECIALIZAÇÃO EM ENGENHARIA BIOMÉDICA COM ÊNFASE EM ENGENHARIA CLÍNICA / Grupo

Campus: UNIDADE PÓS-GRADUAÇÃO **Total de Horas Curso:** 360

Duração: 01/01/2019 a 30/06/2020 **Total de Horas Cumpridas:** 360

Disciplinas e Docentes	Média Final	Carga Horária
ORIENTAÇÃO DE CARREIRA COM COACHING NELSON ROQUE SCHNEIDER - MESTRADO	10	18
CONSULTORIA CLAUDIA MARCIA PEREIRA LOUREIRO - MESTRADO	9	18
GOVERNANÇA CORPORATIVA E EXCELÊNCIA EMPRESARIAL RICARDO BARBOSA DA SILVEIRA - MESTRADO	10	18
MÉTODOS E TÉCNICAS DE PESQUISA ERIKA CONCEICAO GELENSKE CUNHA - MESTRADO	10	36
INTROD. À ENG. BIOMÉDICA E À ENG. CLÍNICA GISELE TEIXEIRA SALEIRO - MESTRADO	10	18
NOÇÕES DE FISIOLOGIA HUMANA GISELE TEIXEIRA SALEIRO - MESTRADO	9,8	36
INSTRUMENTAÇÃO MÉDICO-HOSPITALAR I MIRNA MIGUEL PASSOS - DOUTORADO	10	36
COMPETÊNCIAS DA ENGENHARIA CLÍNICA I GISELE TEIXEIRA SALEIRO - MESTRADO	10	36
INSTRUMENTAÇÃO MÉDICO-HOSPITALAR II MIRNA MIGUEL PASSOS - DOUTORADO	10	36
FUND. DE ELET. BÁSICA E DE INST. ELÉT. HOSPIT. MARCIO JORGE GOMES VICENTE - ESPECIALIZAÇÃO	10	18
INSTRUMENTAÇÃO MÉDICO-HOSPITALAR III MIRNA MIGUEL PASSOS - DOUTORADO	10	36

Disciplinas e Docentes	Média Final	Carga Horária
COMPETÊNCIAS DA ENGENHARIA CLÍNICA II MARCIO JORGE GOMES VICENTE - ESPECIALIZAÇÃO	9,9	36
ENGENHARIA DE MANUTENÇÃO CARLOS ALBERTO MARTINS FERREIRA - DOUTORADO	10	18
TRABALHO DE CONCLUSÃO DE CURSO FABIO MACEDO SIMAS - DOUTORADO	10	-

Forma de Avaliação: A avaliação do aproveitamento será expressa em graus, variando numa escala de zero a dez. O aluno para ser aprovado deverá alcançar média final de aproveitamento não inferior a sete em cada disciplina e frequência mínima de 75% (setenta e cinco por cento) no Curso em processo formal de avaliação.

Observação: O curso foi realizado em cumprimento ao disposto na Resolução n° 1/2018 da Câmara de Educação Superior do Conselho Nacional de Educação, Publicada no D.O.U em 09/04/2018. Credenciada pela Portaria Nº 592, de 29/11/1988, publicada no D.O.U. de 30/11/1988 Recredenciada pela Portaria MEC Nº 1095, de 31/08/2012, publicada no D.O.U. de 04/09/2012. Credenciada pela Portaria MEC Nº 442, de 11/05/2009, publicada no D.O.U. de 12/05/2009

Tema TCC: ANÁLISE GEOESPACIAL DE EQUIPAMENTOS DE DIAGNÓSTICO POR IMAGEM ASSOCIADO ÀS CARACTERÍSTICAS SÓCIODEMOGRÁFICAS NO BRASIL.

Roberta Martins Ramos

Rio de Janeiro - RJ, 30 de novembro de 2020.

Roberta Martins Ramos
Secretário(a) Geral de Alunos

State University of Maringá

RECOGNIZED BY FEDERAL DECREE NO. 77,583 OF 05/11/1976, PUBLISHED IN THE FEDERAL OFFICIAL GAZETTE OF 05/12/1976
MARINGÁ – PARANÁ

The President of the State University of Maringá, in the exercise of his duties and in view of the completion of the

Graduate Program in Health Sciences
Area o Concentration: Human Health
on February 13, 2020, hereby confers the degree of

Master of Health Sciences

to

Isabella Harumi Yonehara Noma

-- Brazilian, born in the State of Paraná, on September 3, 1996, holder of
Identity Document No. 10.873.717-4 – PR --

granting her this diploma so that she may enjoy all the rights and privileges pertaining thereto.

Maringá, May 5, 2021.

-----// signature //-----
Prof. Dr. Julio César Damasceno
President

-----// signature //-----
Prof. Dr. Carlos Humberto
Director of Academic Affairs

-----// signature //-----
Prof. Dr. Luciano de Andrade
Program Coordinator

Isabella Harumi Yonehara Noma
Graduate

Digitalized with CamScanner

STATE UNIVERSITY OF MARINGÁ, maintained by the General Superintendency of Science, Technology, and Higher Education of the State of Paraná – CNPJ: 77.046.951/0001-25.
Reaccredited by Decree No. 4225 of 03/12/2020, published in the State Official Gazette on 03/24/2020.
Graduate Program (Stricto Sensu), recognized by Ministerial Ordinance No. 609/MEC of 03/14/2019, published in the Federal Official Gazette on 03/18/2019.

STATE UNIVERSITY OF MARINGÁ
PRESIDENT'S OFFICE – VICE PRESIDENT'S OFFICE FOR EDUCATION
Directorate of Academic Affairs
Division of Diploma Registration

REGISTERED DIPLOMA under

No.: **0265/2021** Book: **RPG-030**

Page: **175** Process: **0255/2021**

In accordance with the provisions of Article 48, §1, of Law No. 9,394, of 12/20/1996.

Maringá, May 5, 2021.

-----// signature //-----
Vilson Franciscon Jacob
Head of the Division of Diploma Registration

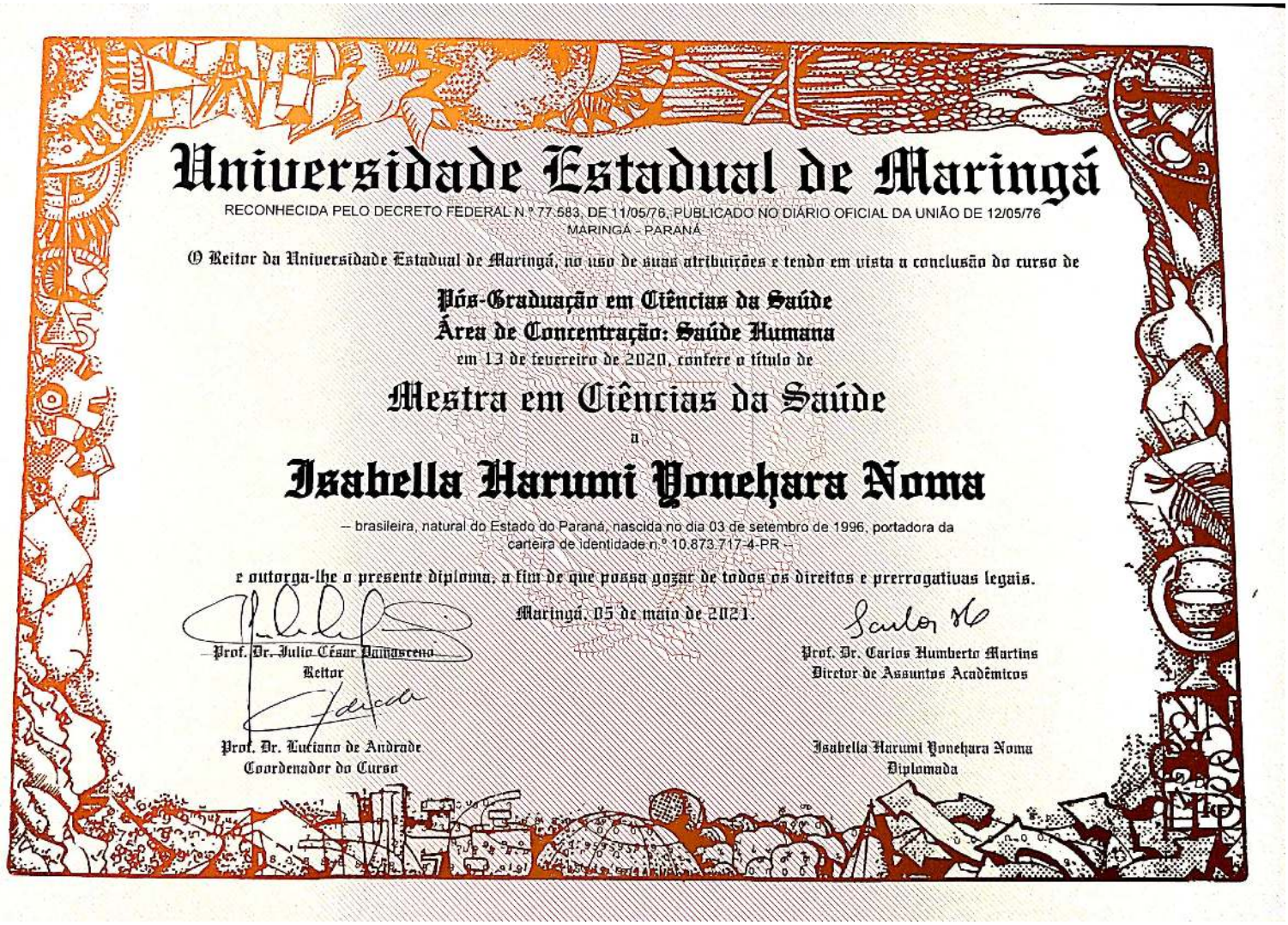
By delegation of authority, in accordance with Ordinance No. 1338-GRE/UEM, of 11/03/2006.

400972

Digitalized with CamScanner

I, Raphael Michels Fantinato de Moura, telephone number 415 425-2508, mailing address P.O. Box 90487, San Diego, CA 92169, certify that the professional translation of this document from Portuguese to English has been performed by myself, a qualified translator fluent in both languages, and that the following is an accurate and complete translation of the document.

Raphael Michels Fantinato de Moura **Date: October 27, 2025.**



Universidade Estadual de Maringá

RECONHECIDA PELO DECRETO FEDERAL N.º 77.583, DE 11/05/76, PUBLICADO NO DIÁRIO OFICIAL DA UNIÃO DE 12/05/76
MARINGÁ - PARANÁ

O Reitor da Universidade Estadual de Maringá, no uso de suas atribuições e tendo em vista a conclusão do curso de

Pós-Graduação em Ciências da Saúde
Área de Concentração: Saúde Humana

em 13 de fevereiro de 2020, confere o título de

Mestra em Ciências da Saúde

a

Isabella Harumi Yonehara Noma

– brasileira, natural do Estado do Paraná, nascida no dia 03 de setembro de 1996, portadora da carteira de identidade n.º 10.873.717-4-PR –

e outorga-lhe o presente diploma, a fim de que possa gozar de todos os direitos e prerrogativas legais.

Maringá, 05 de maio de 2021.

Prof. Dr. Julio César Painaseena
Reitor

Prof. Dr. Carlos Humberto Martins
Diretor de Assuntos Acadêmicos

Prof. Dr. Luciano de Andrade
Coordenador do Curso

Isabella Harumi Yonehara Noma
Diplomada

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UNIVERSIDADE ESTADUAL DE MARINGÁ, mantida pela Superintendência Geral de Ciência, Tecnologia e Ensino Superior do Estado do Paraná - CNPJ: 77.046.951/0001-29
Recredenciada pelo Decreto nº 4225 de 12/03/2020, publicada no D.O.E. de 24/03/2020.
Programa de Pós-Graduação Stricto Sensu, reconhecido pela Portaria Ministerial nº 609/MEC, de 14/03/2019, publicada no D.O.U. de 18/03/2019.

UNIVERSIDADE ESTADUAL DE MARINGÁ
REITORIA - PRÓ-REITORIA DE ENSINO
Diretoria de Assuntos Acadêmicos
Divisão de Registro de Diplomas

DIPLOMA REGISTRADO sob

Nº: **0265/2021** Livro: **RPG-030**

Fls: **175** Processo: **0255/2021**

de acordo com o disposto no artigo 48, §1º, da Lei nº 9.394, de 20/12/1996

Maringá , 05 de maio de 2021.

Vilson Francisconi Jacob
Chefe da Divisão de Registro de Diplomas

Por delegação de competência, de acordo com a Portaria nº 1339/GRE/UEM, de 03/11/2006

400972

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FEDERATIVE REPUBLIC OF BRAZIL
UNIVERSITY OF SÃO PAULO
SCHOOL OF PHARMACEUTICAL SCIENCES

THE PRESIDENT OF THE UNIVERSITY OF SÃO PAULO,
IN THE EXERCISE OF HIS DUTIES,
HEREBY CONFERS UPON

ISABELLA HARUMI YONEHARA NOMA

OF BRAZILIAN NATIONALITY,
HOLDER OF IDENTITY DOCUMENT
NO. 10.873.717-4 PR,
BORN ON SEPTEMBER 3, 1996
AND FROM THE STATE OF PARANÁ,

THE DEGREE OF

DOCTOR OF SCIENCE

OBTAINED ON APRIL 10, 2023,
IN THE PROGRAM: PHARMACY (PATHOPHYSIOLOGY AND TOXICOLOGY),
AREA OF CONCENTRATION: PATHOPHYSIOLOGY.
AND, SO THAT SHE MAY ENJOY ALL LEGAL RIGHTS AND
PRIVILEGES PERTAINING THERETO, HEREBY GRANTS HER THIS DIPLOMA.

SÃO PAULO, APRIL 18, 2023.

----- // signature //-----

PRESIDENT
PROF. DR. CARLOS GILBERTO CARLOTTI JUNIOR

----- // signature //-----

VICE PRESIDENT OF GRADUATE STUDIES

PROF. DR. MÁRCIO DE CASTRO SILVA FILHO

GRADUATE

ISABELLA HARUMI YONEHARA NOMA

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PROGRAM RECOGNIZED ACCORDING TO THE PROVISIONS OF MEC ORDINANCE NO. 609 OF 03/14/2019, PUBLISHED IN THE FEDERAL OFFICIAL GAZETTE ON 03/18/2019.

UNIVERSITY OF SÃO PAULO
GENERAL SECRETARIAT
DIVISION OF ACADEMIC RECORDS

DIPLOMA REGISTERED UNDER NO. **UPG055255**
PROCESS NO. 2023.1.389.9.3
UNDER ARTICLE 48 OF LAW NO. 9394/96.
SÃO PAULO, APRIL 19, 2023.

-----// signature //-----

FLAVIO LUIS DE AVILA LOPES
ACADEMIC TECHNICIAN

APPROVED BY

-----// signature //-----

PROF. DR. MARINA HELENA CURY GALLOTTINI
GENERAL SECRETARY

GENERAL SECRETARIAT
DIVISION OF ACADEMIC RECORDS

THIS DOCUMENT, ISSUED BY THE UNIVERSITY OF SÃO PAULO, IS AUTHENTIC.

SÃO PAULO, APRIL 19, 2023

UNIVERSITY OF SÃO PAULO

-----// signature //-----

TECHNICAL DIRECTOR OF THE DIVISION

ARIOSVALDO BEZERRA DE SOUSA

No.0466088

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I, Raphael Michels Fantinato de Moura, certify that the professional translation of this document from Portuguese to English has been performed by myself, a qualified translator fluent in both languages, and that the following is an accurate and complete translation of the document.

Raphael Michels Fantinato de Moura. **Date: October 27, 2025.**

REPÚBLICA FEDERATIVA DO BRASIL

UNIVERSIDADE DE SÃO PAULO
FACULDADE DE CIÊNCIAS FARMACÊUTICAS

O REITOR DA UNIVERSIDADE DE SÃO PAULO,
NO USO DE SUAS ATRIBUIÇÕES,
CONFERE A

ISABELLA HARUMI YONEHARA NOMA

DE NACIONALIDADE BRASILEIRA,
PORTADORA DA CÉDULA DE IDENTIDADE
RG Nº 10.873.717-4 PR,
NASCIDA EM 03 DE SETEMBRO DE 1996
E NATURAL DO ESTADO DO PARANÁ,

O TÍTULO DE

DOUTORA EM CIÊNCIAS

OBTIDO EM 10 DE ABRIL DE 2023,
NO PROGRAMA: FARMÁCIA (FISIOPATOLOGIA E TOXICOLOGIA),
ÁREA DE CONCENTRAÇÃO: FISIOPATOLOGIA.
E, PARA QUE POSSA GOZAR DE TODOS OS DIREITOS E
PRERROGATIVAS LEGAIS, OUTORGA-LHE O PRESENTE DIPLOMA.

SÃO PAULO, 18 DE ABRIL DE 2023.

REITOR
PROF. DR. CARLOS GILBERTO CARLOTTI JUNIOR

PRÓ-REITOR DE PÓS-GRADUAÇÃO
PROF. DR. MÁRCIO DE CASTRO SILVA FILHO

DIPLOMADA
ISABELLA HARUMI YONEHARA NOMA

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PROGRAMA RECONHECIDO DE ACORDO COM O DISPOSTO NA PORTARIA MEC Nº 609 DE 14/03/2019, D.O.U DE 18/03/2019.

UNIVERSIDADE DE SÃO PAULO
SECRETARIA GERAL
DIVISÃO DE REGISTROS ACADÊMICOS

DIPLOMA REGISTRADO SOB Nº UPG055255
PROCESSO Nº 2023.1.389.9.3
NOS TERMOS DO ARTIGO 48 DA LEI 9394/96.
SÃO PAULO, 19 DE ABRIL DE 2023.

FLAVIO LUIS DE AVILA LOPES
TÉCNICO ACADÊMICO

DE ACORDO.

PROFA. DRA. MARINA HELENA CURY GALLOTTINI
SECRETÁRIA GERAL

SECRETARIA GERAL
DIVISÃO DE REGISTROS ACADÊMICOS

O PRESENTE DOCUMENTO, EXPEDIDO PELA UNIVERSIDADE DE SÃO PAULO É AUTÊNTICO.

SÃO PAULO, 19 DE ABRIL DE 2023.

UNIVERSIDADE DE SÃO PAULO
DIRETOR TÉCNICO DE DIVISÃO

ARIOSVALDO BEZERRA DE SOUSA

№ 0466088

CASA DA MOEDA DO BRASIL

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CERTIFICATE OF COMPLETION

The Vice President of Research and Innovation at the University of São Paulo, in the exercise of his duties, hereby certifies that ISABELLA HARUMI YONEHARA NOMA, BRAZILIAN, holder of an identity document (RG) number 108737174, born on SEPTEMBER 3, 1996, in the State of Paraná, has completed the POSTDOCTORAL PROGRAM carried out in the Department of Clinical and Toxicological Analysis at the School of Pharmaceutical Sciences.

PROJECT: THE ROLE OF HDAC8 IN UVR-DRIVEN MELANOMA INITIATION

SUPERVISOR: PROFESSOR DR. SILVYA STUCHI MARIA ENGLER

PERIOD: 08/01/2023 to 07/31/2024

CNPq - National Council for Scientific and Technological Development

CREDIT HOURS: 1440 HOURS

Paulo Alberto Nussenzveig

VICE PRESIDENT OF RESEARCH AND INNOVATION

Document issued at 1:36:09PM on 07/16/2025 (Brasília date and time).

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Control Code: GJEL-BV1K-6P63-EL2Q

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Activities Performed

Cell culture and animal management (320 hours)

Cloning cells with HDAC8 overexpression (160 hours)

Functional assays (320 hours)

Generation of UVR-driven skin cancers (160 hours)

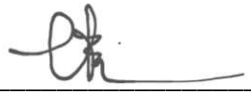
Protein expression (HDAC8, p53, Acetyl-p53) in melanocytes (160 hours)

Protein expression (HDAC8, p53, Acetyl-p53, other markers) in melanoma cells (160 hours)

shRNA-mediated HDAC8 knockdown in melanoma cells (160 hours)

Document issued at 1:36:09PM on 07/16/2025 (Brasília date and time).

I, Carolina Favero da Silva, telephone number 415 425-2508, mailing address P.O. Box 90487, San Diego, CA 92169, certify that the professional translation of this document from Portuguese to English has been performed by myself, a qualified translator fluent in both languages, and that the following is an accurate and complete translation of the document.


_____ Date: August 29, 2025.

ATESTADO DE CONCLUSÃO

O Pró-Reitor de Pesquisa e Inovação da Universidade de São Paulo, no uso de suas atribuições, atesta que ISABELLA HARUMI YONEHARA NOMA, de nacionalidade BRASILEIRA, portador(a) do documento tipo RG número 108737174, nascido(a) em 3 de SETEMBRO de 1996 e natural do Estado de Paraná, concluiu o PROGRAMA DE PÓS-DOUTORADO, realizado no Departamento de Análises Clínicas Toxicológicas da Faculdade de Ciências Farmacêuticas.

PROJETO: THE ROLE OF HDAC8 IN UVR-DRIVEN MELANOMA INITIATION

SUPERVISOR: PROFA. DRA. SILVYA STUCHI MARIA ENGLER

PERÍODO: 01/08/2023 a 31/07/2024

CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico

CARGA HORÁRIA: 1440 HORAS

Paulo Alberto Nussenzveig

PRÓ-REITOR DE PESQUISA E INOVAÇÃO

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Código de Controle: GJEL-BV1K-6P63-EL2Q

A autenticidade deste documento pode ser verificada na página da Universidade de São Paulo https://uspdigital.usp.br/webdoc/

Atividades Desenvolvidas

Cell culture and animal management (320 horas)

Clonning cells with HDAC8 overexpression (160 horas)

Functional assays (320 horas)

Generate UVR-driven skin cancers (160 horas)

Proteins expression (HDAC8, p53, Acetyl-p53) in melanocytes (160 horas)

Proteins expressions (HDAC8, p53, Acetyl-p53, other markers) for melanoma cells (160 horas)

shRNA to HDAC8 knockdown in melanoma cells (160 horas)

Documento emitido às 13:36:09 horas do dia 16/07/2025 (hora e data de Brasilia).

ESALQ
Luiz de Queiroz College of Agriculture
University of São Paulo

USP

DEPARTMENT OF ECONOMICS, ADMINISTRATION, AND SOCIOLOGY
Av. Pádua Dias, 11 | P.O. Box 9 | Piracicaba, SP | 13418-900
Contact for specialization, continuing education, and extension courses
Phone: (19) 2660-3343 / 2650-3220 | secretaria@mbauspesalq.com | www.mbauspesalq.com

Statement of Program Completion

We hereby declare, for academic purposes only, that **Isabella Harumi Yonehara Noma**, holder of identity document **No. 10.873.717-4, USP number 11963986**, and Academic Registration (RA) 280137316427, has completed the Graduate Studies Program titled **MBA in Data Science and Analytics (Distance Learning) – 2nd Semester 2023,** in the field of **Business**, carried out from 10/10/2023 to 08/30/2025, with a total workload of 425 hours.

We further declare that the student submitted a final paper entitled: **"Clustering of Cutaneous and Acral Melanoma: Impact of Genetic Alterations on Immune Response"** and obtained a grade of 9.0.

This program complies with Resolution CNE/CES No. 1, dated April 6, 2018, and is certified by the University of São Paulo, Luiz de Queiroz College of Agriculture, which is accredited by the Ministry of Education (MEC) through Ordinance No. 503, dated July 19, 2022. The official certificate of program completion is in the process of being issued, and this document is valid for one year after its issuance.

Piracicaba, São Paulo, July 30, 2025

Digital Validation:

Use the link below to verify the validity of this document: https://mbxurl.me/Pv71Bs4p

I, Marina Viana Silva, telephone number 415 425-2508, mailing address P.O. Box 90487, San Diego, CA 92169, certify that I have performed the professional translation of this document from Portuguese to English, as a qualified translator fluent in both languages, and that the following is an accurate and complete translation of the document.

Marina Viana

Date: September 12, 2025.

ESALQ
Escola Superior de Agricultura Luiz de Queiroz
Universidade de São Paulo

USP

DEPARTAMENTO DE ECONOMIA, ADMINISTRAÇÃO E SOCIOLOGIA
Av. Pádua Dias, 11 | Caixa Postal 9 | Piracicaba, SP | 13418-900
Contato para cursos de especialização, atualização e difusão
Tel.: (19) 2660-3343/2660-3220 | secretaria@mbauspesalq.com | www.mbauspesalq.com

Declaração de Conclusão de Curso

Declaramos para fins acadêmicos apenas do interessado que **Isabella Harumi Yonehara Noma**, portador (a) do documento de identidade nº **10.873.717-4**, **e do número USP: 11963986**, Registro Acadêmico (RA) 280137316427, concluiu o curso de Pós-Graduação lato sensu intitulado **MBA em Data Science e Analytics EaD - 2º semestre 2023,** área de conhecimento **Administração,** realizado no período de 10/10/2023 a 30/08/2025, com carga horária total de 425 horas.

Declaramos ainda que o aluno apresentou trabalho de conclusão de curso intitulado: "**Clusterização de melanoma cutâneo e acral: Impacto das alterações genéticas na resposta imune**" e obteve nota **9**.

O referido curso atende ao disposto na Resolução CNE/CES nº 1, de 6 de abril de 2018 e é certificado pela Universidade de São Paulo, Escola Superior de Agricultura "Luiz de Queiroz", a qual é credenciada junto ao MEC através da portaria nº503, de 19 de julho de 2022. O certificado de conclusão de curso encontra-se em fase de expedição e o presente documento é válido por um ano após a sua emissão.

Piracicaba, São Paulo, julho, 30, 2025

Validação Digital:

*Utilize o link para comprovar a validade deste documento https://mbxurl.me/Pv71Bs4p

Exhibit B. Evidence that the beneficiary was the recipient of the awards

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UNIVERSITY OF SÃO PAULO
SCHOOL OF PHARMACEUTICAL SCIENCES

SYMPOSIUM CELEBRATING THE
50TH ANNIVERSARY OF THE GRADUATE PROGRAM
IN PHARMACY – PATHOPHYSIOLOGY AND TOXICOLOGY
FCE/USP

CERTIFICATE – Honorable mention

We hereby certify that the author **Isabella Harumi Yonehara Noma**, with the poster titled "Targeting Peroxiredoxin-2 as a potential treatment for NRAS-mutated metastatic melanoma," under the supervision of Prof. Dr. Silvya Stuchi Maria Engler, received an award in the poster presentation category during the **Symposium celebrating the 50th anniversary of the Graduate Program in Pharmacy – Pathophysiology and Toxicology** of FCE/USP, held on 11/30/2022, receiving **Honorable Mention.**

-----// signature //-----

Prof. Dr. Sandra Helena Poliselli Farsky
Coordinator of the Graduate Program in Pharmacy
(Pathophysiology and Toxicology)

UNIVERSITY OF SÃO PAULO
SCHOOL OF PHARMACEUTICAL SCIENCES

I, Raphael Michels Fantinato de Moura, telephone number 415 425-2508, mailing address P.O. Box 90487, San Diego, CA 92169, certify that the professional translation of this document from Portuguese to English has been performed by myself, a qualified translator fluent in both languages, and that the following is an accurate and complete translation of the document.

Raphael Michels Fantinato de Moura **Date: January 13, 2026.**

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UNIVERSIDADE DE SÃO PAULO
FACULDADE DE CIÊNCIAS FARMACÊUTICAS

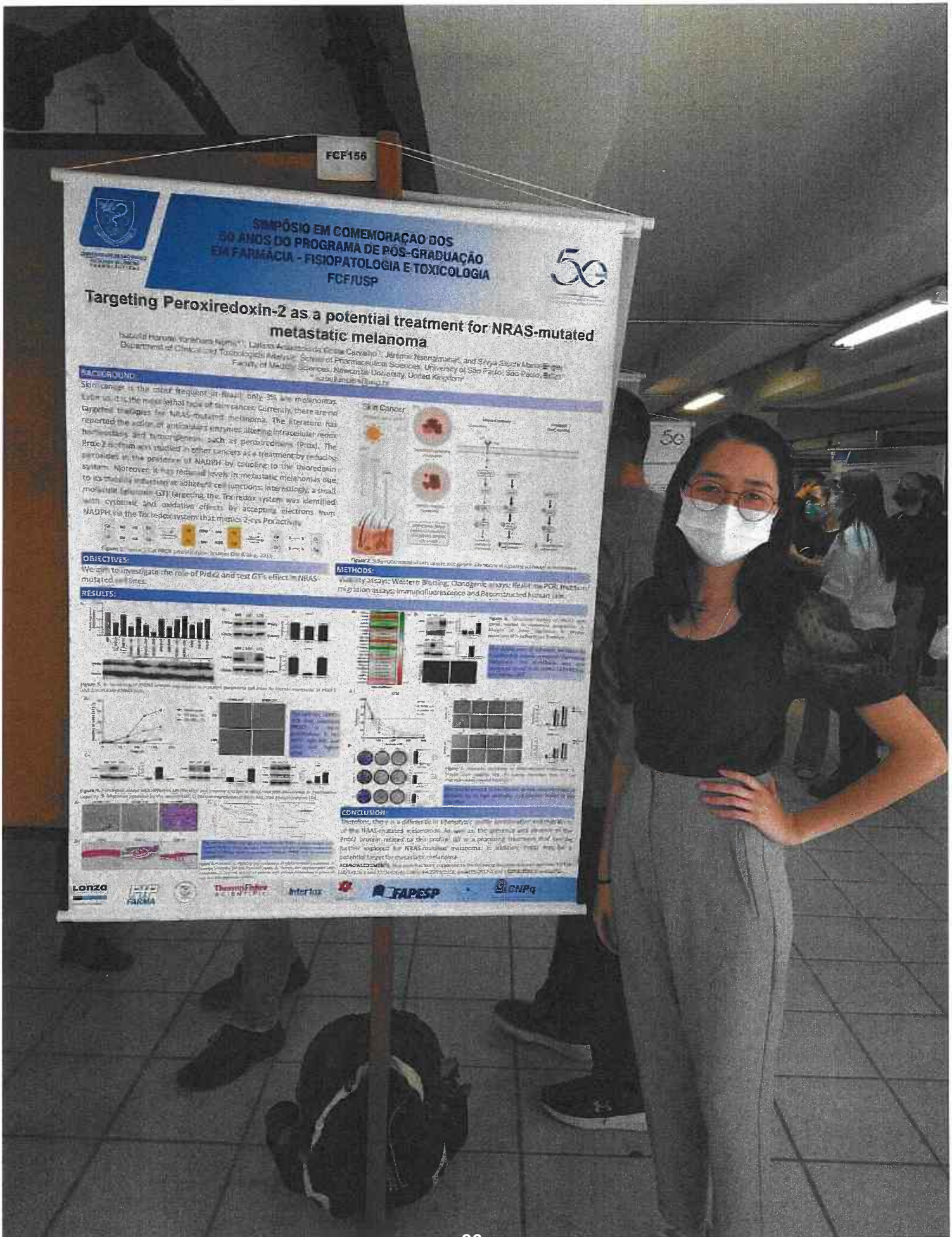
SIMPÓSIO EM COMEMORAÇÃO DOS 50 ANOS DO PROGRAMA DE PÓS-GRADUAÇÃO EM FARMÁCIA - FISIOPATOLOGIA E TOXICOLOGIA
FCF/USP

CERTIFICADO – menção honrosa

Certificamos que a autora **Isabella Harumi Yonehara Noma** com o pôster "*Targeting Peroxiredoxin-2 as a potential treatment for NRAS-mutated metastatic melanoma*", sob a orientação da Profa. Dra. Silvya Stuchi Maria Engler foi premiada na modalidade apresentação de pôster durante o **Simpósio em comemoração dos 50 anos do Programa de Pós-Graduação em Farmácia – Fisiopatologia e Toxicologia** da FCF/USP realizada em 30/11/2022, obtendo **menção honrosa.**

Profa. Dra. Sandra Helena Poliselli Farsky
Coordenadora do Programa de Pós-Graduação em Farmácia (Fisiopatologia e Toxicologia)

UNIVERSIDADE DE SÃO PAULO
FACULDADE DE CIÊNCIAS FARMACÊUTICAS


FCF156
SIMPÓSIO EM COMEMORAÇÃO DOS
50 ANOS DO PROGRAMA DE PÓS-GRADUAÇÃO
EM FARMÁCIA - FISIOPATOLOGIA E TOXICOLOGIA
FCF/USP
Targeting Peroxiredoxin-2 as a potential treatment for NRAS-mutated metastatic melanoma
BACKGROUND
OBJECTIVES:
METHODS:
RESULTS:
CONCLUSION
FAPESP
CNPq

Symposium of 50 Years of Graduate Studies in Pharmacy (Pathophysiology and Toxicology) of the FCF (School of Pharmaceutical Sciences)/ USP (University of São Paulo)

Activity Report

São Paulo
November/2022

The Graduate Program in Pharmacy (Pathophysiology and Toxicology) of the School of Pharmaceutical Sciences at the University of São Paulo completed 50 years and we celebrated this important historical milestone in a scientific and festive activity with various authorities, alumni, and current graduate students of our Program.

We held a Symposium on November 29 and 30, 2022, and were honored by the presence of the following guests: Dean of FCF/USP, Prof. Dr. Humberto Gomes Ferraz; Vice-President for Graduate Studies/USP, Prof. Dr. Márcio de Castro Silva Filho; CAPES, Prof. Dr. Silvia Stanisçuaski Guterres; CNPq, Prof. Dr. Maria Vitória Lopes Badra Bentley; FAPESP, Prof. Dr. Maria José Soares Mendes Giannini; and Coordinator of the USP Agency for Innovation – AUSPIN, Prof. Dr. Luiz Henrique Catalani.

In the event program for November 29, 2022, at 9:00 a.m., the opening was conducted by Prof. Dr. Sandra Helena Poliselli Farsky, Coordinator of the Graduate Program in Pharmacy (Pathophysiology and Toxicology). Subsequently, Prof. Sandra Farsky gave the floor to Prof. Dr. Humberto Gomes Ferraz, Dean of FCF/USP. Following this, Prof. Sandra Farsky spoke about the history of the Program and finally gave the floor to Prof. Dr. Márcio de Castro Silva Filho, Vice-President for Graduate Studies/USP.

From 10:00 a.m. to 11:00 a.m., Moderator Prof. Dr. Irene da Silva Soares and 3 master's students with oral presentations of 15 minutes plus 5 minutes of discussion for each student:

- Carlos Eduardo da Silva Gonçalves – "Aged mesenchymal stem cells contribute towards the development of acute myeloid leukemia", under the supervision of Prof. Dr. Ricardo Ambrósio Fock;
- Gregory Batista Melocco – "*Staphylococcus aureus* genomic data for surveillance, control and management of antimicrobial resistance", under the supervision of Prof. Dr. Nilton Erbet Lincopan Huenuman;
- Stefani Aparecida Rodrigues Minchio – "The role of septin 9 in the regulation of vascular permeability in malaria-associated acute respiratory distress syndrome", under the supervision of Prof. Dr. Sabrina Epiphanio.

From 12:00 p.m. to 2:00 p.m., 22 master's graduate students presented posters at the Community Center (Polytechnic School, School of Pharmaceutical Sciences, and Institute of Chemistry/USP), where they were evaluated by faculty members.

Prof. Dr. Ana Paula de Melo Loureiro (Poster Evaluator):

- Aline de Lima Pessoa Freire – "Effects of cholecalciferol on the autophagic process in raw 264.7 cells stimulated by lps and in treatment with rapamycin", under the supervision of Prof. Dr. Joilson de Oliveira Martins;
- André Berndt Penteado – "Molecular Modeling applied in the Structural Analysis and Comparison of Sirtuin 2 enzymes from Trypanosomatids", under the supervision of Prof. Dr. Gustavo Henrique Goulart Trossini;
- Beatriz Almeida – "ByStander effect of mesenchymal stem cells on aspects related to leukocyte migration control", under the supervision of Prof. Dr. Ricardo Ambrósio Fock;
- Caroline Florêncio – "Perspectives for enteroaggregative Escherichia coli diagnosis using dispersin, AAF and/or AFP fimbriae as targets antigens", under the supervision of Prof. Dr. Roxane Maria Fontes Piazza;
- Cauê Cardoso Silva – "Identification of immunological mechanisms involved with the protective response of the monoclonal anti-GP70 antibody in murine Sporotricosis", under the supervision of Prof. Dr. Sandro Rogério de Almeida;
- Gabriela Dantas de Oliveira Vieira – "Affinity assessment of monoclonal antibodies in the treatment of covid-19 induced by highly transmitted viral variants", under the supervision of Prof. Dr. Eduardo Lani Volpe da Silveira.

Prof. Dr. Jorge Luiz Mello Sampaio (Poster Evaluator):

- Isabelly Alexia Oliveira Salvino – "Identification of Fab structures of anti-trans-sialidase immunoglobulins *in silico* with neutralizing potential against the invasion of the parasite *Trypanosoma cruzi",* under the supervision of Prof. Dr. Eduardo Lani Volpe da Silveira;

- Izabella de Macedo Henrique – "Site-specific PEGylation of the monoclonal antibody FabC11:Stx1/Stx2 as an attempt to increase its half-life", under the supervision of Prof. Dr. Roxane Maria Fontes Piazza;
- Jenniffer Lacerda da Silva – "Study of activation and modulation of immune response against experimental sporotrichosis, from purified extracellular vesicles from dendritic cells", under the supervision of Prof. Dr. Sandro Rogério de Almeida;
- Jéssica Costa Nascimento – "Evaluation of the tryptophan metabolism in the massive generation of antibody-secreting cells in dengue patients through the transcriptome of single blood cells", under the supervision of Prof. Dr. Eduardo Lani Volpe da Silveira;
- Kamilla da Costa Pantoja – "Influence of insulin on the processes of autophagy and phagocytosis in bone marrow-derived macrophages", under the supervision of Prof. Dr. Joilson de Oliveira Martins.

Prof. Dr. Ricardo Ambrósio Fock (Poster Evaluator):

- Karen Rafaela Gonçalves de Araujo – "The mystery behind the apprehensions of the selective cannabinoid receptor type-2 agonist BZO-HEXOXIZID (MDA-19) as a drug of abuse", under the supervision of Prof. Dr. Maurício Yonamine;
- Mariana de Araujo Oliveira – "Evaluation of the autophagic process in subcutaneous mycosis caused by *Sporothrix brasiliensis* in healthy and diabetic animals", under the supervision of Prof. Dr. Carla Taddei de Castro Neves;

- Pedro Augusto Ramos Vanzele – "Early life oral microbiome and the implications of oral feeding introduction in hospitalized preterm newborns"; Rafael dos Santos Barros – "Evaluation the impact of autophagy on the regulation of the senescent phenotype of prone and resistant mice to accelerated senescence", under the supervision of Prof. Dr. Carla Taddei de Castro Neves;
- Raisa Ludmila Calil – "Chemoinformatic studies performed to predict activities of the synthetic canabinoids derivatives", under the supervision of Prof. Dr. Gustavo Henrique Goulart Trossini.

Prof. Dr. Ligia Ferreira Gomes (Poster Evaluator):

- Rosana Lopes Rodrigues Amon – "Irradiated mesenchymal stem cells: influence of the bystander effect on hematopoiesis controls", under the supervision of Prof. Dr. Ricardo Ambrósio Fock;
- Silvia Romano de Assis – "Establishment of *in vitro* model of reconstructed human epidermis for atopic dermatitis", under the supervision of Prof. Dr. Silvya Stuchi Maria Engler;
- Suellen Rodrigues da Silva – "The association between overweight and the familiar historic of prostatic cancer", under the supervision of Prof. Dr. Mario Hiroyuki Hirata;
- Sumara de Freitas – "Effects of cyanidin 3-glucoside on the immunomodulatory action of mesenchymal stem cell", under the supervision of Prof. Dr. Ricardo Ambrósio Fock;

- Ywa Perpétuo Socorro Toda Tavares – "Investigating the role of autophagy on the onset of allergic lung inflammation in both diabetic and healthy mice", under the supervision of Prof. Dr. Joilson de Oliveira Martins.

Continuing the Symposium program, from 2:00 p.m. to 4:00 p.m., Moderator Prof. Dr. Silvya Stuchi Maria Engler invited the speakers to deliver their lectures: first, Prof. Dr. Silvia Stanisçuaski Guterres – CAPES; followed by Prof. Dr. Maria Vitória Lopes Badra Bentley – CNPq; and finally, Prof. Dr. Maria José Soares Mendes Giannini – FAPESP.

The following day, November 30, 2022, starting the activities from 9:00 a.m. to 10:00 a.m., Moderator Prof. Dr. Mario Hiroyuki Hirata invited Prof. Dr. Luiz Henrique Catalani – Coordinator of AUSPIN – to deliver a lecture.

Continuing the program, from 10:00 a.m. to 11:00 a.m., Moderator Prof. Dr. Ana Paula de Melo Loureiro and 3 doctoral students with oral presentations of 15 minutes plus 5 minutes of discussion for each student:

- Julia Rezende da Silva – "Adenosine kinase inhibition as a tool to explore molecular mechanisms related to melanoma progression and resistance", under the supervision of Prof. Dr. Silvya Stuchi Maria Engler;
- Karen Tiemi Akashi "Transcriptomic profile of single B cell subsets in asymptomatic or hospitalized COVID-19 patients", under the supervision of Prof. Dr. Eduardo Lani Volpe da Silveira;
- Vitor Bruno – "Acute administration of ayahuasca prevents cocaine-induced expression of conditioned place preference", under the supervision of Prof. Dr. Tania Marcourakis.

Subsequently, from 11:00 a.m. to 12:00 p.m., Moderator Prof. Dr. Eduardo Lani Volpe da Silveira and 2 postdoctoral fellows – 15-minute oral presentations plus 5 minutes of discussion for each presenter:

- Felipe Rizzetto Santos – "Evaluation of the action of Annexin A1 and neutrophils in patients with chronic kidney disease in conservative treatment", under the supervision of Prof. Dr. Sandra Helena Poliselli Farsky;
- Laura Cristina Lima Diniz – "A promising multi-stage, multi-antigen chimeric recombinant protein as an anti-*Plasmodium vivax* vaccine candidate", under the supervision of Prof. Dr. Irene da Silva Soares.

From 12:45 p.m. to 2:00 p.m., 30 doctoral graduate students presented posters at the Community Center (EP, FCF, and IQ/USP), where they were evaluated by faculty members.

Prof. Dr. Nilton Erbet Lincopan Huenuman (Poster Evaluator):

- Aline Valério de Lima – "Increased resistance to ceftazidime-avibactam and metallo-betalactamase production in *Klebsiella pneumoniae*", under the supervision of Prof. Dr. Jorge Luiz Mello Sampaio;
- Alysson Henrique Urbanski – "Chikungunya infection increases osteoclastogenic genes expression in blood and chronic arthralgia may be related to osteoclast survival", under the supervision of Prof. Dr. Helder Takashi Imoto Nakaya;
- Amanda Pereira Vasconcelos – "Investigative analysis of molecular mechanisms associated with sex dimorphism in sepsis", under the supervision of Prof. Dr. Helder Takashi Imoto Nakaya;

- Amanda Sodré Mota – "Gut Microbiota composition of women with depressive symptoms during perinatal and post partum periods", under the supervision of Prof. Dr. Carla Taddei de Castro Neves;
- André Luis Fabris – "A new eco-friendly microextraction technique based on the principles of Green Analytical Toxicology for the analysis of 31 synthetic cannabinoids in plasma", under the supervision of Prof. Dr. Maurício Yonamine.

Prof. Dr. Maurício Yonamine (Poster Evaluator):

- Augusto Akira Mori – "Functional analyses of LDLR genetic variants found in Familial Hypercholesterolemic patients, using CRISPR/Cas9", under the supervision of Prof. Dr. Mario Hiroyuki Hirata;
- Cilícia Silvério Nascimento – "Bacteria in the vagina: how do they interact?", under the supervision of Prof. Dr. Carla Taddei de Castro Neves;
- Cristiane Silva Morais – "Evaluation of the autophagic and fibrotic mechanism in the peritoneal tissue exposed to the action of rapamycin in diabetic mice with peritonitis induced by *Staphylococcus aureus* and *S. argenteus",* under the supervision of Prof. Dr. Joilson de Oliveira Martins;
- Fernanda Ribeiro dos Santos Esposito – "Successful expansion of healthcare-associated hypervirulent KPC-2-producing *Klebsiella pneumoniae* ST11/KL64 to impacted aquatic environment", under the supervision of Prof. Dr. Nilton Erbet Lincopan Huenuman.

Prof. Dr. Primavera Borelli Garcia (Poster Evaluator):

- Haifa Hassanie – "Construction and validation of SETDB1 catalytic domain by homology modeling", under the supervision of Prof. Dr. Gustavo Henrique Goulart Trossini;
- Herrison Yoshiki Yocida Fontana – "Emergence of carbapenem-resistant nontyphoidal Salmonella serovar Javiana human invasive strain encoding blaKPC-2 in Brazil", under the supervision of Prof. Dr. Nilton Erbet Lincopan Huenuman;
- Isabella Harumi Yonehara Noma – "Targeting Peroxiredoxin-2 as a potential treatment for NRAS-mutated metastatic melanoma", under the supervision of Prof. Dr. Silvya Stuchi Maria Engler;
- Jeevan Giddaluru – "Using location history data from mobile phones of patients to identify transmission patterns of infectious diseases", under the supervision of Prof. Dr. Helder Takashi Imoto Nakaya;
- Jonathan Peña Avila – "Integrative Analysis Of Transcriptomic Profiles Of Covid-19 Patients Using Systems Biology", under the supervision of Prof. Dr. Helder Takashi Imoto Nakaya.

Prof. Dr. Eduardo Lani Volpe da Silveira (Poster Evaluator):

- Juan Carlo Santos e Silva – "Application of genomic tools for the study of epidemiological data", under the supervision of Prof. Dr. Helder Takashi Imoto Nakaya;
- Keila de Oliveira Lima – "Phenotypic test for the detection of serine-betalactamases and metallo-betalactamases co-production", under the supervision of Prof. Dr. Jorge Luiz Mello Sampaio;

- Luana Filippi Xavier – "Role of Annexin A1 on intestinal microbiota: involvement in DSS-induced experimental colitis", under the supervision of Prof. Dr. Sandra Helena Poliselli Farsky;
- Luiz Adriano Damasceno de Queiroz – "Regulation of mTOR receptor by rapamycin in senescence accelerated mice: the role of autophagy in aging process", under the supervision of Prof. Dr. Joilson de Oliveira Martins;
- Luiz Gustavo Sparvoli – "Influence of the hypertensive pathologies on the composition of the microbiome of maternal colostrum and feces of premature newborns", under the supervision of Prof. Dr. Carla Taddei de Castro Neves.

Prof. Dr. Helder Takashi Imoto Nakaya (Poster Evaluator):

- Manoel Oliveira de Moraes Junior – "Cytotoxicity and mechanism of action of copper(II) complexes as a potential treatment to overcome BRAF-inhibitor resistance in melanoma cells", under the supervision of Prof. Dr. Silvya Stuchi Maria Engler;
- Mariana Baldini Prudêncio – "Impact of fecal microbiota transplantation of children with refractory epilepsy under ketogenic diet treatment in an animal model of temporal lobe epilepsy", under the supervision of Prof. Dr. Carla Taddei de Castro Neves;
- Pablo Rhasan dos Santos Scharf – "Heated tobacco vapor and cigarette smoke exposures: immunotoxic outcomes in T cell biology", under the supervision of Prof. Dr. Sandra Helena Poliselli Farsky;
- Pâmela Pacassa Borges – "Toxic mechanisms of tobacco products on the production and mobilization of neutrophils in healthy conditions and

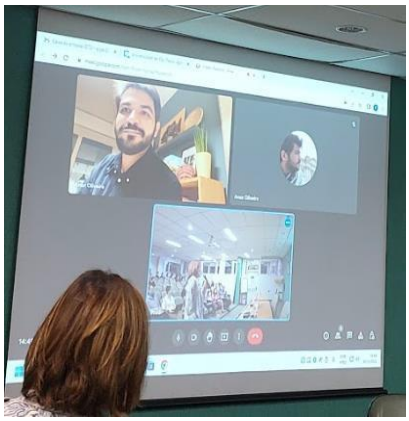
during antigeninduced arthritis (AIA)", under the supervision of Prof. Dr. Silvya Stuchi Maria Engler;

- Pedro Felipe Rodrigues Sousa – "Relation between the repertoire of V(D)J sequences of human immunoglobulins and symptomatology for COVID-19", under the supervision of Prof. Dr. Eduardo Lani Volpe da Silveira.

Prof. Dr. Tania Marcourakis (Poster Evaluator):

- Renaira Oliveira da Silva "Aged human skin reconstructed addressing the invasion of melanoma resistant to treatment with a B-RAF inhibitor", under the supervision of Prof. Dr. Silvya Stuchi Maria Engler;
- Vanessa Barbosa Malaquias – "Biocidal evaluation of surfaces containing AgNPs", under the supervision of Prof. Dr. Mario Hiroyuki Hirata;
- Vanessa Escolano Maso – "Meta-analysis of gene networks associated with human viral infections", under the supervision of Prof. Dr. Helder Takashi Imoto Nakaya;
- Wilson Pascoalino Camargo de Oliveira – "Effects of weight loss on lipid profile and lipid transfer to HDL in grade III obese patients after bariatric surgery", under the supervision of Prof. Dr. Raul Cavalcante Maranhão;
- Yasmin da Silva Santos – "Effect of angiotensin converting enzyme (ACE) inhibitors and/or angiotensin receptor blockers on the pathophysiology and therapy of experimental Covid-19", under the supervision of Prof. Dr. Sabrina Epiphanio.

Next, from 2:00 p.m. to 3:00 p.m., Moderator Prof. Dr. Sandra Helena Poliselli Farsky invited two alumni of the Program to deliver lectures on their graduate experience and professional careers: Dr. Antonio Anáx Falcão de Oliveira, supervised by Prof. Dr. Ana Paula de Melo Loureiro (via Google Meet), and Dr. Selma Andrea Cavalli, supervised by Prof. Dr. Rosario Dominguez Crespo Hirata.



From 3:00 p.m. to 4:00 p.m., Moderator Prof. Dr. Sandra Helena Poliselli Farsky invited Program alumna Dr. Martina Rudnicki to deliver a lecture via Google Meet titled Angiogenesis: basic aspects and pathophysiological implications, supervised by Prof. Dr. Dulcineia Saes Parra Abdalla; subsequently, a tribute was held for Prof. Dulcineia, who retired on October 27, 2022.

After 4:00 p.m., the awards were announced:

Oral Presentation (Master's):

1st place (R$ 1,000.00) Carlos Eduardo da Silva Gonçalves – "Aged mesenchymal stem cells contribute towards the development of acute myeloid leucemia", under the supervision of Prof. Dr. Ricardo Ambrósio Fock.

2nd place (R$ 500.00) Stefani Aparecida Rodrigues Minchio – "The role of septin 9 in the regulation of vascular permeability in malaria-associated acute respiratory distress syndrome", under the supervision of Prof. Dr. Sabrina Epiphanio.

3rd place (R$ 300.00) Gregory Batista Melocco – "*Staphylococcus aureus* genomic data for surveillance, control and management of antimicrobial resistance", under the supervision of Prof. Dr. Nilton Erbet Lincopan Huenuman;

Oral Presentation (Doctoral):

1st place (R$ 1,000.00) Julia Rezende da Silva – "Adenosine kinase inhibition as a tool to explore molecular mechanisms related to melanoma progression and resistance", under the supervision of Prof. Dr. Silvya Stuchi Maria Engler.

2nd place (R$ 500.00) Vitor Bruno – "Acute administration of ayahuasca prevents cocaine-induced expression of conditioned place preference", under the supervision of Prof. Dr. Tania Marcourakis.

3rd place (R$ 300.00) Karen Tiemi Akashi – "Transcriptomic profile of single B cell subsets in asymptomatic or hospitalized COVID-19 patients", under the supervision of Prof. Dr. Eduardo Lani Volpe da Silveira;

Oral Presentation (Postdoctoral Fellows):

1st place (R$ 1,000.00) Laura Cristina Lima Diniz – "A promising multi-stage, multi-antigen chimeric recombinant protein as an anti-*Plasmodium vivax* vaccine candidate", under the supervision of Prof. Dr. Irene da Silva Soares.

2nd place (R$ 500.00) Felipe Rizzetto Santos – "Evaluation of the action of Annexin A1 and neutrophils in patients with chronic kidney disease in conservative treatment", under the supervision of Prof. Dr. Sandra Helena Poliselli Farsky.

Poster Presentation (Master's):

1st place (R$ 300.00) Sumara de Freitas – "Effects of cyanidin 3-glucoside on the immunomodulatory action of Mesenchymal Stem Cell", under the supervision of Prof. Dr. Ricardo Ambrósio Fock.

Honorable Mention (Master's):

- André Berndt Penteado with the poster "Molecular modeling applied in the structural analysis and comparison of sirtuin 2 enzymes from trypanosomatids", under the supervision of Prof. Dr. Gustavo Henrique Goulart Trossini;

- Jéssica Costa Nascimento with the poster "Evaluation of the tryptophan metabolismo in the massive generation of antibody-secreting cells in dengue patients through the transcriptome of single blood cells", under the supervision of Prof. Dr. Eduardo Lani Volpe da Silveira;
- Rosana Lopes Rodrigues Amon with the poster "Irradiated mesenchymal stem cells: influence of the bystander effect on hematopoiesis controls", under the supervision of Prof. Dr. Ricardo Ambrósio Fock;
- Silvia Romano de Assis with the poster "Establishment of *in vitro* model of reconstructed human epidermis for atopic dermatitis", under the supervision of Prof. Dr. Silvya Stuchi Maria Engler;
- Suellen Rodrigues da Silva with the poster "The association between overweight and the familiar historic of prostatic cancer", under the supervision of Prof. Dr. Mario Hiroyuki Hirata;

Poster Presentation (Doctoral):

1st place (R$ 300.00) Renaira de Oliveira da Silva – "Aged human skin reconstructed addressing the invasion of melanoma resistant to treatment with a B-RAF inhibitor", under the supervision of Prof. Dr. Silvya Stuchi Maria Engller.

Honorable Mention (Doctoral):

- Amanda Pereira Vasconcelos with the poster "Investigative analysis of molecular mechanisms associated with sex dimorphism in sepsis", under the supervision of Prof. Dr. Helder Takashi Imoto Nakaya;
- André Luís Fabris with the poster "A new eco-friendly microextraction technique based on the principles of Green Analytical Toxicology for the analysis of 31 synthetic cannabinoids in plasma", under the supervision of Prof. Dr. Maurício Yonamine;
- Isabella Harumi Yonehara Noma with the poster "*Targeting Peroxiredoxin-2 as a potential treatment for NRAS-mutated metastatic melanoma*", under the supervision of Prof. Dr. Silvya Stuchi Maria Engler;
- Jeevan Giddaluru with the poster "Using location history data from mobile phones of patients to identify transmission patterns of infectious diseases", under the supervision of Prof. Dr. Helder Takashi Imoto Nakaya;
- Jonathan Peña Avila with the poster "*Integrative Analysis Of Transcriptomic Profiles Of Covid-19 Patients Using Systems Biology*", under the supervision of Prof. Dr. Helder Takashi Imoto Nakaya;
- Juan Carlo Santos e Silva with the poster "*Application of genomic tools for the study of epidemiological data*", under the supervision of Prof. Dr. Helder Takashi Imoto Nakaya;
- Vanesa Barbosa Malaquias with the poster "Biocidal evaluation of surfaces containing AgNPs", under the supervision of Prof. Dr. Mario Hiroyuki Hirata;
- Vanesa Escolano Maso with the poster "*Meta-analysis of gene networks associated with human viral infections*", under the supervision of Prof. Dr. Helder Takashi Imoto Nakaya.

On November 29 and 30, 2022, the symposium had 114 participants.

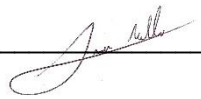
The Graduate Program in Pharmacy (Pathophysiology and Toxicology) of FCF/USP is immensely grateful for the financial donations from faculty members, alumni, and the companies Intertox, Lonza, ThermoFisher, and Veritas, as well as from the Vice-Presidency for Graduate Studies of the University of São Paulo, which made it possible to award the students and cover the costs of airfare for the guest professors, per diems, the event banner, and posters for all graduate students. It also thanks the Pharmaceutical Research Institute Foundation – Fipfarma for the donation of L-folders; the Department of Clinical and Toxicological Analysis of FCF/USP for the gift for the honored professor; and the School of Pharmaceutical Sciences of the University of São Paulo for the kraft paper bags.

The Coordination of the Graduate Program in Pharmacy (Pathophysiology and Toxicology) of the School of Pharmaceutical Sciences at the University of São Paulo thanks everyone for their collaboration, organization, and participation in the Symposium In Celebration of the 50 Years of the Graduate Studies: faculty, students, postdocs, and staff from the Administration (Block 13A) and the Department of Clinical and Toxicological Analysis of FCF/USP.

São Paulo, December 12, 2022.

Prof. Dr. Sandra Helena Poliselli Farsky
Coordinator of the Graduate Program in Pharmacy (Pathophysiology and Toxicology) of FCF/USP

I, André Vinícius Inacio Penna Mello, telephone number 415 425-2508, mailing address P.O. Box 90487, San Diego, CA 92169, certify that the professional translation of this document from Portuguese to English has been performed by myself, a qualified translator fluent in both languages, and that the following is an accurate and complete translation of the document.



Date: March 4, 2026.

Simpósio de 50 anos de Pós-Graduação em Farmácia (Fisiopatologia e Toxicologia) da FCF/USP

Relatório de Atividades

São Paulo
Novembro/2022

O Programa de Pós-Graduação em Farmácia (Fisiopatologia e Toxicologia) da Faculdade de Ciências Farmacêuticas da Universidade de São Paulo, completou 50 anos e comemoramos este importante marco histórico em uma atividade científica e festiva com diversas autoridades, egressos e atuais pós-graduandos de nosso Programa.

Realizamos um Simpósio nos dias 29 e 30 de novembro de 2022 e ficamos honrados com a presença dos seguintes convidados: Diretor da FCF/USP, Prof. Dr. Humberto Gomes Ferraz; Pró-Reitor de Pós-Graduação/USP, Prof. Dr. Márcio de Castro Silva Filho; CAPES, Profa. Dra. Silvia Stanisçuaski Guterres; CNPq, Profa. Dra. Maria Vitória Lopes Badra Bentley; FAPESP, Profa. Dra. Maria José Soares Mendes Giannini – FAPESP e Coordenador da Agência USP e Inovação – AUSPIN, Prof. Dr. Luiz Henrique Catalani.

Na programação do evento do dia 29 de novembro de 2022, às 09h00 abertura pela Profa. Dra. Sandra Helena Poliselli Farsky, Coordenadora do Programa de Pós-Graduação em Farmácia (Fisiopatologia e Toxicologia), na sequência a Profa. Sandra Farsky passou a palavra para o Prof. Dr. Humberto Gomes Ferraz, Diretor da FCF/USP e, em seguida a Profa. Sandra Farsky fala sobre a história do Programa e por fim passa a palavra para o Prof. Dr. Márcio de Castro Silva Filho, Pró-Reitor de Pós-Graduação/USP.

No horário das 10h00 às 11h00, a Moderadora, Profa. Dra. Irene da Silva Soares e 03 mestrandos com apresentação oral de 15 minutos + discussão de 5 minutos para cada aluno:

- Carlos Eduardo da Silva Gonçalves – "Aged mesenchymal stem cells contribute towards the development of acute myeloid leukemia", sob a orientação do Prof. Dr. Ricardo Ambrósio Fock;
- Gregory Batista Melocco – "*Staphylococcus aureus* genomic data for surveillance, control and management of antimicrobial resistance", sob a orientação do Prof. Dr. Nilton Erbet Lincopan Huenuman;
- Stefani Aparecida Rodrigues Minchio – "The role of septin 9 in the regulation of vascular permeability in malaria-associated acute respiratory distress syndrome", sob a orientação da Profa. Dra. Sabrina Epiphanio.

Das 12h00 às 14h00, 22 pós-graduandos de mestrado apresentaram pôsters no Centro de Vivência (Escola Politécnica, Faculdade de Ciências Farmacêuticas e Instituto de Química/USP), onde foram avaliados pelos docentes.

Profa. Dra. Ana Paula de Melo Loureiro (Avaliador de Pôster):

- Aline de Lima Pessoa Freire – "Effects of cholecalciferol on the autophagic process in raw 264.7 cells stimulated by lps and in treatment with rapamycin", sob a orientação do Prof. Dr. Joilson de Oliveira Martins;
- André Berndt Penteado – "Molecular Modeling applied in the Structural Analysis and Comparison of Sirtuin 2 enzymes from Trypanosomatids", sob a orientação do Prof. Dr. Gustavo Henrique Goulart Trossini;
- Beatriz Almeida – "ByStander effect of mesenchymal stem cells on aspects related to leukocyte migration control", sob a orientação do Prof. Dr. Ricardo Ambrósio Fock;
- Caroline Florêncio – "Perspectives for enteroaggregative Escherichia coli diagnosis using dispersin, AAF and/or AFP fimbriae as targets antigens", sob a orientação da Profa. Dra. Roxane Maria Fontes Piazza;
- Cauê Cardoso Silva – "Identification of immunological mechanisms involved with the protective response of the monoclonal anti-GP70 antibody in murine Sporotricosis", sob a orientação do Prof. Dr. Sandro Rogério de Almeida;
- Gabriela Dantas de Oliveira Vieira – "Affinity assessment of monoclonal antibodies in the treatment of covid-19 induced by highly transmitted viral variants", sob a orientação do Prof. Dr. Eduardo Lani Volpe da Silveira.

Prof. Dr. Jorge Luiz Mello Sampaio (Avaliador de Pôster):

- Isabelly Alexia Oliveira Salvino – "Identification of Fab structures of anti-trans-sialidase immunoglobulins *in silico* with neutralizing potential against the invasion of the parasite *Trypanosoma cruzi",* sob a orientação do Prof. Dr. Eduardo Lani Volpe da Silveira;

- Izabella de Macedo Henrique – "Site-specific PEGylation of the monoclonal antibody FabC11:Stx1/Stx2 as an attempt to increase its half-life", sob a orientação da Profa. Dra. Roxane Maria Fontes Piazza;
- Jenniffer Lacerda da Silva – "Study of activation and modulation of immune response against experimental sporotrichosis, from purified extracellular vesicles from dendritic cells", sob a orientação do Prof. Dr. Sandro Rogério de Almeida;
- Jéssica Costa Nascimento – "Evaluation of the tryptophan metabolism in the massive generation of antibody-secreting cells in dengue patients through the transcriptome of single blood cells", sob a orientação do Prof. Dr. Eduardo Lani Volpe da Silveira;
- Kamilla da Costa Pantoja – "Influence of insulin on the processes of autophagy and phagocytosis in bone marrow-derived macrophages", sob a orientação do Prof. Dr. Joilson de Oliveira Martins.

Prof. Dr. Ricardo Ambrósio Fock (Avaliador de Pôster):

- Karen Rafaela Gonçalves de Araujo – "The mystery behind the apprehensions of the selective cannabinoid receptor type-2 agonist BZO-HEXOXIZID (MDA-19) as a drug of abuse", sob a orientação do Prof. Dr. Maurício Yonamine;
- Mariana de Araujo Oliveira – "Evaluation of the autophagic process in subcutaneous mycosis caused by *Sporothrix brasiliensis* in healthy and diabetic animals", sob a orientação da Profa. Dra. Carla Taddei de Castro Neves;

- Pedro Augusto Ramos Vanzele – "Early life oral microbiome and the implications of oral feeding introduction in hospitalized preterm newborns"; Rafael dos Santos Barros – "Evaluation the impact of autophagy on the regulation of the senescent phenotype of prone and resistant mice to accelerated senescence", sob a orientação da Profa. Dra. Carla Taddei de Castro Neves;
- Raisa Ludmila Calil – "Chemoinformatic studies performed to predict activities of the synthetic canabinoids derivatives", sob a orientação do Prof. Dr. Gustavo Henrique Goulart Trossini.

Profa. Dra. Ligia Ferreira Gomes (Avaliador de Pôster):

- Rosana Lopes Rodrigues Amon – "Irradiated mesenchymal stem cells: influence of the bystander effect on hematopoiesis controls", sob a orientação do Prof. Dr. Ricardo Ambrósio Fock;
- Silvia Romano de Assis – "Establishment of *in vitro* model of reconstructed human epidermis for atopic dermatitis", sob a orientação da Profa. Dra. Silvya Stuchi Maria Engler;
- Suellen Rodrigues da Silva – "The association between overweight and the familiar historic of prostatic cancer", sob a orientação do Prof. Dr. Mario Hiroyuki Hirata;
- Sumara de Freitas – "Effects of cyanidin 3-glucoside on the immunomodulatory action of mesenchymal stem cell", sob a orientação do Prof. Dr. Ricardo Ambrósio Fock;

- Ywa Perpétuo Socorro Toda Tavares – “Investigating the role of autophagy on the onset of allergic lung inflammation in both diabetic and healthy mice”, sob a orientação do Prof. Dr. Joilson de Oliveira Martins.

Seguindo a programação do Simpósio, das 14h00 às 16h00, a Moderadora, Profa. Dra. Silvya Stuchi Maria Engler convida para proferir palestra, primeiramente, a Profa. Dra. Silvia Stanisçuaski Guterres – CAPES, na sequência a Profa. Dra. Maria Vitória Lopes Badra Bentley – CNPq e por último a Profa. Dra. Maria José Soares Mendes Giannini – FAPESP.

No dia seguinte, 30 de novembro de 2022, dando início aos trabalhos, das 09h00 às 10h00, o Moderador, Prof. Dr. Mario Hiroyuki Hirata convida para proferir palestra, o Prof. Dr. Luiz Henrique Catalani – Coordenador da AUSPIN.

Dando continuidade, das 10h00 às 11h00, a Moderadora, Profa. Dra. Ana Paula de Melo Loureiro e 03 doutorandos com apresentação oral de 15 minutos + discussão de 5 minutos para cada aluno:

- Julia Rezende da Silva – "Adenosine kinase inhibition as a tool to explore molecular mechanisms related to melanoma progression and resistance", sob a orientação da Profa. Dra. Silvya Stuchi Maria Engler;
- Karen Tiemi Akashi "Transcriptomic profile of single B cell subsets in asymptomatic or hospitalized COVID-19 patients", sob a orientação do Prof. Dr. Eduardo Lani Volpe da Silveira;
- Vitor Bruno – "Acute administration of ayahuasca prevents cocaine-induced expression of conditioned place preference", sob a orientação da Profa. Dra. Tania Marcourakis.

Na sequência, das 11h00 às 12h00, o Moderador, Prof. Dr. Eduardo Lani Volpe da Silveira e 02 pós-doutorandos – apresentação oral 15 min + discussão 5 min para cada aluno:

- Felipe Rizzetto Santos – "Evaluation of the action of Annexin A1 and neutrophils in patients with chronic kidney disease in conservative treatment", sob a supervisão da Profa. Dra. Sandra Helena Poliselli Farsky;
- Laura Cristina Lima Diniz – "A promising multi-stage, multi-antigen chimeric recombinant protein as an anti-*Plasmodium vivax* vaccine candidate", sob a supervisão da Profa. Dra. Irene da Silva Soares.

Das 12h45 às 14h00, 30 pós-graduandos de doutorado apresentaram pôsteres no Centro de Vivência (EP, FCF e IQ/USP), onde foram avaliados pelos docentes.

Prof. Dr. Nilton Erbet Lincopan Huenuman (Avaliador de Pôster):

- Aline Valério de Lima – "Increased resistance to ceftazidime-avibactam and metallo-betalactamase production in *Klebsiella pneumoniae*", sob a orientação do Prof. Dr. Jorge Luiz Mello Sampaio;
- Alysson Henrique Urbanski – "Chikungunya infection increases osteoclastogenic genes expression in blood and chronic arthralgia may be related to osteoclast survival", sob a orientação do Prof. Dr. Helder Takashi Imoto Nakaya;
- Amanda Pereira Vasconcelos – "Investigative analysis of molecular mechanisms associated with sex dimorphism in sepsis", sob a orientação do Prof. Dr. Helder Takashi Imoto Nakaya;

- Amanda Sodré Mota – "Gut Microbiota composition of women with depressive symptoms during perinatal and post partum periods", sob a orientação da Profa. Dra. Carla Taddei de Castro Neves;
- André Luis Fabris – "A new eco-friendly microextraction technique based on the principles of Green Analytical Toxicology for the analysis of 31 synthetic cannabinoids in plasma", sob a orientação do Prof. Dr. Maurício Yonamine.

Prof. Dr. Maurício Yonamine (Avaliador de Pôster):

- Augusto Akira Mori – "Functional analyses of LDLR genetic variants found in Familial Hypercholesterolemic patients, using CRISPR/Cas9", sob a orientação do Prof. Dr. Mario Hiroyuki Hirata;
- Cilícia Silvério Nascimento – "Bacteria in the vagina: how do they interact?", sob a orientação da Profa Dra. Carla Taddei de Castro Neves;
- Cristiane Silva Morais – "Evaluation of the autophagic and fibrotic mechanism in the peritoneal tissue exposed to the action of rapamycin in diabetic mice with peritonitis induced by *Staphylococcus aureus* and *S. argenteus",* sob a orientação do Prof. Dr. Joilson de Oliveira Martins;
- Fernanda Ribeiro dos Santos Esposito – "Successful expansion of healthcare-associated hypervirulent KPC-2-producing *Klebsiella pneumoniae* ST11/KL64 to impacted aquatic environment", sob a orientação do Prof. Dr. Nilton Erbet Lincopan Huenuman.

Profa. Dra. Primavera Borelli Garcia (Avaliador de Pôster):

- Haifa Hassanie – "Construction and validation of SETDB1 catalytic domain by homology modeling", sob a orientação do Prof. Dr. Gustavo Henrique Goulart Trossini;
- Herrison Yoshiki Yocida Fontana – "Emergence of carbapenem-resistant nontyphoidal Salmonella serovar Javiana human invasive strain encoding blaKPC-2 in Brazil", sob a orientação do Prof. Dr. Nilton Erbet Lincopan Huenuman;
- Isabella Harumi Yonehara Noma – "Targeting Peroxiredoxin-2 as a potential treatment for NRAS-mutated metastatic melanoma", sob a orientação da Profa. Dra. Silvya Stuchi Maria Engler;
- Jeevan Giddaluru – "Using location history data from mobile phones of patients to identify transmission patterns of infectious diseases", sob a orientação do Prof. Dr. Helder Takashi Imoto Nakaya;
- Jonathan Peña Avila – "Integrative Analysis Of Transcriptomic Profiles Of Covid-19 Patients Using Systems Biology", sob a orientação do Prof. Dr. Helder Takashi Imoto Nakaya.

Prof. Dr. Eduardo Lani Volpe da Silveira (Avaliador de Pôster):

- Juan Carlo Santos e Silva – "Application of genomic tools for the study of epidemiological data", sob a orientação do Prof. Dr. Helder Takashi Imoto Nakaya;
- Keila de Oliveira Lima – "Phenotypic test for the detection of serine-betalactamases and metallo-betalactamases co-production", sob a orientação do Prof. Dr. Jorge Luiz Mello Sampaio;

- Luana Filippi Xavier – "Role of Annexin A1 on intestinal microbiota: involvement in DSS-induced experimental colitis", sob a orientação da Profa. Dra. Sandra Helena Poliselli Farsky;
- Luiz Adriano Damasceno de Queiroz – "Regulation of mTOR receptor by rapamycin in senescence accelerated mice: the role of autophagy in aging process", sob a orientação do Prof. Dr. Joilson de Oliveira Martins;
- Luiz Gustavo Sparvoli – "Influence of the hypertensive pathologies on the composition of the microbiome of maternal colostrum and feces of premature newborns", sob a orientação da Profa. Dra. Carla Taddei de Castro Neves.

Prof. Dr. Helder Takashi Imoto Nakaya (Avaliador de Pôster):

- Manoel Oliveira de Moraes Junior – "Cytotoxicity and mechanism of action of copper(II) complexes as a potential treatment to overcome BRAF-inhibitor resistance in melanoma cells", sob a orientação da Profa. Dra. Silvya Stuchi Maria Engler;
- Mariana Baldini Prudêncio – "Impact of fecal microbiota transplantation of children with refractory epilepsy under ketogenic diet treatment in an animal model of temporal lobe epilepsy", sob a orientação da Profa. Dra. Carla Taddei de Castro Neves;
- Pablo Rhasan dos Santos Scharf – "Heated tobacco vapor and cigarette smoke exposures: immunotoxic outcomes in T cell biology", sob a orientação da Profa. Dra. Sandra Helena Poliselli Farsky;
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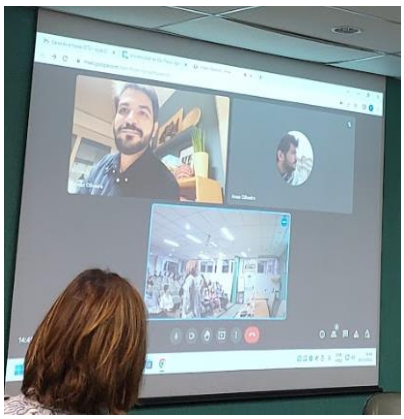
during antigeninduced arthritis (AIA)", sob a orientação da Profa. Dra. Silvya Stuchi Maria Engler;

- Pedro Felipe Rodrigues Sousa – "Relation between the repertoire of V(D)J sequences of human immunoglobulins and symptomatology for COVID-19", sob a orientação do Prof. Dr. Eduardo Lani Volpe da Silveira.

Profa. Dra. Tania Marcourakis (Avaliador de Pôster):

- Renaira Oliveira da Silva "Aged human skin reconstructed addressing the invasion of melanoma resistant to treatment with a B-RAF inhibitor", sob a orientação da Profa. Dra. Silvya Stuchi Maria Engler;
- Vanessa Barbosa Malaquias – "Biocidal evaluation of surfaces containing AgNPs", sob a orientação do Prof. Dr. Mario Hiroyuki Hirata;
- Vanessa Escolano Maso – "Meta-analysis of gene networks associated with human viral infections", sob a orientação do Prof. Dr. Helder Takashi Imoto Nakaya;
- Wilson Pascoalino Camargo de Oliveira – "Effects of weight loss on lipid profile and lipid transfer to HDL in grade III obese patients after bariatric surgery", sob a orientação do Prof. Dr. Raul Cavalcante Maranhão;
- Yasmin da Silva Santos – "Effect of angiotensin converting enzyme (ACE) inhibitors and/or angiotensin receptor blockers on the pathophysiology and therapy of experimental Covid-19", sob a orientação da Profa. Dra. Sabrina Epiphanio.

A seguir, das 14h00 às 15h00, a Moderadora, Profa. Dra. Sandra Helena Poliselli Farsky convida 02 egressos do Programa para proferir palestras (experiência de pós-graduado e a vida profissional): Dr. Antonio Anáx Falcão de Oliveira, orientado da Profa. Dra. Ana Paula de Melo Loureiro (via *Google Meet*) e Dra. Selma Andrea Cavalli, orientado da Profa. Dra. Rosario Dominguez Crespo Hirata.



Das 15h00 às 16h00, a Moderadora, Profa. Dra. Sandra Helena Poliselli Farsky convida a egressa do Programa: a Dra. Martina Rudnicki para proferir a palestra (via *Google Meet*), intitulada: "Angiogênese: aspectos básicos e implicações fisiopatológicas", orientada da Profa. Dra. Dulcineia Saes Parra Abdalla e na sequência foi realizado a homenagem para a Profa. Dulcineia, aposentada em 27 de outubro de 2022.

Depois das 16h00 foram divulgadas as premiações:

Apresentação Oral (Mestrado):

1º. lugar (R$ 1.000,00) Carlos Eduardo da Silva Gonçalves – "Aged mesenchymal stem cells contribute towards the development of acute myeloid leucemia", sob a orientação do Prof. Dr. Ricardo Ambrósio Fock.

2º. lugar (R$ 500,00) Stefani Aparecida Rodrigues Minchio – "The role of septin 9 in the regulation of vascular permeability in malaria-associated acute respiratory distress syndrome", sob a orientação da Profa. Dra. Sabrina Epiphanio.

3º. lugar (R$ 300,00) Gregory Batista Melocco – "*Staphylococcus aureus* genomic data for surveillance, control and management of antimicrobial resistance", sob a orientação do Prof. Dr. Nilton Erbet Lincopan Huenuman;

Apresentação Oral (Doutorado):

1º. lugar (R$ 1.000,00) Julia Rezende da Silva – "Adenosine kinase inhibition as a tool to explore molecular mechanisms related to melanoma progression and resistance", sob a orientação da Profa. Dra. Silvya Stuchi Maria Engler.

2º. lugar (R$ 500,00) Vitor Bruno – "Acute administration of ayahuasca prevents cocaine-induced expression of conditioned place preference", sob a orientação da Profa. Dra. Tania Marcourakis.

3º. lugar (R$ 300,00) Karen Tiemi Akashi – "Transcriptomic profile of single B cell subsets in asymptomatic or hospitalized COVID-19 patients", sob a orientação do Prof. Dr. Eduardo Lani Volpe da Silveira;

Apresentação Oral (Pós-Doutorandos):

1º. lugar (R$ 1.000,00) Laura Cristina Lima Diniz – "A promising multi-stage, multi-antigen chimeric recombinant protein as an anti-*Plasmodium vivax* vaccine candidate", sob a supervisão da Profa. Dra. Irene da Silva Soares.

2º. lugar (R$ 500,00) Felipe Rizzetto Santos – "Evaluation of the action of Annexin A1 and neutrophils in patients with chronic kidney disease in conservative treatment", sob a supervisão da Profa. Dra. Sandra Helena Poliselli Farsky.

Apresentação de Pôster (Mestrado):

1º. lugar (R$ 300,00) Sumara de Freitas – "Effects of cyanidin 3-glucoside on the immunomodulatory action of Mesenchymal Stem Cell", sob a orientação do Prof. Dr. Ricardo Ambrósio Fock.

Menção Honrosa (Mestrado):

- André Berndt Penteado com o pôster "Molecular modeling applied in the structural analysis and comparison of sirtuin 2 enzymes from trypanosomatids", sob a orientação do Prof. Dr. Gustavo Henrique Goulart Trossini;

- Jéssica Costa Nascimento com o pôster "Evaluation of the tryptophan metabolismo in the massive generation of antibody-secreting cells in dengue patients through the transcriptome of single blood cells", sob a orientação do Prof. Dr. Eduardo Lani Volpe da Silveira;
- Rosana Lopes Rodrigues Amon com o pôster "Irradiated mesenchymal stem cells: influence of the bystander effect on hematopoiesis controls", sob a orientação do Prof. Dr. Ricardo Ambrósio Fock;
- Silvia Romano de Assis com o pôster "Establishment of *in vitro* model of reconstructed human epidermis for atopic dermatitis", sob a orientação da Profa. Dra. Silvya Stuchi Maria Engler;
- Suellen Rodrigues da Silva com o pôster "The association between overweight and the familiar historic of prostatic cancer", sob a orientação do Prof. Dr. Mario Hiroyuki Hirata;

Apresentação de Pôster (Doutorado):

1º. lugar (R$ 300,00) Renaira de Oliveira da Silva – "Aged human skin reconstructed addressing the invasion of melanoma resistant to treatment with a B-RAF inhibitor", sob a orientação da Profa. Dra. Silvya Stuchi Maria Engller.

Menção Honrosa (Doutorado):

- Amanda Pereira Vasconcelos com o pôster "Investigative analysis of molecular mechanisms associated with sex dimorphism in sepsis", sob a orientação do Prof. Dr. Helder Takashi Imoto Nakaya;
- André Luís Fabris com o pôster "A new eco-friendly microextraction technique based on the principles of Green Analytical Toxicology for the analysis of 31 synthetic cannabinoids in plasma", sob a orientação do Prof. Dr. Maurício Yonamine;
- Isabella Harumi Yonehara Noma com o pôster "*Targeting Peroxiredoxin-2 as a potential treatment for NRAS-mutated metastatic melanoma*", sob a orientação da Profa. Dra. Silvya Stuchi Maria Engler;
- Jeevan Giddaluru com o pôster "Using location history data from mobile phones of patients to identify transmission patterns of infectious diseases", sob a orientação do Prof. Dr. Helder Takashi Imoto Nakaya;
- Jonathan Peña Avila com o pôster "*Integrative Analysis Of Transcriptomic Profiles Of Covid-19 Patients Using Systems Biology*", sob a orientação do Prof. Dr. Helder Takashi Imoto Nakaya;
- Juan Carlo Santos e Silva com o pôster "*Application of genomic tools for the study of epidemiological data*", sob a orientação do Prof. Dr. Helder Takashi Imoto Nakaya;
- Vanesa Barbosa Malaquias com o pôster "Biocidal evaluation of surfaces containing AgNPs", sob a orientação do Prof. Dr. Mario Hiroyuki Hirata;
- Vanesa Escolano Maso com o pôster "*Meta-analysis of gene networks associated with human viral infections*", sob a orientação do Prof. Dr.

Helder Takashi Imoto Nakaya.

Nos dias 29 e 30 de novembro de 2002, o simpósio contou com 114 participantes.

O Programa de Pós-Graduação em Farmácia (Fisiopatologia e Toxicologia) da FCF/USP agradece imensamente pelas doações de verba pelos docentes, ex-alunos, empresas: Intertox, Lonza, ThermoFisher e Veritas e da Pró-Reitoria de Pós-Graduação da Universidade de São Paulo, no qual foi possível premiar os alunos, adquirir passagens aéreas das professoras convidadas, diárias, faixa e banners para todos os pós-graduandos. Agradece, também, a Fundação Instituto Pesquisas Farmacêuticas - Fipfarma pela doação de pastas L; o Departamento de Análises Clínicas e Toxicológicas da FCF/USP com o presente para a professora homenageada e a Faculdade de Ciências Farmacêuticas da Universidade de São Paulo com as sacolas craft.

A Coordenação do Programa de Pós-Graduação em Farmácia (Fisiopatologia e Toxicologia) da Faculdade de Ciências Farmacêuticas da Universidade de São Paulo, agradece a colaboração, realização e participação no “Simpósio comemorativo de 50 anos de Pós-Graduação” de todos: docentes, alunos, pós-docs e funcionários da Administração (Bloco 13A) e do Departamento de Análises Clínicas e Toxicológicas da FCF/USP.

São Paulo, 12 de dezembro de 2022.

Profa. Dra. Sandra Helena Poliselli Farsky
Coordenadora do Programa de Pós-Graduação em Farmácia
(Fisiopatologia e Toxicologia) da FCF/USP

NATIONAL COUNCIL FOR SCIENTIFIC AND TECHNOLOGICAL DEVELOPMENT (CNPQ)
Setor de Autarquias Sul (SAUS), Block 01, Lot 06, Building H – Asa Sul Neighborhood - ZIP Code 70070-010 – Brasília – DF – www.gov.br/cnpq
Telemundi II Building

LETTER

Isabella Harumi Yonehara Noma Rua
Biagi Rivellino, 29, Vila Almeida
04756150 – São Paulo – SP – Brazil

Directorate for Institutional, International, and Innovation Cooperation
Support for International Programs

Official Letter SN/SEABE/COAFO/CNPq

Brasília, July 18th, 2023

Process Number: 200083/2023-6

Host Institution: H. Lee Moffitt Cancer Center and Research Institute/United States

As an addendum to the electronic message in which you were informed of the award of a fellowship abroad, we are writing to inform you of the benefits, duration, and terms and conditions of the fellowship, in accordance with with applicable CNPq regulations..

Program Type: Postdoctoral Fellowship Abroad (PDE)

Term: 08/01/2023 to 07/31/2024

Benefits:

Monthly stipend: USD 2,100.00 (base amount: USD 2,100.00)

Maintenance costs (single payment): USD 2.100,00

Health insurance fees up to: USD 1.080,00

Aid for Transportation: US$ 1.604,00

CONDITIONS

Within 30 (thirty) days from the start of activities, the fellowship holder must submit scanned copies of the **proof of boarding**; **proof of enrollment** or a **letter from the host institution abroad certifying the start of activities**; and **proof of health insurance coverage**, through the form "Submission of Supplementary Documentation for Abroad Fellowships," available on the CNPq website, Carlos Chagas Platform.

If the documents are not submitted within the stipulated deadline, the fellowship will be suspended by CNPq.

IMPORTANT NOTES:

1 - Carefully read the Fellowship Abroad Regulations, available at http://www.cnpq.br/web/guest/view/-/journal_content/56_INSTANCE_OoED/10157/515690 , and the booklet "Support Information for Students Abroad with a CNPq Fellowship" http://www.cienciasemfronteiras.gov.br/documents/214072/bd912c91-1df4-4515-98de-1ba20a369651.

2 - Payment of the first three monthly stipends and of the educational materials allowance will be made by credit to the Fellowship Card, in advance of the start of the fellowship term.

2.1 - For fellowship holders who reside in or are located in Brazil, CNPq will pay, according to the fellowship modality, the maintenance costs, health insurance, and aid for transportation by deposit into the personal checking account provided at the time of acceptance of the fellowship in the online form "Supplementary Data."

2.2 - If the fellowship holder is residing abroad, the benefits due will be paid through the Fellowship Card.

3 - Payment of tuition fees and/or bench fees, when applicable, will be made directly to the host institution.

3.1- Split-Site Undergraduate Abroad (SWG) fellowship holders must contact the partner institution in the destination country for additional information regarding this benefit. Access http://www.cienciasemfronteiras.gov.br/web/csf to obtain the list/contact information of partner institutions.

3.2- For Full Doctorate Abroad (GDE) and Split-Site Doctorate Abroad (SWE) fellowship holders, payment of tuition fees and/or bench fees will be made upon submission of an invoice to the email atendimento@cnpq.br .

4- Payment of monthly stipends abroad is made quarterly, in accordance with the schedule below:

Payroll month	**Monthly stipends to be paid**	**Credit month**
December	February/March/April	January
March	May/June/July	April
June	August/September/October	July
September	November/December/January	October

If the monthly stipend to be paid falls outside this schedule, payment will be made through a supplemental payroll.

Any information related to this award must be obtained through the Customer Service Center, by email at atendimento@cnpq.br , or by telephone at +55 (61) 3211-9977.

Roberta Wilbert Emmel
Head of the Office for Support to Fellowships Abroad and Alumni – SEABE
PO 196/2021

TO WHOM IT MAY CONCERN

Brasília, July 18th, 2023

We hereby certify that the CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico, of the Ministry of Science, Technology and Innovation from Brazil has granted a fellowship for 12 months to Isabella Harumi Yonehara Noma to pursue Post-Doctoral Training starting in August/2023 up to July/2024 at H.Lee Moffitt Cancer Center and Research Institute.

This fellowship consists of:

Monthly stipend: US 2.100,00

Maintenance costs (single payment): USD 2.100,00

Health insurance fees up to: USD 1.080,00

Aid for Transportation: US$ 1.604,00

Roberta Wilbert Emmel
Head of the Office for Support to Fellowships Abroad and Alumni – SEABE
PO 196/2021

Document electronically signed by **ROBERTA WILBERT EMMEL, Head of the Office for Support to Fellowships Abroad and Alumni - PO No. 196/2021 / 97/2022**, on 07/18/2023, at 4:40 p.m., in accordance with Article 6 of Decree No. 8,539, of October 8, 2015.

The authenticity of this document may be verified on the website http://sei.cnpq.br/verifica.html by entering verification code **1782594** and CRC code **C60DC258**.

I, Raphael Michels Fantinato de Moura, telephone number 415 425-2508, mailing address P.O. Box 90487, San Diego, CA 92169, certify that the professional translation of this document from Portuguese to English has been performed by myself, a qualified translator fluent in both languages, and that the following is an accurate and complete translation of the document.

Raphael Michels Fantinato de Moura. **Date: January 14, 2026.**

CONSELHO NACIONAL DE DESENVOLVIMENTO CIENTÍFICO E TECNOLÓGICO
Setor de Autarquias Sul (SAUS), Quadra 01, Lote 06, Bloco H - Bairro Asa Sul - CEP 70070-010 - Brasília - DF - www.gov.br/cnpq
Edifício Telemundi II

CARTA

Isabella Harumi Yonehara Noma
Rua Biágio Rivellino, 29, Vila Almeida
04756150 - São Paulo - SP - Brasil

Diretoria de Cooperação Institucional, Internacional e Inovação
Fomento a Programas Internacionais

Ofício SN/SEABE/COAFO/CNPq

Brasília, 18 de julho de 2023

Processo número: 200083/2023-6

Inst.: H. Lee Moffitt Cancer Center and Research Institute/Estados Unidos

Em aditamento a mensagem eletrônica na qual lhe foi comunicada a concessão de uma bolsa de estudos no exterior, vimos informar-lhe os benefícios, prazos e condições da referida bolsa, em conformidade com as normas em vigor no CNPq.

Modalidade: Pós-doutorado no Exterior - PDE

Vigência: 01/08/2023 até 31/07/2024

Benefícios:

Mensalidade: USD 2.100,00 (valor-base: USD 2.100,00)

Auxílio Instalação (parcela única): USD 2.100,00

Seguro-Saúde: USD 1.080,00

Auxilio deslocamento: US$ 1.604,00

CONDIÇÕES

No prazo de 30 (trinta) dias contados a partir do início das atividades, o bolsista deverá enviar as cópias digitalizadas do **comprovante de embarque**; do **comprovante de matrícula** ou **carta da Instituição de destino no exterior atestando o início das atividades**; **e comprovante da contratação do seguro-saúde**, por meio do Formulário "Envio de Documentação Complementar de Bolsa no Exterior", disponível na página do CNPq, Plataforma Carlos Chagas.

Caso os documentos não sejam enviados no prazo previsto, a bolsa será suspensa pelo CNPq.

OBSERVAÇÕES IMPORTANTES:

1 - Leia atentamente a Norma de Bolsas no Exterior, disponível no endereço http://www.cnpq.br/web/guest/view/-/journal_content/56_INSTANCE_0oED/10157/515690 , e a cartilha "Informações de Apoio ao Estudante no Exterior com bolsa do CNPq" http://www.cienciasemfronteiras.gov.br/documents/214072/bd912c91-1df4-4515-98de1ba20a369651.

2 - O pagamento das 3 primeiras mensalidades e do auxílio material didático da bolsa será realizado mediante crédito no Cartão-Bolsista, antecipadamente ao início da vigência da bolsa.

2.1 - Ao bolsista que resida ou se encontre no Brasil, o CNPq pagará, de acordo com a modalidade da bolsa, o auxílio instalação, o seguro saúde e o auxílio deslocamento por meio de depósito em conta corrente pessoal, informada no momento da aceitação da bolsa no formulário on-line "Dados Complementares".

2.2 - Caso o bolsista se encontre residindo no exterior, os benefícios devidos serão pagos por meio do Cartão-Bolsista.

3 - O pagamento de taxas escolares e/ou taxas de bancada, quando for o caso, será efetuado diretamente à instituição de destino.

3.1 - Os bolsistas de Graduação Sanduíche no Exterior (SWG) deverão entrar em contato com a instituição parceira do país de destino, para informações adicionais a respeito deste benefício. Acesse o endereço http://www.cienciasemfronteiras.gov.br/web/csf para obter a relação/contato das instituições parceiras.

3.2 - Para os bolsistas de Doutorado Pleno no Exterior (GDE) e Doutorado Sanduíche no Exterior (SWE), o pagamento das taxas escolares e/ou taxas de bancada será realizado mediante envio de fatura para o email atendimento@cnpq.br .

4 - O pagamento das mensalidades no exterior é efetuado trimestralmente, de acordo com o cronograma abaixo:

Mês da Folha de Pagamento	Mensalidades a serem pagas	Mês de Crédito
Dezembro	fevereiro/março/abril	Janeiro
Março	maio/junho/julho	Abril
Junho	agosto/setembro/outubro	Julho
Setembro	novembro/dezembro/janeiro	Outubro

Caso a mensalidade a ser paga esteja fora desse cronograma, o pagamento será efetivado por meio de folha suplementar.

Quaisquer informações relacionadas a esta concessão deverão ser obtidas por meio da Central de Atendimento, e-mail atendimento@cnpq.br , ou pelo telefone 061-3211-9977.

Roberta Wilbert Emmel
Chefe do Serviço de Apoio às Bolsas no Exterior e Egressos - SEABE
PO 196/2021

TO WHOM IT MAY CONCERN

Brasilia, July 18th, 2023

We hereby certify that the CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico, of the Ministry of Science, Technology and Innovation from Brazil has granted a scholarship for 12 months to Isabella Harumi Yonehara Noma to pursue Post-Doctoral Trainning starting in August/2023 up to July/2024 at H.Lee Moffitt Cancer Center and Research Institute.

This scholarship consists of:

Monthly stipend: USD 2.100,00

Maintenance costs(single payment): USD 2.100,00

Health insurance fees up to: USD 1.080,00

Aid for Transportation: US$ 1.604,00

Roberta Wilbert Emmel
Chefe do Serviço de Apoio às Bolsas no Exterior e Egressos - SEABE
PO 196/2021

Documento assinado eletronicamente por **ROBERTA WILBERT EMMEL, Chefe de Serviço de Apoio às Bolsas no Exterior e Egressos - PO Nº 196/2021 / 97/2022**, em 18/07/2023, às 16:40, conforme o art. 6º do Decreto nº 8.539, de 08 de outubro de 2015.

A autenticidade do documento pode ser conferida no site http://sei.cnpq.br/verifica.html informando o código verificador **1782594** e o código CRC **C60DC258**.

SCIENCE

CNPq to invest BRL 215 million in international scientific cooperation scholarships, projects

Funds represent 26% increase over the BRL 170 million (USD 30.8 million) invested last year. Applications must be submitted by August 9, with final results scheduled for publication in November

Published in Jun 26, 2024 10:12 PM

Secretaria de Comunicação Social

Image: Freepik.

The National Council for Scientific and Technological Development (*Conselho Nacional de Desenvolvimento Científico e Tecnológico*/CNPq) will invest BRL 215 million (USD 39 million) in international scientific cooperation projects and scholarships for Brazilian researchers abroad. This represents a 26% increase from last year's BRL 170 million investment. Applications must be submitted by August 9, with final results expected to be published in November.

Prospective applicants may compete in three categories: 1) collaborative research projects with established international partners; 2) individual proposals from researchers within 10 years of earning their doctoral degrees; and 3) individual proposals from researchers currently holding active CNPq Junior Postdoctoral (*Pós-Doutorado Júnior*/PDJ) or Senior Postdoctoral (*Pós-Doutorado Sênior*/PDS) scholarships.

This public call specifies the investment of at least 10% of funds in cooperation projects involving research groups and networks from Latin American, Caribbean and African countries. Additionally, 20% of the funds will be earmarked for projects associated with institutions in Brazil's North, Northeast and Central-West regions.

Out of the total allocated funds, BRL 200 million (USD 36.2 million) will be allocated to

for Category (1) applicants, starting in 2025.

Category (1) collaborative research projects may receive up to BRL 300,000 (USD 54,400) per proposal. Individual proposals in Category (2) are capped at BRL 200,000 (USD 36,200), within the 12-month duration limit for the Postdoctoral Fellowship Abroad (*Pós-Doutorado no Exterior*/PDE). For Category (3), individual proposals can receive up to BRL 80,000 (USD 14,500), with a 3-month duration limit for the Senior Technological Development and Innovation Abroad (*Desenvolvimento Tecnológico e Inovação no Exterior Sênior*/DES) fellowship. During this period, the PDJ or PDS fellowship is temporarily suspended and will be resumed upon the researcher's return.

Learn more about Public Call MCTI/CNPq No. 16/2024.

Check the scholarship amounts from CNPq.

Source: CNPq

Category

Science and Technology

Tags: CNPq Science Technology Research

Share:

RN-007/2018

FELLOWSHIPS ABROAD

Establishes the general and specific regulations for the modalities of fellowships abroad: Senior Visiting Researcher (ESN), Postdoctoral Fellowship Abroad (PDE), Split-Site Doctorate Abroad (SWE), Full Doctorate Abroad (GDE), Technological Development and Innovation Abroad (Junior – DEJ) and (Senior – DES), and Professional Master's Degree Abroad (MPE).

The President of the NATIONAL COUNCIL FOR SCIENTIFIC AND TECHNOLOGICAL DEVELOPMENT – CNPq, in the exercise of the powers conferred upon him by Decree No. 8,866 of October 3, 2016, considering the Legal Framework for Science, Technology and Innovation (CT&I) — Constitutional Amendment No. 85/2015, of 02/26/2015, Law No. 13,243/2016, of 01/11/2016, and Decree No. 9,283/2018, of 02/07/2018 — and in accordance with the decision of the Executive Board at its 3rd (third) meeting held on 03/01/2018,

Revokes: RN-029/2012 and its amendments

RESOLVES:

To establish the general and specific regulations for the following fellowship modalities abroad:

- Senior Visiting Researcher (ESN)
- Postdoctoral Fellowship Abroad (PDE)
- Split-Site Doctorate Abroad (SWE)
- Full Doctorate Abroad (GDE)
- Technological Development and Innovation Abroad (Junior – DEJ) and (Senior – DES)
- Professional Master's Degree Abroad (MPE)

I – GENERAL REGULATIONS

1. Application

1.1. Applications shall be submitted by researchers or students through the *Electronic Proposal Submission Form*, in accordance with the *Fellowship Application Calendar* or a specific call for proposals.

2. Evaluation

2.1. The evaluation and ranking of proposals shall be carried out in the following stages:

a) review by the technical area;

b) review by *ad hoc* consultants, when applicable;

c) comparative merit analysis and ranking of proposals by Advisory or Review Committees;

d) final decision by the Board of Directors, subject to CNPq's financial availability.

2.2. The opinion issued by the Advisory or Review Committee must take into account the following aspects:

a) the merit of the proposal;

b) the opinions of the technical area and of *ad hoc* consultants, when applicable;

c) the specific characteristics of the fellowship modalities.

2.3. The curricula to be considered in the evaluation shall be those available on the Lattes Platform.

3. Award

3.1. The results of the evaluations will be published in the Federal Official Gazette (DOU), on the CNPq website, and by electronic notification to the applicant.

3.2. From the date of publication of the result in the DOU and on the CNPq website, the applicant may file an appeal through a specific online form, available on the CNPq Electronic Platform, within 10 consecutive days.

3.3. The award of fellowships abroad requires that the beneficiary have acceptance from the project host institution for the development of the proposed activities.

3.4. For approved proposals, the communication of the evaluation result will include the access link to the *Supplementary Data Form* and to the *Grant Agreement*.

3.4.1. After receipt of the *Supplementary Data Form* and the *Grant Agreement*, CNPq will send the *Benefits Letter* to the email address registered in the fellowship holder's Lattes Curriculum, including a key for electronic verification of the document's authenticity.

3.4.2. The applicant must issue a *Power of Attorney* (template – Annex VII) granting authority to a person in Brazil to represent him/her in matters related to the CNPq fellowship. The document must be kept in the custody of the fellowship holder's attorney-in-fact.

3.5. The duration of the fellowship shall be determined by the period, in months, approved by CNPq and must comply with the provisions of the *Fellowship Application Calendar* or of specific calls or programs published on the CNPq Portal. Once the implementation deadline has expired, the award will be canceled.

3.6. Any requests for extension of the fellowship must be submitted before the end of the award period, in compliance with the deadline established in the specific regulation of the modality.

3.7. Fellowships granted through institutional quotas or to coordinators shall follow differentiated implementation procedures, to be defined in specific calls.

4. Payment of Fellowships

4.1. The amounts of the monthly stipends shall be established by CNPq in a specific regulation, the Table of Values for Fellowships Abroad.

4.2. For payment of the initial benefits, the approved applicant must have completed and electronically submitted to CNPq the *Supplementary Data Form* and the *Grant Agreement*, within the deadline stipulated in the approval notification.

4.3. Payments to the fellowship holder shall be made quarterly, by credit abroad, in accordance with the schedule linked to the start date of the fellowship term. The schedule is published in the Fellowship Abroad Application Calendar on the CNPq website.

4.3.1. For fellowship holders who reside in or are located in Brazil, CNPq shall pay, according to the fellowship modality, the maintenance costs, health insurance, and aid for transportation, by deposit into a personal checking account, to be provided at the time of acceptance of the fellowship in the online *Supplementary Data Form*. Other benefits, such as the first three monthly stipends and other modality-specific allowances, shall be credited to the fellowship card prior to the start of the fellowship term.

4.3.1.1. In the case of deposits made into a checking account, the amount shall be credited in Brazilian currency, using the buy exchange rate published by the Central Bank of Brazil for the day immediately preceding authorization of payment by CNPq. Amounts transferred to the fellowship card shall be deposited in the currency of the destination country or, when unavailable, in US dollars.

4.3.1.2. CNPq shall not be responsible for any exchange rate fluctuations or taxes, and the beneficiary shall be responsible for any necessary foreign currency exchange transactions carried out in Brazil.

4.3.1.3. In Brazil, the fellowship card shall be sent by mail to the mailing address registered in the Lattes Curriculum, with delivery subject to signature upon receipt (Acknowledgment of Receipt – A.R.).

4.3.1.4. A fellowship holder who is already residing in the destination country at the time of approval shall not be entitled to the outbound transportation aid or to the relocation allowance. There shall also be no advance payment of the benefits listed in subitem **4.3.1**. Payments shall be credited to the fellowship card, which shall be sent to the foreign address previously registered in the Lattes Curriculum as the mailing address.

4.4. The fellowship term shall be preliminarily determined based on the start date of activities abroad reported by the fellowship holder in the *Supplementary Data Form*. If activities abroad begin by the 15th day of the month, the fellowship term shall begin in the same month. If they begin on or after the 16th day of the month, the fellowship term shall begin in the following month.

4.5. Within 30 days after the start of activities, the fellowship holder must submit, through the CNPq Electronic Platform, scanned copies of the following documents, under penalty of suspension of the fellowship:

a) proof of travel/boarding;

b) proof of enrollment or a letter from the institution certifying the start of activities; and

c) proof of health insurance coverage.

5. Fellowship Benefits

The benefits of fellowships abroad include payment of monthly stipends, transportation aid, maintenance costs, health insurance, and fees, in accordance with the specific characteristics of each modality. No benefits are provided to dependents, except for Full Doctorate, in accordance with the applicable specific regulation.

5.1. Monthly Stipends

Monthly stipends shall be calculated in accordance with the Table of Values for Fellowships Abroad.

5.2. Transportation Aid

5.2.1. CNPq shall deposit, into the fellowship holder's bank account in Brazil, the amount corresponding to the transportation aid intended for the purchase of airline tickets. The allowance amounts shall be defined based on market prices and set forth in the Table of Values for Transportation Aids Abroad. It shall be the responsibility of the fellowship holder to purchase the airline ticket from the airline/company or agency of their choice.

5.2.1.1. Beneficiaries of fellowships with a duration equal to or shorter than six months shall receive the transportation aid amount in a single installment, corresponding to round-trip airline tickets.

5.2.2. For fellowships with a duration longer than six months, the portion of the transportation aid corresponding to the return to the home country shall be deposited together with the final monthly stipend.

5.2.3. No outbound transportation aid shall be granted to fellowship holders residing in the country where the activities set forth in the approved project/work plan will be carried out. In such cases, the return transportation aid at the end of the fellowship period may be granted upon request by the beneficiary at least 60 days prior to the end of the fellowship term.

5.3. Maintenance cost

The maintenance cost aid is intended to cover part of the fellowship holder's personal expenses related to relocation abroad and shall be paid in accordance with the Table of Values for Fellowships Abroad, exclusively to fellowship holders residing in Brazil.

5.4. Health Insurance

The health insurance is intended for the contracting of a company that provides coverage for medical and hospital expenses for the fellowship holder and, in the case of Full Doctorate fellowships, for their dependents.

5.4.1. The contracting of health insurance by the fellowship holder is mandatory and must be evidenced in accordance with item **4.5**, letter c.

5.4.2. Health insurance amounts are established in the Table of Values for Fellowships Abroad.

5.4.3. For fellowships with a duration shorter than 12 months, the health insurance amount shall be proportional to the duration of the fellowship.

5.5. Tuition Fees

Tuition fees are intended to cover expenses required by the institution that are necessary for the effective enrollment. The estimated annual amounts must be informed by the applicant in the proposal submission form.

5.6. Bench Fees

Bench fees, when applicable, are intended to cover expenses required by the institution for the conduct of scientific and technological research activities by split-site doctorate or full doctorate fellowship holders. The amounts must be informed by the applicant in the proposal submission form.

6. Supplementation of Fellowships from Other Institutions

6.1. CNPq allows the supplementation of a fellowship granted by a foreign or international institution, provided that it is previously authorized by the Area Director.

6.2. CNPq shall not supplement amounts or periods of fellowships granted with funds from the National Treasury.

6.3. The fellowship may be held concurrently with temporary and/or partial remuneration received by the fellowship holder in the form of a Teaching or Research Fellowship, provided that the advisor declares that such activities do not impair the progress of the program.

7. Obligations of the Fellowship Holder

7.1. To devote themselves to the activities set forth in the project or approved work plan by CNPq during the term of the fellowship.

7.1.1. Travel by the fellowship holder to Brazil during the term of the fellowship may be authorized by CNPq under exceptional circumstances, duly justified.

7.1.1.1. For the assessment of the request, a justification must be submitted, accompanied by the relevant documentation. If the duration exceeds 15 days, payment of the fellowship shall be suspended.

7.2. To act as an *ad hoc* consultant by issuing opinions on research projects, when requested. Failure to comply with this provision shall result in suspension of the fellowship.

7.3. To request prior authorization for any change related to the research project, the work plan, or the fellowship itself, which shall be subject to analysis by CNPq.

7.4. To reimburse CNPq for any benefits unduly paid, within a maximum period of 30 days.

7.5. To return to Brazil within 30 days after the end of the fellowship term and to remain in the country for a period not shorter than the duration of the fellowship.

7.5.1. At the request of the fellowship holder, and upon demonstration that their stay abroad will be of strategic relevance to the development of Science, Technology, and Innovation in Brazil, CNPq may replace the obligation to return to and remain in Brazilian territory with other obligations that ensure reimbursement of the investment made by the country in the training of the fellowship holder.

7.6. Works produced or published, in any medium, as a result of activities supported by CNPq must necessarily acknowledge the support received, using one of the following expressions, in the language of the work:

a) “This work was carried out with the support of CNPq, the National Council for Scientific and Technological Development – Brazil”; or

b) “CNPq Fellow – Brazil”.

7.7. Failure to comply with the regulatory provisions obliges the fellowship holder to fully reimburse CNPq for all expenses incurred for their benefit, as provided for in the specific CNPq Normative Resolution on reimbursement, under penalty of registration of the resulting debt in the Federal Active Debt and in the Federal Public Sector Outstanding Credits Information System – CADIN.

7.7.1. CNPq may, exceptionally, grant a discharge of liability in cases where failure in the training process is established. Such cases shall be decided by the Area Director.

8. Suspension and Cancellation

8.1. Temporary suspension of the fellowship, with subsequent reactivation, may be requested, provided that its end date is

respected.

8.2. Suspension or cancellation of the fellowship may occur at the request of the fellowship holder or their advisor, or at the initiative of CNPq, and must always be duly justified.

8.2.1. It shall be the responsibility of the technical staff of CNPq, assisted by *ad hoc* consultants, when necessary, to analyze the fellowship holder's situation and to issue an opinion on the need for suspension or cancellation of the fellowship. The final decision shall fall within the competence of the Office of the General Coordination.

8.3. Reactivation of a suspended fellowship, when applicable, must be requested at least 30 days in advance.

9. Monitoring, Evaluation, and Closure

9.1. The performance of the fellowship holder shall be monitored by CNPq through the analysis of reports or other forms of follow-up defined in accordance with the specific characteristics of each modality.

9.1.1. The report on execution of the object must be submitted by the fellowship holder through the CNPq electronic platform no later than 60 days after the end of the fellowship.

9.2. The process shall be closed when the beneficiary:

a) submits proof of the tickets used;

b) submits the modality-specific documents required for closure of the process;

c) has the report on execution of the object approved by CNPq;

d) has no outstanding financial liabilities with CNPq related to the process; and

e) complies with the terms agreed upon in the *Grant Agreement*.

10. Final Provisions

10.1. These rules apply to all fellowship modalities granted with budgetary resources from CNPq. Fellowships granted under agreements and arrangements with other institutions, within strategic programs, or with resources from Sectoral Funds may be subject to different provisions.

10.1-A. Public Call Notices may not provide for equivalence between different academic degrees, whether in admissibility criteria or in evaluation criteria.[2]

10.2. Advisors and/or coordinators are prohibited from awarding a fellowship to a spouse, partner, or relative by blood or affinity, in the direct or collateral line, up to the third degree, inclusive.

10.3. The accumulation of a CNPq fellowship with another fellowship granted with resources from the National Treasury is prohibited.

10.4. Omitted or exceptional cases shall be analyzed by the Area Board of Directors.

10.5. This Normative Resolution shall enter into force as of the date of its publication, and Normative Resolutions RN-029/2012, RN-025/2013, RN-010/2013, RN-051/2014, RN-009/2014, RN-030/2015, RN-024/2015, RN-017/2015, RN-012/2015, RN-009/2015, RN-028/2016, RN-005/2017, and all other provisions to the contrary are hereby revoked.

II – SPECIFIC RULES

Annexes:

I – Senior Visiting Researcher

II – Postdoctoral Fellowship Abroad

III – Split-Site Doctorate Abroad

IV – Full Doctorate Abroad

V – Technological Development and Innovation Abroad (Junior – DEJ) and (Senior – DES)

VI – Professional Master's Degree Abroad

III – TEMPLATE

Annex:

VII – Power of Attorney

Brasília, April 12, 2018.

MARIO NETO BORGES

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Annex I

Senior Visiting Researcher (ESN)

1. Purpose

To enable researchers to carry out a research project, or part thereof, at a foreign institution.

2. Requirements and Conditions

2.1. For the applicant:

a) be a CNPq Level 1 researcher;

b) have an affiliation with a research institution in Brazil.

2.2. For the host institution:

– be internationally recognized in the applicant's field of interest.

2.3. For the host researcher:

– have recognized expertise in the project's field.

3. Duration

– From three to six months.

4. Benefits

a) Monthly stipends, calculated in accordance with the Table of Values for Fellowships Abroad;

b) Transportation aid;

c) Maintenance cost;

d) Health insurance.

5. Mandatory documents for application:

a) Electronic Proposal Submission Form;

b) Curriculum Vitae registered on the Lattes Platform;

c) Project / Work Plan.

6. Selection criteria

Applicants will be selected based on their curriculum vitae, the international standing of the host institution, and the quality of the work plan, and ranked in comparison with other applicants.

7. Documents to be obtained by the fellowship holder by the application submission deadline and kept on file until the process is concluded:

a) Formal acceptance from the host institution;

b) Written agreement from the host researcher regarding the proposed activities; and

c) Valid permanent residence status in Brazil, in the case of foreign researchers.

8. Mandatory documents for closing the process:

Annex II

Postdoctoral Fellowship Abroad (PDE)

1. Purpose

To enable researchers to enhance and update their knowledge through an internship and the development of a research project with innovative scientific or technological content at a foreign institution.

2. Requirements and Conditions

2.1. For the applicant:

a) hold a doctoral degree at the time the fellowship is implemented;

b) devote full-time dedication to the activities scheduled at the host institution;

c) for former recipients of doctoral fellowships abroad funded by a national agency, comply with the minimum period of residence in Brazil required by that agency;

d) observe a minimum interval of three years between two Postdoctoral Fellowships Abroad funded by CNPq; and

e) be Brazilian or a foreign national holding a permanent residence status in Brazil.

2.2. For the host institution:

– be internationally recognized in the applicant's field of interest.

2.3. For the advisor:

– have recognized expertise in the project's field.

3. Duration

3.1. The fellowship duration is from six to twelve months, with extensions permitted provided the total duration does not exceed 24 months.

3.2. In the event of childbirth or adoption occurring during the fellowship period and formally reported by the fellowship holder to CNPq, the fellowship duration may be extended by up to four months.

4. Benefits

a) Monthly stipends, calculated in accordance with the Table of Values for Fellowships Abroad;

b) Transportation aid;

c) Maintenance costs; and

d) Health insurance.

5. Mandatory documents for application:

a) Electronic Proposal Submission Form;

b) Applicant's Curriculum Vitae registered on the Lattes Platform;

c) Advisor's Curriculum Vitae;

d) Research project;

e) Advisor's written agreement with the proposed activities; and

f) Confirmation by the advisor that the applicant's proficiency in the language of the host country is sufficient for the execution of the planned activities.

6. Selection criteria

Applicants will be selected based on their curriculum vitae, the advisor's curriculum vitae, the international standing of the host institution, and the quality of the project, and ranked in comparison with other applicants.

7. Document to be obtained by the fellowship holder by the proposal submission deadline and kept on file until the process is concluded:

– Valid permanent residence status in Brazil, in the case of foreign researchers.

8. Fellowship extension

Any request for extension must be formally submitted at least 60 days before the end of the fellowship.

9. Mandatory documents for closing the process:

– Specific documents, including the advisor's statement regarding the activities carried out by the fellow.

Annex III

[IRRELEVANT CONTENT]

[IRRELEVANT CONTENT]

Split-Site Doctoral Abroad (SWE)

1. Purpose

To support students formally enrolled in a doctoral program in Brazil who demonstrate the qualifications to benefit, abroad, from the opportunity for theoretical deepening, data collection and/or processing, or partial development of the experimental component of their dissertation to be defended in Brazil.

2. Requirements and Conditions

2.1. For the applicant:

a) be formally enrolled in a doctoral program in Brazil recognized by CAPES;

c) have proficiency in the language used at the host institution;

d) have the consent of the graduate program coordinator and of the advisors in Brazil and abroad; and

e) be Brazilian or a foreign national holding permanent residence status in Brazil.

2.2. For the host institution advisor:

– be a researcher with an established academic record relevant to the complementary development of the doctoral dissertation.

3. Duration

3.1. From 3 to 12 months. An extension is permitted, subject to the maximum duration limit of the fellowship.

3.1.1. The total duration of the Doctorate in Brazil, combined with the Split-Site Doctorate Abroad, may not exceed 48 months.

3.1.1.1. In the event that the doctoral degree is not obtained due to withdrawal or abandonment at the recipient's own initiative, without force majeure, or due to failure to comply with the applicable regulations, the amounts invested in the Split-Site Doctorate Abroad shall be added to those of the Doctorate in Brazil for purposes of reimbursement to CNPq.

3.1.2. In cases of cotutelle between Brazilian and foreign universities, provided for in specific agreements, the period abroad may be extended up to 24 months. Cotutelle is understood as a situation in which the doctoral degree is awarded by both participating institutions.

4. Benefits:

a) Monthly stipends, calculated in accordance with the Table of Values for Fellowships Abroad;

b) Transportation aid;

c) Maintenance costs;

d) Health insurance;

e) Tuition fees; and

f) Bench fees.

5. Mandatory documents for application:

a) Electronic Proposal Submission Form;

b) Applicant's Curriculum Vitae registered on the Lattes Platform;

c) Curriculum vitae of the advisor abroad;

d) Work plan and/or research project for the fellowship period;

e) Formal consent from the coordinator of the graduate program in which the applicant is enrolled;

f) Formal acceptance from the host institution, issued by the body responsible for admitting the applicant, including information on the language and minimum proficiency level required;

g) Agreement from the advisors in Brazil and abroad regarding the proposed activities; and

h) Confirmation from the advisor abroad that the applicant has sufficient proficiency in the required language for the execution of the planned activities.

6. Selection criteria

Applicants will be selected based on their curriculum vitae, the curriculum vitae of the advisor abroad, the merit and relevance of the proposal, the international standing of the research group abroad, and ranked in comparison with other candidates.

7. Document to be obtained by the fellowship holder prior to signing the Grant Agreement and kept on file until

the process is closed:

– Valid permanent residence status in Brazil, in the case of a foreign researcher.

8. Fellowship extension

Any request for extension must be submitted through a specific online form at least 30 days before the end of the fellowship.

9. Mandatory documents for closing the process:

– Specific: evaluations by the advisors in Brazil and abroad.[1]

Annex IV

Full Doctorate Abroad (GDE)

1. Purpose

To train holders of a doctorate abroad at institutions of recognized excellence, in fields of knowledge considered at the forefront of scientific and technological development and in strategic areas defined by the CNPq Deliberative Council.

2. Requirements and Conditions for the Applicant:

a) be Brazilian or a foreign national with permanent residence status in Brazil;

b) demonstrate proficiency in the language required for the program.

3. Duration

3.1. The duration of the fellowship is up to 48 months.

3.2. In the event of childbirth or adoption occurring during the fellowship period and formally reported by the fellowship holder to CNPq, the fellowship duration may be extended by up to four months.

4. Benefits:

a) Monthly stipends, calculated in accordance with the provisions of subitems 4.1 and 4.2 below and the Table of Values for Fellowships Abroad;

b) Transportation aid;

I. CNPq shall deposit, into the fellowship holder's personal bank account in Brazil, the amount corresponding to the **outbound** transportation aid for the fellowship holder and up to two dependents, when applicable.

II. The transportation aid corresponding to the return to Brazil (including up to two dependents) must be requested by the fellowship holder from the Graduate and Research Fellowships Abroad Support Service – SEBPE, by email at sebpe@cnpq.br, at least 30 days prior to the expected date of travel.

III. A fellowship holder who gets married during the fellowship period shall not be entitled to the outbound transportation aid for the dependent, but only to the return transportation aid upon his or her return to Brazil.

c) Maintenance costs;

d) Health Insurance;

I. When a dependent is added to an already implemented Full Doctorate fellowship, the health insurance benefit shall be paid proportionally to the remaining period of the fellowship term.1

e) Tuition Fees;

f) Bench Fees; and

g) Field Research, when provided for in the original proposal, for a maximum period of 12 months if carried out in Brazil, and six months if carried out abroad.

4.1. Parameters for Calculating the Fellowship Amount

For the calculation of the value of the Full Doctorate fellowship, the fellowship holder's family situation and the host country shall be considered.

4.1.1. A maximum of two dependents may be included.

4.1.2. Dependents are defined as:

a) the spouse;

b) the partner who proves a stable union, upon presentation of documents recognized/valid under current legislation.

I. The existence of a common child waives the requirement to prove a stable union, with presentation of the birth certificate being sufficient;

c) an unmarried child or stepchild under 18 years of age;

d) an unmarried child or stepchild over 18 and up to 24 years of age, enrolled in an undergraduate program in the same host country as the fellowship holder and who lives under the fellowship holder's financial dependency;

e) a child or stepchild over 18 years of age who is disabled or legally incapacitated and who lives under the fellowship holder's financial dependency;

f) a minor who lives under the financial dependency of the fellowship holder, spouse, or partner under the terms of a judicial order and who, as applicable, meets the requirements set forth in items "c," "d," or "e."

4.1.3. When both spouses are beneficiaries of Full Doctorate fellowships from CNPq, only one of them shall be entitled to the dependent-related allowance, as well as to health insurance, transportation aid, and maintenance costs benefits.

4.1.3.1. When the fellowships begin simultaneously, the couple must indicate which fellowship the dependents, if any, will be linked to.

4.1.3.2. When the fellowships end at different times, the dependents may be linked to the other fellowship for the remaining period. Such linkage does not exempt the fellowship holders from their obligation to return to Brazil upon completion of the fellowship of the spouse who continues pursuing his or her studies.

4.1.4. When one spouse receives a Full Doctorate fellowship from CNPq and the other receives a fellowship from any agency, only the CNPq fellowship holder shall be entitled to the travel allowance.

4.1.5. A fellowship holder who marries a foreign national shall be entitled to include the spouse as a dependent, upon presentation of:

a) a marriage certificate or document issued/legalized by the competent consular authority; and

b) a declaration stating the absence of an employment relationship and that the foreign spouse does not receive a fellowship.

4.2. Calculation of the Fellowship Amount

4.2.1. To the basic value of the Full Doctorate fellowship shall be added the amounts resulting from the family situation, which shall only be implemented upon the candidate's declaration that the dependents will in fact accompany the fellow during

the fellowship term for an uninterrupted period equal to or greater than nine months.

4.2.2. CNPq shall make payment of the corresponding additional amount as of the date informed by the fellowship holder for the dependent's travel, which must be evidenced by submission of a scanned copy of the travel proof within a maximum period of 60 days.

4.2.3. After implementation, the fellowship amount may be changed due to changes in the family situation or by determination of CNPq. It is the fellowship holder's obligation to inform CNPq of any and all changes in his or her family situation.

4.2.3.1. When the change results in an increase in the fellowship amount, its implementation shall be retroactive to the date of occurrence of the fact, and the fellowship holder shall have a period of 90 days to submit the marriage and birth certificates to CNPq.

4.2.3.2. When the change results in a decrease in the fellowship amount, its implementation shall be retroactive to the date of occurrence of the fact that gave rise to it, upon the fellowship holder's declaration or upon verification by CNPq of the change in the family situation.

4.2.4. Any functional or employment relationship of any dependent, even if obtained abroad, shall result in the exclusion of such dependent from the calculation of the fellowship amount.

5. Mandatory documents for Application:

a) Electronic Proposal Submission Form;

b) Curriculum vitae registered on the Lattes Platform;

c) Curriculum vitae of the advisor abroad;

d) Research Project;

e) Statement of agreement by the advisor abroad with the execution of the proposed project;

f) Formal acceptance from the host institution, issued by the body responsible for admitting the applicant, including information on the language and minimum proficiency level required for the doctorate;

g) Proof of proficiency in the language to be used in the doctoral program, as required by the host institution;

h) Academic transcript from the applicant's most recent stage of education (undergraduate or master's degree).

6. Selection Criteria

6.1. Applicants will be selected based on their curriculum vitae, the curriculum vitae of the advisor abroad, the merit of the proposal, the international standing of the research group abroad, and ranked in comparison with other candidates.

6.2. The alignment of the project with the strategic areas defined by the CNPq Deliberative Council or with a specific program, as well as the existence of an employment relationship of the applicant in Brazil, are favorable factors for the granting of the fellowship.

7. Documents to be obtained by the fellowship holder by the application submission deadline and kept on file until the process is concluded:

– Valid permanent residence status in Brazil, in the case of a foreign national.

8. Monitoring and Evaluation

8.1. The fellowship holder's performance shall be evaluated annually. Continuation of the fellowship is conditional upon submission to CNPq of the documents listed in the following subitem at least 60 days prior to the end

of each fellowship year, as mentioned in the benefits letter sent to the fellowship holder.

8.2. Documents required for monitoring and evaluation, to be submitted to CNPq through CNPq's electronic platform, under penalty of cancellation of the fellowship:

a) Technical report on the activities carried out;

b) Work plan for the subsequent period;

c) Doctoral academic transcript, when applicable; and

d) Confidential assessment by the advisor abroad regarding the fellowship holder's performance, together with approval of the work plan for the next period, using the specific form.

8.3. If the fellowship holder's performance is deemed unsatisfactory, the fellowship may be cancelled.

9. Field Research

9.1. Purpose

To support CNPq Full Doctorate Abroad fellowship holders who need to carry out data collection and processing or partial development of their dissertation.

9.2. Requirements and Conditions

The need for field research must be provided for in the initial work plan. Cases not foreseen in the original proposal, when duly justified and with the consent of the advisor abroad, will be decided by the CNPq Board of Directors.

9.3. Duration

Up to 12 months if conducted in Brazil, and up to six months if conducted abroad.

9.3.1. The total duration of the doctoral fellowship, including the field research period, may not exceed 48 months.

9.4. Benefits:

a) Transportation aid, in accordance with the approved work plan;

b) Monthly stipend:

I. Field research lasting up to three months: maintenance of payment of the originally granted fellowship stipend;

II. Field research lasting more than three months: payment of a monthly stipend equivalent to a domestic doctoral fellowship in the country where the field research is conducted.

9.5. Documents required to request Field Research, to be submitted to CNPq through its electronic platform at least 60 days prior to the planned travel date:

a) Schedule of activities to be carried out, including travel dates;

b) Letter from the host institution agreeing to the implementation of the proposed plan at its facilities, when applicable;

c) Consent of the advisor abroad.

10. Extension of the Fellowship

Any requests for extension must be submitted through a specific online form at least 60 days before the end

of the fellowship period.

11. Mandatory documents for closing the process:

– Specific: scanned copy of the certificate or diploma attesting to the award of the degree.

12. Junior Postdoctoral Fellowship in Brazil

A CNPq Full Doctorate Abroad fellow with no employment relationship in Brazil shall be given priority in the award of a Junior Postdoctoral Fellowship in Brazil, subject to the Fellowship Application Calendar and the criteria and requirements of the modality set forth in the relevant specific regulation.

Annex V

Technological Development and Innovation Abroad (Junior – DEJ) and Senior – DES)

1. Purpose

To support the participation of specialists, technologists, and technical-scientific personnel in the development of research projects, studies, training, and capacity-building at centers of excellence abroad, through internships and courses.

2. Requirements and Conditions for the Applicant

a) be Brazilian or a foreign national with regular migratory status in Brazil;

b) have academic training compatible with the level and purpose of the internship or course;

c) have knowledge of the language used in the course/host institution; and

3. Duration

Maximum of 12 months.

4. Benefits:

a) Monthly stipends, calculated in accordance with the Table of Values for Fellowships Abroad;

b) Transportation aid;

c) Maintenance costs, except for applicants residing abroad;

d) Health Insurance.

5. Minimum eligibility criteria

DEJ – Professional holding a higher education degree.

DES – Professional holding a higher education degree with at least five years of proven experience in research, development, or innovation activities, or who, even without the required length of experience and as provided for in a specific call, demonstrates outstanding scientific and technological production.

6. Mandatory Documents for application:

a) Electronic Proposal Submission Form;

b) Curriculum vitae registered on the Lattes Platform;

c) Internship work plan or course program;

d) Letter of acceptance from the host institution abroad; and

e) Document evidencing proficiency in the language required for the performance of the activities, or a letter from the host institution certifying that the applicant has sufficient knowledge of the language to carry out the activities.

7. Selection criteria

Applicants will be selected based on their curriculum vitae, the merit of the proposal, and the benefits and results that may arise from the training abroad.

8. Document to be obtained by the fellowship holder by the proposal submission deadline and kept on file until the process is closed:

– Documentary proof of regular migratory status in Brazil, in the case of a foreign researcher.

9. Mandatory documents for closing the process:

– Specific: statement from the host institution abroad certifying completion of the activities provided for in the work plan, or certificate of course completion.

Annex VI

Professional Master's Degree Abroad (MPE)

1. Purpose

To train professionals abroad at the master's level, at institutions of excellence focused on quality, entrepreneurship, and competitiveness in areas of knowledge considered to be at the forefront of scientific and technological development and in strategic areas defined by CNPq.

2. Requirements and Conditions for the Applicant:

a) be Brazilian or a foreign national with permanent residence status in Brazil;

b) have completed an undergraduate degree program;

c) not hold a doctoral degree;

3. Duration

3.1. Up to 12 months, with extensions permitted, provided that the total duration does not exceed 24 months.

3.2. In the event of childbirth or adoption occurring during the fellowship period and formally reported by the fellowship holder to CNPq, the fellowship term may be extended for up to four months.

4. Benefits:

a) Monthly stipends, in accordance with the Table of Values for Fellowships Abroad;

b) Transportation aid;

I. CNPq shall deposit, into the fellowship holder's bank account in Brazil, the amount corresponding to the outbound transportation aid;

II. The transportation aid corresponding to the return to Brazil shall be requested by the fellowship holder from the Graduate Studies and Research Abroad Fellowship Service (SEBPE), by email at sebpe@cnpq.br , at least 30 days prior to the scheduled travel date;

c) Maintenance costs;

d) Health Insurance.

e) Tuition Fees.

5. Mandatory documents for application:

a) Electronic Proposal Submission Form;

b) The applicant's curriculum vitae registered on the Lattes Platform;

c) Curriculum vitae of the advisor abroad;

d) Undergraduate degree completion certificate;

e) Work Plan;

f) Statement of agreement from the advisor abroad with the execution of the proposed work plan;

g) Proof of proficiency in the language required by the host institution; and

h) Formal acceptance from the host institution, issued by the body responsible for the applicant's admission, including information on the language and minimum proficiency level required for the master's program and, where applicable, the amount of tuition fees during the fellowship period.

6. Selection criteria

6.1. Applicants will be selected based on their curriculum vitae, the curriculum vitae of the advisor abroad, the merit of the proposal, and the technical-scientific quality of the host institution, and ranked in comparison with other applicants.

6.2. The existence of an employment relationship in Brazil, as well as exemption from tuition fees, are favorable factors for the granting of the fellowship.

7. Extension of the Fellowship

7.1. Any requests for extension must be submitted through a specific online form at least 60 days prior to the end of the fellowship term.

8. Mandatory documents for closing the process:

Specific: scanned copy of the certificate or diploma evidencing award of the degree.

Annex VII

POWER OF ATTORNEY

(TEMPLATE)

GRANTOR:

_____ _____

(Name) (Nationality)

_____ holder of identity card no. _____, _____ issued on ___/___/___

(Marital Status) (State)

registered with the CPF/MF under no. _____, resident and domiciled at _____

_____

(Full Address)

ATTORNEY-IN-FACT:

_____ _____

(Name) (Nationality)

_____ holder of identity card no. _____, _____ issued on ___/___/___

(Marital Status) (State)

registered with the CPF/MF under no. _____, resident and domiciled at _____

_____

(Full Address)

POWERS:

The Grantor hereby grants the Attorney-in-Fact specific powers to resolve matters related to the benefit granted to the Grantor in the records of CNPq Process No. _____, before the National Council for Scientific and Technological Development, as well as before any public or private body or entity, including the Federal Court of Accounts (TCU), as well as the special powers to settle, withdraw, waive the right on which such benefit is based, receive funds and give receipt and discharge, enter into commitments, file appeals, amend declarations, receive notices, service of process and summons of any kind, retain legal counsel for the specific purpose of this power of attorney, without the power of substitution, and to perform all acts necessary for the proper and faithful performance of this mandate.

_____, ___ of _____, _____.

(Place and Date)

_____

(Signature)

NOTE: Signature must be notarized.

[1] Amended by RN-029/2018 of 09/12/2018, published in the Federal Official Gazette (DOU) of 09/13/2018, Section 1, page 13.

[2] Added by PO-1182/2022 of 12/26/2022, published in the Federal Official Gazette (DOU) of 12/28/2022, Section 1, page 28.

Read in full

Permanent link to the regulation

http://www.cnpq.br/web/gue

I, Raphael Michels Fantinato de Moura, telephone number 415 425-2508, mailing address P.O. Box 90487, San Diego, CA 92169, certify that the professional translation of this document from Portuguese to English has been performed by myself, a qualified translator fluent in both languages, and that the following is an accurate and complete translation of the document.

Raphael Michels Fantinato de Moura. **Date: January 14, 2026.**

RN-007/2018

BOLSAS NO EXTERIOR

Estabelece as normas gerais e específicas para as modalidades de bolsas no exterior: Estágio Sênior (ESN), Pós-Doutorado no Exterior (PDE), Doutorado Sanduíche no Exterior (SWE), Doutorado Pleno no Exterior (GDE), Desenvolvimento Tecnológico e Inovação no Exterior (Junior - DEJ) e (Sênior - DES), e Mestrado Profissional no Exterior (MPE).

O Presidente do CONSELHO NACIONAL DE DESENVOLVIMENTO CIENTÍFICO E TECNOLÓGICO - CNPq, no uso das atribuições que lhe são conferidas pelo Decreto nº 8.866 de 3 de outubro de 2016, considerando o Marco Legal de CT&I - Emenda Constitucional nº 85/2015, de 26/02/2015, Lei nº 13.243/2016, de 11/01/2016 e Decreto nº 9.283/2018, de 07/02/2018 - e em conformidade com decisão da Diretoria Executiva em sua 3ª (terceira) reunião de 1º/03/2018,

Revoga: RN-029/2012 e suas alterações

R E S O L V E:

Estabelecer as normas gerais e específicas para as seguintes modalidades de bolsas no exterior:

- Estágio Sênior (ESN)
- Pós-Doutorado no Exterior (PDE)
- Doutorado Sanduíche no Exterior (SWE)
- Doutorado Pleno no Exterior (GDE)
- Desenvolvimento Tecnológico e Inovação no Exterior (Junior - DEJ) e (Sênior - DES)
- Mestrado Profissional no Exterior (MPE)

I - NORMAS GERAIS

1. Solicitação

1.1. É feita por pesquisadores ou estudantes por meio do *Formulário Eletrônico de Propostas*, de acordo com o *Calendário de Solicitação de Bolsas* ou chamada específica.

2. Julgamento

2.1. O julgamento e a classificação das propostas são feitos nas seguintes etapas:

a) análise pela área técnica;

b) análise por consultores *ad hoc*, quando for o caso;

c) análise comparativa de mérito e classificação das propostas por Comitês de Assessoramento ou Julgadores;

d) decisão final pela Diretoria, em função da disponibilidade financeira do CNPq.

2.2. O parecer emitido pelo Comitê de Assessoramento ou Julgador deve levar em consideração os seguintes aspectos:

a) o mérito da proposta;

b) os pareceres da área técnica e dos consultores *ad hoc*, quando for o caso;

c) as especificidades das modalidades.

2.3. Os currículos a serem considerados no julgamento serão aqueles disponíveis na Plataforma Lattes.

3. Concessão

3.1. Os resultados dos julgamentos serão divulgados no Diário Oficial da União (DOU), na página do CNPq na Internet e por meio de notificação eletrônica ao candidato.

3.2. Do resultado, o candidato poderá interpor recurso em formulário *online* específico, disponível na Plataforma Eletrônica do CNPq, no prazo de 10 dias corridos, após a publicação do resultado no DOU e na página do CNPq.

3.3. As concessões de bolsa no exterior requerem que o beneficiário possua aceite da instituição de execução do projeto para o desenvolvimento de sua proposta.

3.4. Para as propostas aprovadas, a comunicação do resultado do julgamento incluirá o *link* de acesso ao *Formulário de Dados Complementares* e ao *Termo de Outorga*.

3.4.1. Após o recebimento do *Formulário de Dados Complementares* e do *Termo de Outorga*, o CNPq enviará a *Carta de Benefícios* ao endereço eletrônico do bolsista cadastrado em seu Currículo Lattes, com chave para consulta eletrônica da autenticidade do documento.

3.4.2. O candidato deverá emitir *Procuração* (modelo - anexo VII) outorgando poderes a pessoa no Brasil, para representá-lo nas questões relacionadas à bolsa do CNPq. O documento deverá ser mantido sob a guarda do procurador do bolsista.

3.5. A vigência da bolsa será determinada pelo período, em meses, aprovado pelo CNPq e deverá obedecer ao disposto no *Calendário para Solicitação de Bolsas* ou em chamada ou programa específicos, publicados no Portal do CNPq. Expirado o prazo para implementação, a concessão será cancelada.

3.6. Eventuais pedidos de prorrogação da bolsa deverão ser feitos antes do término da concessão, observado o prazo estabelecido na norma específica da modalidade.

3.7. Bolsas concedidas por intermédio de cotas institucionais ou a coordenadores terão procedimentos de implementação diferenciados, a serem definidos em chamadas específicas.

4. Pagamento das Bolsas

4.1. Os valores das mensalidades serão fixados pelo CNPq em norma específica Tabela de Valores de Bolsas no Exterior.

4.2. Para o pagamento dos benefícios iniciais é necessário que o candidato aprovado tenha preenchido e enviado eletronicamente ao CNPq o *Formulário de Dados Complementares* e o *Termo de Outorga*, no prazo estipulado na comunicação de aprovação da concessão.

4.3. O pagamento ao bolsista será realizado trimestralmente, mediante crédito no exterior, conforme cronograma vinculado ao início da vigência da bolsa. O cronograma é publicado no Calendário de Solicitação de Bolsas no Exterior na página do CNPq.

4.3.1. Ao bolsista que resida ou encontra-se no Brasil, o CNPq pagará, de acordo com a modalidade da bolsa, o auxílio-instalação, o seguro-saúde e o auxílio-deslocamento, por meio de depósito em conta corrente pessoal, a ser informada, no momento da aceitação da bolsa, no formulário *online Dados Complementares*. Os demais benefícios, tais como: as três primeiras mensalidades da bolsa e outros auxílios específicos de cada modalidade serão creditados no cartão bolsista antecipadamente ao início da vigência da bolsa.

4.3.1.1. No caso dos depósitos realizados em conta corrente, o valor será creditado em moeda corrente brasileira, adotando-se a cotação de câmbio (compra) divulgada pelo Banco Central referente ao dia imediatamente anterior ao da autorização do pagamento pelo CNPq. No caso dos valores transferidos ao cartão bolsista, estes serão depositados em moeda corrente do país de destino ou, quando não disponível, em dólar americano.

4.3.1.2. O CNPq não se responsabiliza por eventuais variações cambiais e impostos, ficando o beneficiário responsável pelas transações necessárias no Brasil de troca por moeda estrangeira.

4.3.1.3. No Brasil, o cartão bolsista será encaminhado via correio ao endereço de correspondência cadastrado no Currículo Lattes, sendo a entrega condicionada a assinatura de Aviso de Recebimento (A.R.).

4.3.1.4. O bolsista que se encontre residindo no país de destino, quando da aprovação da bolsa, não fará jus ao valor correspondente ao auxílio-deslocamento relativo ao trecho de ida e nem ao auxílio-instalação. Não haverá também a antecipação dos benefícios constantes do subitem **4.3.1**. O pagamento será creditado no cartão bolsista, o qual será encaminhado para o endereço no exterior previamente cadastrado no Currículo Lattes como endereço de correspondência.

4.4. A vigência da bolsa será determinada, preliminarmente, com base na data de início das atividades no exterior informada pelo bolsista no *Formulário de Dados Complementares*. Quando as atividades no exterior começarem até o 15º dia do mês, a vigência da bolsa iniciará no mesmo mês. Caso comecem a partir do 16º dia do mês, a vigência da bolsa iniciará no mês subsequente.

4.5. Até 30 dias após o início das atividades, o bolsista deverá enviar por meio da Plataforma eletrônica do CNPq, cópias digitalizadas dos seguintes documentos, sob pena de suspensão da bolsa:

a) comprovante de embarque;
b) comprovante de matrícula ou carta da instituição atestando o início das atividades; e
c) comprovante da contratação do seguro-saúde.

5. Benefícios da Bolsa

Os benefícios da bolsa no exterior compreendem o pagamento de mensalidades, auxílio-deslocamento, auxílio-instalação, seguro-saúde e taxas, de acordo com as especificidades de cada modalidade. Não há benefícios a dependentes, exceto para o Doutorado Pleno, conforme norma específica.

5.1. Mensalidades

As mensalidades serão calculadas conforme Tabela de Valores de Bolsas no Exterior.

5.2. Auxílio-Deslocamento

5.2.1. O CNPq depositará, na conta corrente do bolsista no Brasil, a importância respectiva ao auxílio-deslocamento destinado à aquisição da passagem aérea. Os valores do auxílio serão definidos, considerando os preços praticados pelo mercado, e fixados na Tabela de Valores de Auxílio-Deslocamento ao Exterior. Caberá ao bolsista adquirir a passagem aérea na empresa /agência de sua preferência.

5.2.1.1. Os beneficiários de bolsas com vigência igual ou inferior a seis meses receberão o valor do auxílio-deslocamento, em uma única parcela, no valor correspondente às passagens aéreas de ida e de volta.

5.2.2. Para bolsas com duração superior a seis meses, o valor do auxílio-deslocamento correspondente ao retorno ao país, será depositado juntamente com a última mensalidade.

5.2.3. Não será concedido auxílio-deslocamento de ida a bolsista residente no país onde serão executadas as atividades previstas no projeto/plano de trabalho aprovado. Nestes casos, o auxílio-deslocamento ao final da bolsa poderá ser concedido mediante solicitação do beneficiário pelo menos 60 dias antes do término da vigência da bolsa.

5.3. Auxílio-Instalação

O auxílio-instalação visa cobrir parte dos gastos pessoais do bolsista, com sua mudança para o exterior e será pago conforme Tabela de Valores de Bolsas no Exterior apenas para bolsistas que estejam residindo no Brasil.

5.4. Seguro-Saúde

O seguro-saúde destina-se à contratação de empresa que ofereça cobertura de despesas médicas e hospitalares ao bolsista e a seus dependentes no caso de Doutorado Pleno.

5.4.1. É obrigatória a contratação de seguro-saúde pelo bolsista e sua comprovação conforme subitem **4.5**, alíena c.

5.4.2. Os valores do seguro-saúde são estabelecidos na Tabela de Valores de Bolsas no Exterior.

5.4.3. Para bolsas com vigência inferior a 12 meses, o seguro-saúde será proporcional à duração da bolsa.

5.5. Taxas Escolares

As taxas escolares destinam-se à cobertura de despesas exigidas pela instituição, necessárias à efetivação da matrícula. Os valores anuais estimados deverão ser informados pelo candidato no formulário de submissão de propostas.

5.6. Taxas de Bancada

As taxas de Bancada, quando aplicável, destinam-se a custear as despesas exigidas pela instituição, para a realização das atividades de pesquisa científica e tecnológica pelo bolsista de doutorado sanduíche ou de doutorado pleno. Os valores deverão ser informados pelo candidato no formulário de submissão de propostas.

6. Complementação de Bolsa de Outras Instituições

6.1. O CNPq admite coadjuvar bolsa concedida por instituição estrangeira ou internacional, desde que previamente autorizado pelo Diretor da área.

6.2. O CNPq não complementará valores ou períodos de bolsas concedidas com recursos do Tesouro Nacional.

6.3. É permitida a simultaneidade da bolsa com remuneração temporária e/ou parcial percebida pelo bolsista a título de *Teaching* ou *Research Fellowship*, desde que o orientador do bolsista declare que tais atividades não prejudicam o andamento do curso.

7. Obrigações do Bolsista

7.1. Dedicar-se às atividades previstas no projeto ou plano de trabalho aprovado pelo CNPq, durante a vigência da bolsa.

7.1.1. A vinda do bolsista ao Brasil no período de vigência da bolsa é autorizada pelo CNPq em condições excepcionais, devidamente justificadas.

7.1.1.1. Para avaliação da solicitação, deverá ser apresentada motivação, acompanhada da documentação pertinente. Caso a duração seja superior a 15 dias, o pagamento da bolsa será suspenso.

7.2. Atuar como consultor *ad hoc* emitindo parecer sobre projeto de pesquisa, quando solicitado. O não cumprimento deste dispositivo implicará suspensão da bolsa.

7.3. Solicitar previamente autorização para qualquer mudança relativa ao projeto de pesquisa, ao plano de trabalho ou à própria bolsa, a qual será objeto de análise pelo CNPq.

7.4. Devolver ao CNPq eventuais benefícios pagos indevidamente, no prazo máximo de 30 dias.

7.5. Retornar ao Brasil, até 30 dias após o término da vigência da bolsa, e permanecer no País por período não inferior ao da vigência da bolsa.

7.5.1. A pedido do bolsista e demonstrado que sua permanência fora do País terá relevância estratégica para o desenvolvimento da Ciência, Tecnologia e Inovação no Brasil, o CNPq poderá substituir a obrigação de retorno e permanência no território brasileiro por outras que assegurem o ressarcimento do investimento feito pelo País na formação do bolsista.

7.6. Os trabalhos produzidos ou publicados, em qualquer mídia, em decorrência das atividades apoiadas pelo CNPq, deverão, necessariamente, fazer referência ao apoio recebido, com as seguintes expressões, no idioma do trabalho.
a) "O presente trabalho foi realizado com apoio do CNPq, Conselho Nacional de Desenvolvimento Científico e Tecnológico - Brasil" ou
b) "Bolsista do CNPq - Brasil".

7.7. O não cumprimento das disposições normativas, obriga o bolsista a ressarcir integralmente o CNPq de todas as despesas realizadas em seu proveito, conforme disposto na Resolução Normativa do CNPq específica de ressarcimento, sob pena de inscrição do débito decorrente na Dívida Ativa da União e no Cadastro Informativo de Créditos Não Quitados do Setor Público Federal - CADIN.

7.7.1. O CNPq poderá, excepcionalmente, conceder baixa de responsabilidade nos casos em que se configure insucesso na capacitação. Os casos de insucesso serão deliberados pelo Diretor da área.

8. Suspensão e Cancelamento

8.1. A suspensão temporária da bolsa, com posterior reativação, poderá ser solicitada, desde que respeitada sua data de

término.

8.2. A suspensão ou cancelamento da bolsa poderá ocorrer a pedido do bolsista ou de seu orientador ou, ainda, por iniciativa do CNPq, sempre devidamente justificada.

8.2.1. Caberá ao corpo técnico do CNPq, assessorado por consultores *ad hoc*, quando necessário, analisar a situação do bolsista e manifestar-se pela necessidade de suspensão ou cancelamento da bolsa. A decisão final será da competência da Coordenação-Geral.

8.3. A reativação da bolsa suspensa, quando for o caso, deve ser solicitada com antecedência mínima de 30 dias.

9. Acompanhamento, Avaliação e Encerramento

9.1. O desempenho do bolsista será acompanhado pelo CNPq mediante análise de relatórios ou outras formas de acompanhamento definidas de acordo com as especificidades da modalidade.

9.1.1. O relatório de execução do objeto deve ser apresentado pelo bolsista por intermédio da Plataforma eletrônica do CNPq até, no máximo, 60 dias após o término da bolsa.

9.2. O encerramento do processo ocorrerá quando o beneficiário:

a) encaminhar os comprovantes de passagens utilizados;

b) encaminhar os documentos específicos da modalidade exigidos para o encerramento do processo;

c) tiver o relatório de execução do objeto aprovado pelo CNPq;

d) não possuir quaisquer pendências financeiras com o CNPq, relativas ao processo; e

e) cumprir o pactuado no *Termo de Outorga*.

10. Disposições Finais

10.1. As presentes normas aplicam-se a todas as modalidades de bolsas concedidas com recursos orçamentários do CNPq. Bolsas concedidas no âmbito de convênios e acordos com outras instituições, de programas estratégicos, ou com recursos oriundos dos Fundos Setoriais poderão ter disposições diferentes.

10.1-A. Os editais de Chamadas Públicas não poderão prever equivalência de titulações diferentes, seja nos critérios de admissibilidade, seja nos critérios de julgamento.[2]

10.2. É vedado aos orientadores e/ou coordenadores conceder bolsa a cônjuge, companheiro ou parente em linha reta, colateral ou por afinidade, até o terceiro grau, inclusive.

10.3. É vedado o acúmulo de bolsa do CNPq com outra bolsa concedida com recursos do Tesouro Nacional.

10.4. Casos omissos ou excepcionais serão analisados pela Diretoria da área.

10.5. Esta Resolução Normativa entra em vigência a partir da data da sua publicação e ficam revogadas as RN-029/2012, RN-025/2013, RN-010/2013, RN-051/2014, RN-009/2014, RN-030/2015, RN-024/2015, RN-017/2015, RN-012/2015, RN-009/2015, RN-028/2016; RN-005/2017 e demais disposições em contrário.

II - NORMAS ESPECÍFICAS

Anexos:

I - Estágio Sênior

II - Pós-Doutorado no Exterior

III - Doutorado Sanduíche no Exterior

IV - Doutorado Pleno no Exterior

V - Desenvolvimento Tecnológico e Inovação no Exterior (Junior - DEJ) e (Sênior- DES)

VI - Mestrado Profissional no Exterior

III - MODELO

Anexo:

VII - Procuração

Brasília, 12 de abril de 2018.

MARIO NETO BORGES

Ref.: 01300.012551/2017-51

Publicado do DOU de 17/04/2018, Seção 1, página 13.

Anexo I

Estágio Sênior (ESN)

1. Finalidade

Propiciar ao pesquisador o desenvolvimento de projeto de pesquisa ou parte dele em instituição estrangeira.

2. Requisitos e Condições

2.1. Para o candidato:

a) ser pesquisador nível 1 do CNPq;

b) ter vínculo com instituição de pesquisa no Brasil;

2.2. Para a instituição de destino:

- ser reconhecida internacionalmente na área de interesse do candidato.

2.3. Para o pesquisador anfitrião:

- ter reconhecida competência na área do projeto.

3. Duração

- De três a seis meses.

4. Benefícios

a) Mensalidades, calculadas conforme Tabela de Valores de Bolsas no Exterior;

b) Auxílio-Deslocamento;

c) Auxílio-Instalação;

d) Seguro-Saúde.

5. Documentos indispensáveis para inscrição:

a) Formulário Eletrônico de Propostas;

b) Currículo cadastrado na Plataforma Lattes;

c) Projeto / Plano de trabalho.

6. Critérios para seleção dos candidatos

Os candidatos serão selecionados em função do seu currículo, do conceito internacional da instituição de destino, da qualidade do plano de trabalho e classificados em comparação com os demais candidatos.

7. Documentos a serem obtidos pelo bolsista até a data limite de envio das propostas e mantidos sob sua guarda até o encerramento do processo:

a) Anuência formal da instituição de destino;

b) Concordância do pesquisador anfitrião com as atividades propostas; e

c) Visto permanente no Brasil atualizado, no caso de pesquisador estrangeiro.

8. Documentos indispensáveis para encerramento do processo:

Anexo II

Pós-Doutorado no Exterior (PDE)

1. Finalidade

Possibilitar ao pesquisador a capacitação e atualização de seus conhecimentos por meio de estágio e desenvolvimento de projeto com conteúdo científico ou tecnológico inovador, em instituição no exterior.

2. Requisitos e Condições

2.1 . Para o candidato:

a) possuir o título de doutor quando da implementação da bolsa;

b) dedicar-se integralmente às atividades programadas na instituição de destino;

c) para ex-bolsista de doutorado no exterior de agência nacional, observar o tempo mínimo de permanência no Brasil exigido pela agência;

d) cumprir interstício mínimo de 3 anos entre dois Pós-Doutorados no Exterior com bolsa do CNPq e

e) ser brasileiro ou estrangeiro com visto permanente no Brasil.

2.2. Para a instituição de destino:

- ser reconhecida internacionalmente na área de interesse do candidato.

2.3. Para o orientador:

- ter reconhecida competência na área do projeto.

3. Duração

3.1. A duração da bolsa é de seis a 12 meses, sendo permitida prorrogação, desde que não ultrapasse o tempo total de 24 meses.

3.2. No caso de parto ou adoção ocorrido durante o período da bolsa formalmente comunicado pela bolsista ao CNPq, a vigência da bolsa será prorrogada por até quatro meses.

4. Benefícios

a) Mensalidades, calculadas conforme Tabela de Valores de Bolsas no Exterior;

b) Auxílio-Deslocamento;

c) Auxílio-Instalação e

d) Seguro-Saúde.

5. Documentos indispensáveis para inscrição:

a) Formulário Eletrônico de Propostas;

b) Currículo do candidato cadastrado na Plataforma Lattes;

c) Curriculo do orientador;

d) Projeto de pesquisa;

e) Concordância do orientador com as atividades propostas; e

f) Confirmação por parte do orientador de que o conhecimento do idioma do país de destino é suficiente para o desenvolvimento das atividades previstas.

6. Critérios para seleção dos candidatos

Os candidatos serão selecionados em função do seu currículo, do currículo do orientador, do conceito internacional da instituição de destino e da qualidade do projeto e classificados em comparação com os demais candidatos.

7. Documento a ser obtido pelo bolsista até a data limite de envio das propostas e mantido sob sua guarda até o encerramento do processo:

- visto permanente no Brasil atualizado, no caso de pesquisador estrangeiro.

8. Prorrogação da Bolsa

Eventuais pedidos de prorrogação devem ser solicitados em específico, até 60 dias antes do término da bolsa.

9. Documentos indispensáveis para encerramento do processo:

- Específicos: manifestação do orientador sobre as atividades desenvolvidas pelo bolsista.

Anexo III

Doutorado Sanduíche no Exterior (SWE)

1. Finalidade

Apoiar aluno formalmente matriculado em curso de doutorado no Brasil que comprove qualificação para usufruir, no exterior, da oportunidade de aprofundamento teórico, coleta e/ou tratamento de dados ou desenvolvimento parcial da parte experimental de sua tese a ser defendida no Brasil.

2. Requisitos e Condições

2.1. Para o candidato:

a) estar formalmente matriculado em curso de doutorado no Brasil reconhecido pela CAPES;

b) não ser aposentado;

c) ter conhecimento do idioma utilizado na instituição de destino;

d) ter anuência do coordenador do curso de pós-graduação e dos orientadores no País e no exterior e

e) ser brasileiro ou estrangeiro com visto permanente no Brasil.

2.2. Para o orientador da instituição de destino:

- ser pesquisador com produção acadêmica consolidada e relevante para desenvolvimento complementar da tese de doutorado.

3. Duração

3.1. De três a 12 meses. É permitida a prorrogação, respeitado o limite máximo de duração da bolsa.

3.1.1. O prazo total da bolsa de Doutorado no País, somado ao período do Doutorado Sanduíche no Exterior, não poderá ultrapassar 48 meses.

3.1.2. Nos casos de cotutela entre as Universidades brasileira e estrangeira, previstos em acordos específicos, o período poderá se estender até 24 meses no exterior. Entende-se por cotutela a situação em que o diploma de doutorado é emitido pelas duas instituições participantes.

4. Benefícios:

a) Mensalidades, calculadas conforme Tabela de Valores de Bolsas no Exterior;

b) Auxílio-Deslocamento;

c) Auxílio-Instalação;

d) Seguro-Saúde;

e) Taxas Escolares e

f) Taxas de Bancada.

5. Documentos indispensáveis para inscrição:

a) Formulário Eletrônico de Propostas;

b) Currículo do candidato à bolsa cadastrado na Plataforma Lattes;

c) Currículo do orientador no exterior;

d) Plano de trabalho e/ou projeto de pesquisa para o período da bolsa;

e) Anuência formal do coordenador do curso de pós-graduação no qual está matriculado;

f) Anuência formal da instituição de destino emitida pelo órgão responsável pela admissão do candidato, incluindo informação sobre o idioma e nível de proficiência mínimo exigido;

g) Concordância dos orientadores no Brasil e no exterior com as atividades propostas e

h) Confirmação por parte do orientador no exterior de que o candidato tem conhecimento suficiente do idioma exigido para o desenvolvimento das atividades previstas.

6. Critérios para seleção dos candidatos

Os candidatos serão selecionados em função de seu currículo, do currículo do orientador no exterior, do mérito da proposta, do conceito internacional do grupo de pesquisa no exterior e classificados em comparação com os demais candidatos.

7. Documento a ser obtido pelo bolsista antes da assinatura do Termo de Outorga e mantido sob sua guarda até o encerramento do processo:

- visto permanente no Brasil atualizado, no caso de pesquisador estrangeiro.

8. Prorrogação da Bolsa

Eventuais pedidos de prorrogação devem ser solicitados em formulário *online* específico, até 30 dias antes do término da bolsa.

9. Documentos indispensáveis para encerramento do processo:

- Específicos: avaliações dos orientadores no País e no exterior.

Doutorado Sanduíche no Exterior (SWE)

1. Finalidade

Apoiar aluno formalmente matriculado em curso de doutorado no Brasil que comprove qualificação para usufruir, no exterior, da oportunidade de aprofundamento teórico, coleta e/ou tratamento de dados ou desenvolvimento parcial da parte experimental de sua tese a ser defendida no Brasil.

2. Requisitos e Condições

2.1. Para o candidato:

a) estar formalmente matriculado em curso de doutorado no Brasil reconhecido pela CAPES;

c) ter conhecimento do idioma utilizado na instituição de destino;

d) ter anuência do coordenador do curso de pós-graduação e dos orientadores no País e no exterior e

e) ser brasileiro ou estrangeiro com visto permanente no Brasil.

2.2. Para o orientador da instituição de destino:

- ser pesquisador com produção acadêmica consolidada e relevante para desenvolvimento complementar da tese de doutorado.

3. Duração

3.1. De 3 a 12 meses. É permitida a prorrogação, respeitado o limite máximo de duração da bolsa.

3.1.1. O prazo total da bolsa de Doutorado no País, somado ao período do Doutorado Sanduíche no Exterior, não poderá ultrapassar 48 meses.

3.1.1.1 No caso de não obtenção do título de doutor por abandono ou desistência de própria iniciativa, sem motivo de força maior ou pelo não cumprimento das disposições normativas, os valores investidos no Doutorado Sanduíche no Exterior serão somados ao de bolsa de Doutorado no País, para efeito de ressarcimento ao CNPq.

3.1.2. Nos casos de cotutela entre as Universidades brasileira e estrangeira, previstos em acordos específicos, o período poderá se estender até 24 meses no exterior. Entende-se por cotutela a situação em que o diploma de doutorado é emitido pelas duas instituições participantes.

4. Benefícios:

a) Mensalidades, calculadas conforme Tabela de Valores de Bolsas no Exterior;

b) Auxílio-Deslocamento;

c) Auxílio-Instalação;

d) Seguro-Saúde;

e) Taxas Escolares e

f) Taxas de Bancada.

5. Documentos indispensáveis para inscrição:

a) Formulário Eletrônico de Propostas;

b) Currículo do candidato à bolsa cadastrado na Plataforma Lattes;

c) Currículo do orientador no exterior;

d) Plano de trabalho e/ou projeto de pesquisa para o período da bolsa;

e) Anuência formal do coordenador do curso de pós-graduação no qual está matriculado;

f) Anuência formal da instituição de destino emitida pelo órgão responsável pela admissão do candidato, incluindo informação sobre o idioma e nível de proficiência mínimo exigido;

g) Concordância dos orientadores no Brasil e no exterior com as atividades propostas e

h) Confirmação por parte do orientador no exterior de que o candidato tem conhecimento suficiente do idioma exigido para o desenvolvimento das atividades previstas.

6. Critérios para seleção dos candidatos

Os candidatos serão selecionados em função de seu currículo, do currículo do orientador no exterior, do mérito e relevância da proposta, do conceito internacional do grupo de pesquisa no exterior e classificados em comparação com os demais candidatos.

7. Documento a ser obtido pelo bolsista antes da assinatura do Termo de Outorga e mantido sob sua guarda até

o encerramento do processo:

- visto permanente no Brasil atualizado, no caso de pesquisador estrangeiro.

8. Prorrogação da Bolsa

Eventuais pedidos de prorrogação devem ser solicitados em formulário *online* específico, até 30 dias antes do término da bolsa.

9. Documentos indispensáveis para encerramento do processo:

- Específicos: avaliações dos orientadores no País e no exterior.[1]

Anexo IV

Doutorado Pleno no Exterior (GDE)

1. Finalidade

Formar doutores no exterior em instituições de reconhecido nível de excelência, em áreas do conhecimento consideradas de vanguarda científico-tecnológica e naquelas estratégicas definidas pelo Conselho Deliberativo do CNPq.

2. Requisitos e Condições para o Candidato:

a) ser brasileiro ou estrangeiro com visto permanente no Brasil;

b) ter proficiência em idioma requerido para o curso;

3. Duração

3.1. A duração da bolsa é até 48 meses.

3.2. No caso de parto ou adoção ocorrido durante o período da bolsa formalmente comunicado pela bolsista ao CNPq, a vigência da bolsa será prorrogada por até quatro meses.

4. Benefícios:

a) Mensalidades, calculadas conforme disposições dos subitens 4.1 e 4.2 adiante e Tabela de Valores de Bolsas no Exterior;

b) Auxílio-Deslocamento;

I. O CNPq depositará, na conta corrente do bolsista no Brasil, a importância correspondente ao auxílio deslocamento de **ida** do bolsista e de até dois dependentes, quando for o caso.

II. O valor do auxílio-deslocamento correspondente ao regresso ao Brasil (incluindo até dois dependentes) deverá ser solicitado pelo bolsista ao Serviço de Bolsas de Pós-Graduação e Pesquisa no Exterior - SEBPE, e-mail: sebpe@cnpq.br, com antecedência mínima de 30 dias da data prevista da viagem.

III. O bolsista que vier a se casar durante a vigência da bolsa não terá direito ao auxílio- deslocamento de ida do dependente, mas apenas ao de volta, por ocasião de seu retorno ao Brasil.

c) Auxílio-Instalação;

d) Seguro-Saúde;

I. Quando ocorrer a inclusão de dependente em bolsa de Doutorado Pleno já implementada, o seguro-saúde será pago proporcionalmente ao período faltante para o final da vigência.1

e)Taxas Escolares;

f)Taxas de Bancada; e

g)Pesquisa de campo, quando prevista na proposta original, pelo período máximo de 12 meses, se realizada no Brasil, e de seis meses no exterior.

4.1. Parâmetros para Cálculo do Valor da Bolsa

Para cálculo do valor da bolsa de Doutorado Pleno serão considerados a situação familiar do bolsista e o país de destino.

4.1.1. Poderão ser incluídos no máximo dois dependentes.

4.1.2. Consideram-se dependentes:

a) o cônjuge;

b) o companheiro que comprove a união estável, mediante a apresentação de documentos reconhecidos/válidos na legislação vigente.

I. A existência de filho em comum dispensa a comprovação de união estável, sendo suficiente a apresentação da certidão de nascimento.

c) filho ou enteado solteiro menor de 18 anos;

d) filho ou enteado solteiro maior de 18 anos e até 24 anos, matriculado em curso de graduação no mesmo país de destino do bolsista e que viva sob a dependência econômica deste;

e) filho ou enteado maior de 18 anos, inválido ou incapaz, assim considerado em lei, que viva sob a dependência econômica do bolsista;

f) menor que viva sob a dependência econômica do bolsista, cônjuge ou companheiro sob o amparo de termo judicial que preencha, no que couber, os requisitos mencionados em "c", "d" ou "e".

4.1.3. Quando os cônjuges forem beneficiários de bolsas de Doutorado Pleno do CNPq, somente a um deles caberá o adicional relativo a dependentes, bem como os valores do seguro-saúde, auxílio-deslocamento e auxílio-instalação.

4.1.3.1. Quando as bolsas tiverem inícios simultâneos, o casal deve se manifestar a qual das bolsas se vincularão os dependentes, se houver.

4.1.3.2. Quando as bolsas tiverem términos diferentes, os dependentes poderão ser vinculados à outra bolsa, para o período restante. Essa vinculação não isenta os bolsistas de seu compromisso de retorno ao Brasil, ao término da bolsa do cônjuge que permaneça desenvolvendo seus estudos.

4.1.4. Quando um dos cônjuges receber bolsa de Doutorado Pleno do CNPq e o outro receber bolsa de qualquer agência, somente o bolsista do CNPq terá direito ao auxílio-deslocamento.

4.1.5. O bolsista que contrair matrimônio com estrangeiro terá direito a sua inclusão como dependente, mediante apresentação de:

a) certidão de casamento ou documento emitido/legalizado pelo órgão consular competente; e

b) declaração de ausência de vínculo empregatício e de não recebimento de bolsa pelo cônjuge estrangeiro.

4.2 . Cálculo do Valor da Bolsa

4.2.1. Ao valor básico da bolsa de Doutorado Pleno serão acrescidos os valores advindos da situação familiar, que só serão implementados mediante declaração do candidato de que os dependentes efetivamente o acompanharão durante

a vigência da bolsa, por um período ininterrupto igual ou superior a nove meses.

4.2.2. O CNPq efetuará o pagamento do acréscimo correspondente a partir da data informada pelo bolsista para deslocamento do dependente, que deverá ser comprovado mediante a apresentação de cópia digitalizada do comprovante da viagem no prazo máximo de 60 dias.

4.2.3. Após sua implementação, o valor da bolsa poderá ser alterado em função de mudanças na situação familiar ou por determinação do CNPq. É obrigação do bolsista comunicar ao CNPq toda e qualquer alteração na sua situação familiar.

4.2.3.1. Quando a variação implicar acréscimo ao valor da bolsa, sua implementação retroagirá à data da ocorrência do fato, tendo o bolsista o prazo de 90 dias para enviar ao CNPq as certidões de casamento e nascimento.

4.2.3.2. Quando a variação implicar decréscimo do valor da bolsa, sua implementação retroagirá à data da ocorrência do fato que lhe tiver dado causa, mediante declaração do bolsista ou constatação pelo CNPq da alteração da situação familiar.

4.2.4. A vinculação funcional ou empregatícia de qualquer dos dependentes, mesmo que adquirida no exterior, resultará na exclusão deste do cômputo do valor da bolsa.

5. Documentos Indispensáveis para inscrição:

a) Formulário Eletrônico de Propostas;
b) Currículo cadastrado na Plataforma Lattes;
c) Currículo do orientador no exterior;
d) Projeto de Pesquisa;
e) Concordância do orientador no exterior com a execução do projeto proposto;
f) Anuência formal da instituição de destino emitida pelo órgão responsável pela admissão do candidato, incluindo informação sobre o idioma e nível de proficiência mínimo exigido para o doutorado;
g) Comprovante de proficiência no idioma a ser utilizado no doutoramento exigido pela instituição de destino;
h) Histórico Escolar da última etapa de formação do candidato (graduação ou mestrado).

6. Critérios para seleção dos candidatos.

6.1. Os candidatos serão selecionados em função de seu currículo, do currículo do orientador no exterior, do mérito da proposta, do conceito internacional do grupo de pesquisa no exterior e classificados em comparação com os demais candidatos.

6.2. A inserção do projeto nas áreas estratégicas definidas pelo Conselho Deliberativo do CNPq ou em Programa específico, bem como a existência de vínculo empregatício do candidato no Brasil são fatores favoráveis à concessão da bolsa.

7. Documento a ser obtido pelo bolsista até a data limite de envio das propostas e mantido sob sua guarda até o encerramento do processo:

- Visto permanente no Brasil atualizado, no caso de estrangeiro.

8. Acompanhamento e Avaliação

8.1. A avaliação do bolsista será efetuada anualmente. A manutenção da bolsa fica condicionada à apresentação ao CNPq dos documentos relacionados no subitem seguinte, no prazo mínimo de 60 dias antes do término de cada ano de

bolsa, conforme mencionado na carta de benefícios encaminhada ao bolsista.

8.2. Documentos indispensáveis para o acompanhamento e a avaliação a serem enviados ao CNPq por meio da Plataforma eletrônica do CNPq, sob pena de cancelamento da bolsa:

a) Relatório técnico das atividades desenvolvidas;

b) Plano de trabalho para o período subsequente;

c) Histórico Escolar do doutorado, quando houver; e

d) Parecer sigiloso do orientador no exterior, sobre o desempenho do bolsista, e concordância com o plano de trabalho para o próximo período, em formulário específico.

8.3. Caso o desempenho do bolsista seja considerado insatisfatório, a bolsa poderá ser cancelada.

9. Pesquisa de Campo

9.1. Finalidade

Apoiar bolsista de Doutorado Pleno no Exterior do CNPq que necessite proceder à coleta e tratamento de dados ou ao desenvolvimento parcial de sua tese.

9.2. Requisitos e Condições

Estar prevista no plano de trabalho inicial a necessidade de pesquisa de campo. Casos não previstos na proposta original, devidamente justificados e com anuência do orientador no exterior, serão deliberados pela Diretoria do CNPq.

9.3. Duração

Até 12 meses se realizada no Brasil, e até seis meses se realizada no exterior.

9.3.1. A duração total da bolsa de doutorado, incluindo o período da pesquisa de campo, não poderá ultrapassar 48 meses.

9.4. Benefícios:

a) Auxílio-Deslocamento conforme plano de trabalho aprovado.

b) Mensalidade:

I. pesquisa de campo com duração de até três meses: manutenção do pagamento da mensalidade da bolsa concedida;

II. pesquisa de campo com duração superior a três meses: pagamento da mensalidade equivalente à bolsa de doutorado no país em que será realizada a pesquisa de campo.

9.5. Documentos indispensáveis para solicitação da Pesquisa de Campo a serem enviados ao CNPq, por meio da Plataforma eletrônica do CNPq, no prazo mínimo de 60 dias da data prevista para a viagem:

a) Cronograma das atividades a serem desenvolvidas, incluindo as datas das viagens;

b) Carta da instituição de destino, concordando com o desenvolvimento do plano proposto em suas instalações, quando pertinente;

c) Anuência do orientador no exterior.

10. Prorrogação da Bolsa

Eventuais pedidos de prorrogação deverão ser solicitados em formulário *online* específico 60 dias antes do término da

bolsa.

11. Documentos indispensáveis para encerramento do processo:

- Específicos: cópia digitalizada do certificado ou diploma de obtenção do título.

12. Bolsa de Pós-Doutorado Júnior no País

O bolsista de Doutorado Pleno no Exterior do CNPq, sem vínculo empregatício no Brasil, terá prioridade na concessão de bolsa de Pós-Doutorado Júnior no País, observados o Calendário de Solicitação de Bolsas e os critérios e requisitos da modalidade dispostos em norma específica.

Anexo V

Desenvolvimento Tecnológico e Inovação no Exterior (Junior - DEJ) e Sênior - DES)

1. Finalidade

Apoiar a participação de especialistas, tecnólogos, pessoal técnico-científico para o desenvolvimento de projetos de pesquisa, estudos, treinamentos e capacitação em instituições de excelência no exterior, por meio da realização de estágios e cursos.

2. Requisitos e Condições para o Candidato

a) ser brasileiro ou estrangeiro com situação migratória regular no Brasil;

b) ter formação compatível com o nível e a finalidade do estágio ou curso;

c) ter conhecimento do idioma utilizado no curso/instituição de destino; e

3. Duração

Máxima de 12 meses.

4. Benefícios:

a) Mensalidades, calculadas conforme Tabela de Valores de Bolsas no Exterior;

b) Auxílio-Deslocamento;

c) Auxílio-Instalação, exceto a candidatos residentes no exterior;

d) Seguro-Saúde.

5. Critérios mínimos para enquadramento

DEJ - Profissional de nível superior.

DES - Profissional de nível superior com, no mínimo, cinco anos de efetiva experiência em atividades de pesquisa, desenvolvimento ou inovação, ou que apresente, mesmo não tendo o tempo de experiência exigido e conforme previsto em chamada específica, produção científica e tecnológica de destaque.

6. Documentos indispensáveis para inscrição:

a) Formulário Eletrônico de Propostas;

b) Currículo cadastrado na Plataforma Lattes;

c) Plano de trabalho do estágio ou programação do curso;

d) Carta de aceitação da instituição no exterior; e

e) Documento que comprove a proficiência no idioma requerido para o desenvolvimento das atividades ou carta da instituição de destino atestando que o candidato tem conhecimento suficiente do idioma para realização das atividades.

7. Critérios para seleção dos candidatos

Os candidatos serão selecionados em função de seu curriculo, do mérito da proposta e dos benefícios e resultados que poderão advir da capacitação no exterior.

8. Documento a ser obtido pelo bolsista até a data limite de envio das propostas e mantido sob sua guarda até o encerramento do processo:

- Documento comprobatório da situação migratória regular no Brasil, no caso de pesquisador estrangeiro.

9. Documentos indispensáveis para encerramento do processo:

- Específicos: declaração da instituição no exterior atestando a realização das atividades previstas no plano de trabalho ou certificado de conclusão do curso.

Anexo VI

Mestrado Profissional no Exterior (MPE)

1. Finalidade

Formar profissionais no exterior em nível de mestrado, em instituições de excelência, voltadas para a qualidade, o empreendedorismo e a competitividade nas áreas do conhecimento consideradas de vanguarda científico-tecnológica e naquelas estratégicas definidas pelo CNPq.

2. Requisitos e Condições para o Candidato:

a) ser brasileiro ou estrangeiro com visto permanente no Brasil;

b) ter concluído a graduação em curso de nível superior;

c) não possuir título de doutor;

3. Duração

3.1. De até 12 meses, sendo permitida prorrogação, desde que não ultrapasse o tempo total de 24 meses.

3.2. No caso de parto ou adoção ocorrido durante o período da bolsa formalmente comunicado pela bolsista ao CNPq, a vigência da bolsa será prorrogada por até 4 meses.

4. Benefícios:

a) Mensalidades, conforme Tabela de Valores de Bolsas no Exterior;

b) Auxílio-Deslocamento;

I. O CNPq depositará, na conta corrente do bolsista no Brasil, a importância correspondente ao auxílio-deslocamento de **ida** do bolsista.

II. O valor do auxílio-deslocamento correspondente ao **regresso** ao Brasil deverá ser solicitado pelo bolsista ao Serviço de Bolsas de Pós-Graduação e Pesquisa no Exterior (SEBPE), e-mail: sebpe@cnpq.br , com antecedência mínima de 30 dias da data prevista da viagem.

c) Auxílio-Instalação;

d) Seguro-Saúde;

e) Taxas Escolares.

5. Documentos indispensáveis para inscrição:

a) Formulário Eletrônico de Propostas;

b) Currículo do candidato cadastrado na Plataforma Lattes;

c) Currículo do orientador no exterior;

d) Certificado de conclusão da graduação;

e) Plano de Trabalho;

f) Concordância do orientador no exterior com a execução do plano de trabalho proposto;

g) Comprovante de proficiência no idioma exigido pela instituição de destino; e

h) Anuência formal da instituição de destino emitida pelo órgão responsável pela admissão do candidato, incluindo informação sobre o idioma e nível de proficiência mínimo exigido para o mestrado e valores de taxas escolares durante o período da bolsa, se couber.

6. Critérios para seleção dos candidatos

6.1. Os candidatos serão selecionados em função de seu currículo, do currículo do orientador no exterior, do mérito da proposta, da qualidade técnico-científica da instituição de destino e classificados em comparação com os demais candidatos.

6.2. A existência de vínculo empregatício do candidato no Brasil, bem como a isenção de taxas escolares são fatores favoráveis à concessão da bolsa.

7. Prorrogação da Bolsa

7.1. Eventuais pedidos de prorrogação devem ser solicitados em formulário *online* específico, até 60 dias antes do término da vigência da bolsa.

8. Documentos indispensáveis para encerramento do processo:

Específicos: cópia digitalizada do certificado ou diploma de obtenção do título

Anexo VII

PROCURAÇÃO

(MODELO)

OUTORGANTE:

_____, _____

(Nome) (Nacionalidade)

_____, portador da cédula de identidade nº _____, ___ emitida em __ / __/____

(Estado Civil) (UF)

inscrito no CPF/MF sob o nº _____ , residente e domiciliado à _____

_____.

(Endereço Completo)

OUTORGADO:

_____, _____

(Nome) (Nacionalidade)

_____, portador da cédula de identidade nº _____, ___ emitida em __ / __/___

(Estado Civil) (UF)

inscrito no CPF/MF sob o nº _____ , residente e domiciliado à _____

_____.

(Endereço Completo)

PODERES:

O Outorgante confere ao Outorgado poderes específicos para solucionar questões relacionadas ao benefício concedido ao Outorgante nos autos do Processo CNPq nº_____, junto ao Conselho Nacional de Desenvolvimento Científico e Tecnológico, bem como a qualquer órgão ou entidade pública ou privada, inclusive o Tribunal de Contas da União ¿TCU, além dos poderes especiais para transigir, desistir, renunciar ao direito sobre o qual se funda tal benefício, receber e dar quitação, firmar compromisso, recorrer, retificar declarações, receber notificação, citação e intimação de qualquer espécie, contratar advogado para o fim específico desta outorga, não podendo substabelecer estes poderes, praticando, ademais, todos os atos necessários ao bom e fiel desempenho deste mandato.

_____, ____ de _____de _____.

(Local e Data)

_____

(Assinatura)

OBS: Reconhecer firma.

[1] Alterado pela RN-029/2018 de 12/09/2018, publicada no DOU de 13/09/2018, Seção 1, pág. 13.

[2] Incluído pela PO-1182/2022, de 26/12/2022, publicada no DOU, de 28/12/2022, Seção 1, página 28.

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Exhibit C. Evidence that the beneficiary participated as a judge of the work of others in the field of scientific research

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05-Apr-2024

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I realize that our expert reviewers greatly contribute to the high standards of the Journal, and I thank you for your present and/or future participation.

Sincerely,
Dr. SILVYA STUCHI MARIA-ENGLER
Brazilian Journal of Pharmaceutical Sciences Associate Editor
editorchiefbjps@usp.br, editorchiefbjps@usp.br

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MANUSCRIPT DETAILS

TITLE: Dry powder inhalation formulation co-loaded with erlotinib and curcumin for treatment of non-small cell lung cancer

AUTHORS: Rahman, Md

ABSTRACT: Erlotinib is used as first-line chemotherapy for the treatment of non-small cell lung carcinoma which accounts for approximately 85% of lung cancers.. Curcumin has potential antitumor effects with additional protective effect. The dry powder inhalation formulation co-loaded with erlotinib and curcumin resulted in suitable particle size (313.28 nm), polydispersity index (0.156) and zeta potential (29.4 mV). Carr's index and Hausner ratio demonstrated suitable flowability of powder. High FPF (64.7 %) and appropriate MMAD (2.98 µm) values demonstrated good aerosol performance and suitable candidate for direct delivery to lungs. In-vitro cytotoxic potential of three DPI formulations were determined against lung cancer cells (A549). The IC50 value for E-DPI and C-DPI were found to be 36.6 ± 2.4 µM and 24.4 ± 2.7 µM, respectively. The IC50 value for EC-DPI was 20.5 ± 2.1 µM and demonstrated the synergistic effect of both the drugs against lung cancer cells. The internalization of the drug inside A549 cells was detected by cellular uptake study. The EC-DPI demonstrated the highest cellular uptakes compared to formulation containg curcumin and erlotinib alone. Results demonstrated suitability of DPI formulation for direct delivery of curcumin and erlotinib to lungs with preferential cytotoxic effect against non-small-cell lung carcinoma.

Outlook

Reminder: Brazilian Journal of Pharmaceutical Sciences

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19-Apr-2024

Dear Dr. Noma:

Recently, you agreed to review Manuscript ID BJPS-2024-0245, entitled "Dry powder inhalation formulation co-loaded with erlotinib and curcumin for treatment of non-small cell lung cancer." The manuscript is located in your Reviewer Center at https://mc04.manuscriptcentral.com/bjps-scielo.

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I appreciate your help in accomplishing our goal of having an expedited reviewing process. Please do not hesitate to contact me if I can be of any assistance.

Best regards,

Dr. SILVYA STUCHI MARIA-ENGLER
Associate Editor, Brazilian Journal of Pharmaceutical Sciences

> Dry powder inhalation formulation co-loaded with erlotinib and curcumin for treatment of non-small cell lung cancer

Reviewer Affiliation
University of São Paulo Institute of Biomedical Sciences
Manuscript ID:
BJPS-2024-0245
Manuscript Type
Original Article
Keyword
Non-small cell lung cancer., Erlotinib, Curcumin, A549, Cellular uptake
Date Submitted:
25-Mar-2024
Date Assigned:
05-Apr-2024
Date Review Returned:
21-Apr-2024
M-Score for this manuscript:
2.25
Author(s)
Rahman, Md

Questionnaire	Yes	No	Not applicable
req Does the manuscript contain new and significant information to justify publication?	✓		
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Minor Revision

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Confidential Comments to the Editor

Comments to the Author

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Thank you very much for submitting your manuscript to Brazilian Journal of Pharmaceutical Sciences. We have finished the first round of peer review and sent you the review comments for revision just now. Please check the comments and revise the paper accordingly.
1- We suggest revising the English writing because we detected some mistakes that could be clearer for the readers.
2-We noticed that your abstract needs to include your aim, discussion, and conclusion.
3- Introduction: You can include if there have been other studies with a combination of curcumin and erlotinib or testing dry power. Why is this article original? Moreover, include the objective. Do not include methods in your introduction.
4-Results and Discussion: We noticed that your discussion could have been stronger if you had explored your references. You also need to compare your results with those of other studies. We suggest that shows who refer to this phrase: Line 60. The IC50 value was observed to be 20.6 ± 2.1 μM. In all results, you need to show the comparison test between the groups and whether these results have some statistical difference.
5- Conclusion: Explain how your results demonstrated this conclusion: Line 55. "protecting healthy cells from any damages."
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PROGRAMA DE PÓS-GRADUAÇÃO EM BIOCIÊNCIAS E FISIOPATOLOGIA

PBF
Programa de Pós-Graduação em Biociências e Fisiopatologia

CERTIFICADO

Certificamos que, **ISABELLA HARUMI YONEHARA NOMA**

Atuou como revisora *ad hoc* de resumo simples no *VII International Meeting of Biosciences and Physiopathology* e XI Simpósio de Biociências Aplicadas à Farmácia (Processo nº 3352/2020), realizados no período de 16 a 18 de Setembro de 2020 pelo Programa de Pós-Graduação em Biociências e Fisiopatologia (PBF), do Centro de Ciências da Saúde (CCS), da Universidade Estadual de Maringá – PR.

Maringá, 30 de outubro de 2020.

Prof.ª Dra. Gessilda de Alcantara Nogueira de Melo
Coordenadora do Programa de Pós-Graduação em Biociências e Fisiopatologia &
Organizadora do *VII International Meeting of Biosciences and Physiopathology* e XI Simpósio de Biociências Aplicadas à Farmácia

VII *International Meeting of Biosciences and Physiopathology*
XI *Simpósio de Biociências Aplicadas à Farmácia*

Registrado no processo nº 3352/2020 - PRO/PBF/UEM

CERTIFICATE

We certify that Isabella Harumi Yonehara Noma served as ad roc reviewer of a simple abstract at the VII International Meeting of Biosciences and Physiopathology and XI Symposium of Biosciences Applied to Pharmacy (Process no. 3352/2020), held from September 16 to 18, 2020 by the Graduate Program in Biosciences and Physiopathology (PBF), of the Health Sciences Center (CCS), of the State University of Maringá - PR.

CERTIFICATE OF TRANSLATION

I hereby certify that I am familiar with both the English and Portuguese language and that the translation of the attached document is an accurate translation from the Portuguese language into English, and that the same is a true and complete translation to the best of my knowledge, ability and belief.

Signature: Isabella H. Y. Noma Date: 08-19-2024

Name: Isabella Harumi Yonehara Noma

International Meeting of Biosciences & Physiopathology

Simpósio de Biociências Aplicadas à Farmácia

CERTIFICADO

Certificamos que Isabella Harumi Yonehara Noma atuou como revisor *ad hoc* de Resumos Simples do *VI International Meeting of Biosciences and Physiopathology - X Simpósio de Biociência Aplicadas à Farmácia*, realizado de 18 a 20 de setembro de 2019 em Ivatuba – PR.

Maringá, 21 de setembro de 2019

Registrado no Processo
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Prof.ª Dr.ª Débora de Mello Gonçales Sant'Ana
Coordenadora do Evento

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Realização: Programa de Pós-Graduação em Biociências e Fisiopatologia

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Signature: Isabella H. Y. Noma Date: 08-19-2024

Name: Isabella Harumi Yonehara Noma

Re: Orientações para Correção de Resumos Simples (2)

De: Simpósio PBF - UEM simposiopbf@gmail.com
Para: Amanda Gubert amanda_gubert@hotmail.com, andressambrito@hotmail.com , Aryadne Almeida aryadne_almeida@hotmail.com, blautons@gmail.com , Bruna Renata bruna-emilio@hotmail.com, Camila Barros Galinari camisgalinari@gmail.com, CAROLINA TREVISOLLI PALOMO carolina_trevisolli@hotmail.com, Carla Betania carla_betania@hotmail.com, Daniella Faria renata.daniella@gmail.com, Deborah Scanferla deborahscanferla@gmail.com, glaucia arita glauciasayuria@gmail.com, bellanoma@hotmail.com , Jan Eduardo Meneguello jan.meneguello@gmail.com, João Vítor Perez Souza jvperezsouza@gmail.com, Karina Sakita karina.msakita@gmail.com, Lainy Leiny lainyleiny@gmail.com, Lais Marques laissmarques@outlook.com, Laise Elpidio laise_nayana@hotmail.com, le.murase@gmail.com , Lincoln Luís sirlincolnls@gmail.com, lyvia.fmeirelles@gmail.com , vitoria_caioba@hotmail.com , Mariana Aparecida Oliveira Madia mari.madia@hotmail.com, Natalia Lourenço Mari nlmari95@gmail.com, Pollyanna Conrado pollyconrado@gmail.com, Raquel Melo raquelcmelo97@gmail.com, Regivaldo Florentino regivaldo.florentino@gmail.com, renatalini23@gmail.com , Stefani Panza stefani.panza@gmail.com, Thais Silva thais.silva8912@gmail.com, Valéria Baquetti Mosca valeria.bmosca@gmail.com, Vanessa Baldin vanessapbaldin@gmail.com, Victor Souza victoruem@gmail.com, Waylla Albuquerque w.albuquerque11@gmail.com
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Boa tarde Caro(a) Revisor(a) de Resumo Simples,

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Desde já, agradecemos sua colaboração,

--
Comissão Organizadora
VI International Meeting of Biosciences and Physiopathology e X Simpósio de Biociências Aplicadas à Farmácia

---

De: Isabella Noma bellanoma@hotmail.com
Para: Simpósio PBF - UEM simposiopbf@gmail.com
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Recebido.

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Isabella Harumi Yonehara Noma
Biomédica/ Mestranda do Programa de Pós-Graduação em Ciências da Saúde
Universidade Estadual de Maringá

De: Pollyanna Conrado pollyconrado@gmail.com

Para: Amanda Gubert amanda_gubert@hotmail.com, Aryadne Almeida aryadne_almeida@hotmail.com, Bruna Renata bruna-emilio@hotmail.com, CAROLINA TREVISOLLI PALOMO carolina_trevisolli@hotmail.com, Camila Barros Galinari camisgalinari@gmail.com, Carla Betania carla_betania@hotmail.com, Daniella Faria renata.daniella@gmail.com, Deborah Scanferla deborahscanferla@gmail.com, Jan Eduardo Meneguello jan.meneguello@gmail.com, João Vítor Perez Souza jvperezsouza@gmail.com, Karina Sakita karina.msakita@gmail.com, Lainy Leiny lainyleiny@gmail.com, Lais Marques laissmarques@outlook.com, Laise Elpidio laise_nayana@hotmail.com, Lincoln Luís sirlincolnls@gmail.com, Mariana Aparecida Oliveira Madia mari.madia@hotmail.com, Natalia Lourenço Mari nlmari95@gmail.com, Raquel Melo raquelcmelo97@gmail.com, Regivaldo Florentino regivaldo.florentino@gmail.com, Simpósio PBF - UEM simposiopbf@gmail.com, Stefani Panza stefani.panza@gmail.com, Thais Silva thais.silva8912@gmail.com, Valéria Baquetti Mosca valeria.bmosca@gmail.com, Vanessa Baldin vanessapbaldin@gmail.com, Victor Souza victoruem@gmail.com, Waylla Albuquerque w.albuquerque11@gmail.com, andressambrito@hotmail.com , bellanoma@hotmail.com , blautons@gmail.com , glaucia arita glauciasayuria@gmail.com, le.murase@gmail.com , lyvia.fmeirelles@gmail.com , renatalini23@gmail.com , vitoria_caioba@hotmail.com

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Boa noite!

Confirmo recebimento.

De: Deborah Scanferla deborahscanferla@gmail.com

Para: Pollyanna Conrado pollyconrado@gmail.com

Cc: Amanda Gubert amanda_gubert@hotmail.com, Aryadne Almeida aryadne_almeida@hotmail.com, Bruna Renata bruna-emilio@hotmail.com, CAROLINA TREVISOLLI PALOMO carolina_trevisolli@hotmail.com, Camila Barros Galinari camisgalinari@gmail.com, Carla Betania carla_betania@hotmail.com, Daniella Faria renata.daniella@gmail.com, Jan Eduardo Meneguello jan.meneguello@gmail.com, João Vítor Perez Souza jvperezsouza@gmail.com, Karina Sakita karina.msakita@gmail.com, Lainy Leiny lainyleiny@gmail.com, Lais Marques laissmarques@outlook.com, Laise Elpidio laise_nayana@hotmail.com, Lincoln Luís sirlincolnls@gmail.com, Mariana Aparecida Oliveira Madia mari.madia@hotmail.com, Natalia Lourenço Mari nlmari95@gmail.com, Raquel Melo raquelcmelo97@gmail.com, Regivaldo Florentino regivaldo.florentino@gmail.com, Simpósio

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De: Renata Sano Lini renatalini23@gmail.com

Para: Deborah Scanferla deborahscanferla@gmail.com

Cc: Pollyanna Conrado pollyconrado@gmail.com, Amanda Gubert amanda_gubert@hotmail.com, Aryadne Almeida aryadne_almeida@hotmail.com, Bruna Renata bruna-emilio@hotmail.com, CAROLINA TREVISOLLI PALOMO carolina_trevisolli@hotmail.com, Camila Barros Galinari camisgalinari@gmail.com, Carla Betania carla_betania@hotmail.com, Daniella Faria renata.daniella@gmail.com, Jan Eduardo Meneguello jan.meneguello@gmail.com, João Vítor Perez Souza jvperezsouza@gmail.com, Karina Sakita karina.msakita@gmail.com, Lainy Leiny lainyleiny@gmail.com, Lais Marques laissmarques@outlook.com, Laise Elpidio laise_nayana@hotmail.com, Lincoln Luís sirlincolnls@gmail.com, Mariana Aparecida Oliveira Madia mari.madia@hotmail.com, Natalia Lourenço Mari nlmari95@gmail.com, Raquel Melo raquelcmelo97@gmail.com, Regivaldo Florentino regivaldo.florentino@gmail.com, Simpósio PBF - UEM simposiopbf@gmail.com, Stefani Panza stefani.panza@gmail.com, Thais Silva thais.silva8912@gmail.com, Valéria Baquetti Mosca valeria.bmosca@gmail.com, Vanessa Baldin vanessapbaldin@gmail.com, Victor Souza victoruem@gmail.com, Waylla Albuquerque w.albuquerque11@gmail.com, andressambrito@hotmail.com , bellanoma@hotmail.com , Bruna Lauton blautons@gmail.com, glaucia arita glauciasayuria@gmail.com, le.murase@gmail.com , lyvia.fmeirelles@gmail.com , vitoria_caioba@hotmail.com

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Bom dia! E-mail recebido!
Att,
Renata Sano Lini

---

5 Emails

Revisão final dos resumos simples

De: Simpósio PBF - UEM simposiopbf@gmail.com
Para: Adriano Araujo Ferreira prof.adriano.af@gmail.com, Aléia Harumi aleiayamanaka@gmail.com, Aline Aguiar alineliliaguiar@hotmail.com, amandacarvalhodutra@gmail.com , AMANDA GUBERT ALVES DOS SANTOS agsgubert@gmail.com, AMAURI DONADON LEAL JUNIOR amaurilealjunior@gmail.com, ANDRESSA SULAMITA SIQUEIRA MENEZES DE BRITO andressambrito@hotmail.com, Beatriz Ferreira ff.bia95@gmail.com, BRUNA LAUTON SIMÕES blautons@gmail.com, Bruna Tiyo brunatiyo@gmail.com, Camila Barros Galinari camisgalinari@gmail.com, Cinthia Akemi Tanoshi cinthiaatanoshi@gmail.com, CLAUDIA NARA DE ALMEIDA LINO BRATTI claudiaalino@gmail.com, Deborah Scanferla deborahscanferla@gmail.com, glaucia arita glauciasayuria@gmail.com, ISABELLA HARUMI YONEHARA NOMA bellanoma@hotmail.com, joão Paulo jaodospaulo@hotmail.com, JULIA CALVI MORI juliacmori@gmail.com, Karile Costa karileccosta@gmail.com, Karina Sakita karina.msakita@gmail.com, Lainy Leiny lainyleiny@gmail.com, Lincoln Luís sirlincolnls@gmail.com, LUCIMARA RODRIGUES CAROBELI lucimaracarobeli@gmail.com, LYVIA ELOIZA DE FREITAS MEIRELLES lyvia.fmeirelles@gmail.com, Maria Vitoria F. de Souza vitoria_caioba@hotmail.com, MARIANA BURANELO mariana_buranello@hotmail.com, MARIANA TEIXEIRA DA SILVA mariteixeira181116@gmail.com, Mateus Thomazella thomazella16@gmail.com, Natalia Lourenço Mari nlmari95@gmail.com, Pollyanna Conrado pollyconrado@gmail.com, RAUL GOMES AGUERA raul1994_gomes@hotmail.com, Regivaldo Florentino regivaldo.florentino@gmail.com, Renata Sano Lini renatalini23@gmail.com, Stefani Panza stefani.panza@gmail.com
Data: dom., 30 de ago. de 2020, 1:29 PM

Prezados revisores,

Primeiramente, gostaríamos de agradecer pelo apoio e empenho na correção dos resumos simples, sua ajuda foi fundamental para o nosso evento!

Gostaríamos de pedir que olhem no seu perfil de avaliador, pois os trabalhos que foram "Aprovados com Ressalvas" retornaram para a próxima etapa de correções e precisamos finalizar essa etapa final de correções até amanhã. Aqueles trabalhos que ainda não estão nas normas do evento ou com falhas GRAVES na escrita, por favor aprovem com correções.

Em breve seus certificados serão enviados.

Obrigada novamente!

--

Comissão Organizadora

VII International Meeting of Biosciences and Physiopathology e XI Simpósio de Biociências Aplicadas à Farmácia

To whom it may concern,

I am Marcia Edilaine Lopes Consolaro- Coordinator of the Postgraduate Program in Biosciences and Physiopathology - PBF at the State University of Maringá. This statement is being issued to confirm the participation of Isabella Harumi Yonehara Noma in the academic and scientific activities organized by our institution.

Ms. Noma served as a reviewer and evaluator of scientific abstracts during the following events:

- VI International Meeting of Biosciences and Physiopathology
- X Simpósio de Biociências Aplicadas à Farmácia

Her responsibilities included:

- Reviewing abstracts submitted by authors;
- Providing technical comments, recommendations, and linguistic corrections;
- Participating in the evaluation system, using the platform provided by the Organizing Committee;
- Confirming receipt of communications and following correction guidelines issued to reviewers.

Her collaboration contributed directly to the quality control and scientific integrity of the event, supporting the successful organization and publication of the accepted abstracts.

This participation was voluntary and academic in nature, and was carried out in accordance with the scientific standards established by the committee. Evidence of her involvement includes official e-mails exchanged between the organizing committee and reviewers, which can be provided if necessary.

We issue this statement upon request, for whatever legal or administrative purposes it may serve.

Marcia Edilaine Lopes Consolaro
[Associate Professor and Coordinator]

[Department of Clinical Analysis and Biomedicine /
State University of Maringa]
Date: [12/15/2025]

Exhibit D. Evidence of the original scientific contributions of major significance in the field

Biomedicine & Pharmacotherapy 177 (2024) 116953

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Peroxiredoxin-2 represses NRAS-mutated melanoma cells invasion by modulating EMT markers

Isabella Harumi Yonehara Noma [a,1], Larissa Anastacio da Costa Carvalho [a,1], Denisse Esther Mallaupoma Camarena [a], Renaira Oliveira Silva [a], Manoel Oliveira de Moraes Junior [a], Sophia Tavares de Souza [a], Julia Newton-Bishop [b], Jérémie Nsengimana [c], Silvya Stuchi Maria-Engler [a,*]

[a] Department of Clinical and Toxicological Analysis, School of Pharmaceutical Sciences, University of São Paulo, Avenida Professor Lineu Prestes, 580, São Paulo, SP 05508-00, Brazil
[b] Leeds Institute of Medical Research, School of Medicine, University of Leeds, Leeds LS9 7TF, UK
[c] Biostatistics Research Group, Population Health Sciences Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne NE2 4BN, UK

ARTICLE INFO

Keywords:
Cutaneous malignant melanoma
Peroxiredoxin 2
Phenotype switching
Chemical Compounds studied in this article:
Gliotoxin (PubChem CID: 6223)

ABSTRACT

The second most common mutation in melanoma occurs in NRAS oncogene, being a more aggressive disease that has no effective approved treatment. Besides, cellular plasticity limits better outcomes of the advanced and therapy-resistant patients. Peroxiredoxins (PRDXs) control cellular processes through direct hydrogen peroxide oxidation or by redox-relaying processes. Here, we demonstrated that PRDX2 could act as a modulator of multiple EMT markers in NRAS-mutated melanomas. PRDX2 knockdown lead to phenotypic changes towards invasion in human reconstructed skin and the treatment with a PRDX mimetic (gliotoxin), decreased migration in PRDX2-deficient cells. We also confirmed the favorable clinical outcome of patients expressing PRDX2 in a large primary melanoma cohort. This study contributes to our knowledge about genes involved in phenotype switching and opens a new perspective for PRDX2 as a biomarker and target in NRAS-mutated melanomas.

1. Introduction

Melanoma of the skin is the fifth most common type of cancer [1] and the deadliest form of skin cancer due to its high metastatic potential, development of resistance and tumor recurrence [2]. The second most frequent mutation in melanoma occurs in NRAS oncogene, found in approximately 25 % of cutaneous melanoma cases, after BRAF, which affects 40–45 % of all patients [3]. NRAS-mutated melanomas are more aggressive, resulting in lower patient overall survival, and it has no specific therapy approved [4]. Immunotherapy is the first-line treatment for surgically incurable NRAS-mutated melanoma at high stages, such as anti-PD-1(nivolumab or pembrolizumab), and the combination of anti-PD-1 and CTLA-4 blockade (ipilimumab). Second-line treatment includes MEK inhibitors and combination of targeted-therapies as BRAF and MEK inhibitors. Altogether, the therapeutic success is limited for these patients by drug resistance and clinical relapse, emphasizing the need for new therapeutic approaches and new targeted combinations [3, 5].

One of the major challenges in treating metastatic melanoma is the residual disease associated with intratumoral heterogeneity and cellular plasticity. These well-characterized phenotypic switches between differentiated (proliferative) and undifferentiated (invasive) occur as cells undergo phenotypic transitions as an adaptative mechanism under stressful conditions. These phenotypes have different gene signatures, therapeutic sensitivity, and metastatic potential [6].

A common stressful condition in cancer, is when the physiological balance in the redox state is disrupted. The transient production of ROS is necessary for cell signaling, but its persistent production and exposure, can trigger tumor initiation and/or progression and also be cytotoxic. Once ROS have a dual role in cancer, agents that modulates its levels are interesting targets [7]. In this sense, cells regulate the expression of cellular peroxidase enzymes to maintain the redox homeostasis [8].

Peroxiredoxins (PRDXs) are one of the major hydrogen peroxide

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Table 1
Identification of NRAS-mutated melanoma cell lines.

Cell lines	Mutation	Short-tandem-repeat
SK-MEL-147	NRAS p.Gln61Arg (c.182 A>G); Q61R (PubMed=24576830; PubMed=25728708).	Amelogenin (X); CSF1PO (10, 12); D13S317 (9, 11); D16S539 (12, 13); D5S818 (10, 13); D7S820 (9, 11); THO1 (6, 9.3); TPOX (8, 12); vWA (17, 18).
SK-MEL-173	NRAS p.Gln61Lys (c.181 C>A): Q61K (PubMed=21725359; PubMed=24576830).	Amelogenin (X, Y); CSF1PO (10, 11); D13S317 (9, 11); D16S539 (11, 12); D5S818 (9, 11); D7S820 (9, 11); THO1 (7, 9.3); TPOX (8); vWA (18).

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consumers, controlling cellular processes through direct hydrogen peroxide oxidation or by redox-relaying processes. The PRDX family is classified according to the number of active-site cysteine residues into the 1-Cys subfamily and 2-Cys (PRDX1, 2, 3, and 4), with PRDX1 and 2 being more abundant in the cytosol and nucleus [8]. Our group have previously demonstrated that PRDXs could act as biomarkers and are involved in melanoma heterogeneity and resistance [9,10]. The importance of redox regulation and the use of PRDXs as biomarkers, have also been highlighted before [11]. It has been demonstrated that PRDX2 suppresses BRAF-mutated melanoma cell proliferation and migration by increasing the E-cadherin/β-catenin complexes in adherens junctions, a critical step for metastatic ability [12]. Studies in invasive melanoma cells and metastatic tumors have already demonstrated a downregulation or silencing of PRDX2 [8,9,13,14].

Once NRAS-mutated melanomas have no effective treatment, we investigated the role of PRDX2 in this context and demonstrated that it could modulate the invasion profile, impacting the overall patient survival and also can act as a biomarker or possible target.

2. Materials and methods

2.1. Cell culture

The cell lines SK-MEL-147, SK-MEL-173 (kindly donated by Dr. María Soengas, Centro Nacional de Investigaciones Oncológicas, Madrid, Spain) were cultured in DMEM (Gibco, USA) with 10 % FBS, 25 μg/mL ampicillin, and 100 μg/mL streptomycin). Cells were cultured at 37 °C with 5 % CO_2 in a humidified chamber and passage numbers were less than 100. Cells were assayed for mycoplasma contamination every two weeks with conventional PCR. The short-tandem-repeat profiling (DNA fingerprinting) was performed using PowerPlex® 16 System (Promega) on following short-tandem-repeat loci. The results in table showed the perfect match with known profile (Table 1).

2.2. Cell viability assays

Cell viability was evaluated using the death curve assay after exposure to Gliotoxin - GT (Sigma-Aldrich /G9893–5MG) treatment. GT was solubilized in DMSO (10 mM). In a 24-well plate, 2×10^4 cells were seeded and, after adherence, they were treated with different concentrations of GT (0, 50, 100, 200 and 400 nM) for 24 hours of incubation. After staining using Trypan Blue dye (T6146, Sigma-Aldrich, St. Louis, MO, USA), viable cells were counted in a Neubauer chamber.

2.3. Growth curve

The growth curve was evaluated by counting cells in a Neubauer chamber, using the Trypan Blue dye exclusion method. Cell lines were plated at a concentration of 10^4 cells/well in triplicate. After a period of 24, 72, 120, and 168 hours the cells were collected. Then, they were incubated with Trypan Blue solution (0.4 % in PBS) for approximately 5 minutes. In this assay, non-viable cells can be identified by blue staining and counted under an optical microscope, while viable cells are not permeable to the dye [15].

2.4. Western blotting

Cell lysates for Western Blotting were prepared in RIPA buffer with a cocktail of protease inhibitors (Roche, Penzberg, Upper Bavaria, Germany) and with a cocktail of phosphatase inhibitors I and II (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Total protein (40 μg) run to SDS gel electrophoresis with a 4–20 % gradient under reducing conditions and subsequent transfer to a PVDF membrane (Hybond-P, Amersham Pharmacia Biotech, Piscataway, NJ, USA). The membrane was blocked with 5 % BSA diluted in TBS-Tween 20 (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1 % Tween-20) for 1 hour and the antibodies were

Table 2
shRNA sequences for PRDX2 inhibition.

Bacterial stock code	ID	Sequences	PRDX2- 201	PRDX2- 202
TRCN0000064905	PRDX2–0	CAGACGCTTGTCTGAGGATTA	Éxon 4	Éxon 4
TRCN0000064906	PRDX2–1	GTGAAGCTGTCGGACTACAAA	Éxon 2	Éxon 2
TRCN0000064907	PRDX2–2	GCCTGGCAGTGACACGATTAA	Éxon 6	Éxon 5

tested at concentrations recommended by the manufacturer (Table S1). Protein bands were detected by enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech, Piscataway, NJ, USA) and semi-quantified using a Image J software.

2.5. Gene expression by real-time PCR

Total RNA from cell lines was extracted using the RNeasy Plus Mini Kit (Qiagen), according to the manufacturer's instructions. The RNA was heated to 65°C for 5 minutes, and quantified using a spectrophotometer at 260 nm to evaluate the sample's quality. RT-PCR reaction (real-time polymerase chain reaction) was performed using the Taqman® method (Life Technologies, USA). The cDNAs obtained from the samples were diluted (2 μg/μL). Reactions had a final volume of 10 μL containing 0.5 μL of primer mix (forward and reverse) for each gene of interest (Table S2), 1 μL of cDNA, 5 μL of 2x Taqman® Gene Expression Master Mix (Life Technologies, USA), and 3.5 μL of RNAse-free water. The relative level of gene expression was calculated using the delta delta Ct method (cycle threshold method) with GAPDH as endogenous controls [16].

2.6. Clonogenic assay

The cells were seeded at 10^3 cells/well in 6-well dishes and cultured in DMEM for 2 and 3 weeks. After cell adhesion (24 hours), they were treated at different concentrations, based on the results obtained in the death curve. The culture medium was changed every 2–3 days and gliotoxin was added for a period of 15 days, varying according to the cell line used. After the colony formation period, the wells were washed with PBSA. The colonies were then fixed and stained with a solution of 49.75 % methanol, 49.75 % deionized water, and 0.5 % crystal violet. The plates were photographed to compare the treatment effects. The analysis of the number of colonies and the area they occupied was carried out according to Guzmán et al.[17].

2.7. Transwell assay

The invasion capacity of melanoma cells was evaluated after treatment of the cell lines on Transwell membranes (8 μm pore size, Becton Dickinson). These were coated with 30 μL of Matrigel (BD Biosciences, San Jose, CA, diluted 1:6 in FBS-free DMEM). Cells were resuspended (5×10^4 cells/well) in FBS-free DMEM and added to the upper compartment of the chamber. Then, 750 μL of DMEM supplemented with 10 % FBS was added to the lower chamber. Treatments were used in both upper and lower compartments. After 24 h, cells that migrated through Matrigel were fixed in 4 % formaldehyde (in 1% PBS), stained with 1 % crystal violet (in methanol) for 20 minutes before counting in an inverted microscope (Axiovert S100, Zeiss, Germany). In each well, 5 representative and independent random fields were counted (20x magnification).

2.8. Wound-healing assay

Evaluation of the ability of melanoma cells to migrate after treatment: 5×10^4cells/well were seeded in 24-well plates. After reaching approximately 90 % confluence, slits ("wounds") were made in the center of each well using a 200 μL pipette tip. The cells were then washed with 1x PBS, and treatments with DMEM culture medium + 1 % FBS were added. The experiment was carried out until the slits were closed in the controls, with pictures taken through a microscope. Analysis of cell-free areas was performed using ImageJ software.

2.9. PRDX2 gene silencing through lentiviral vectors

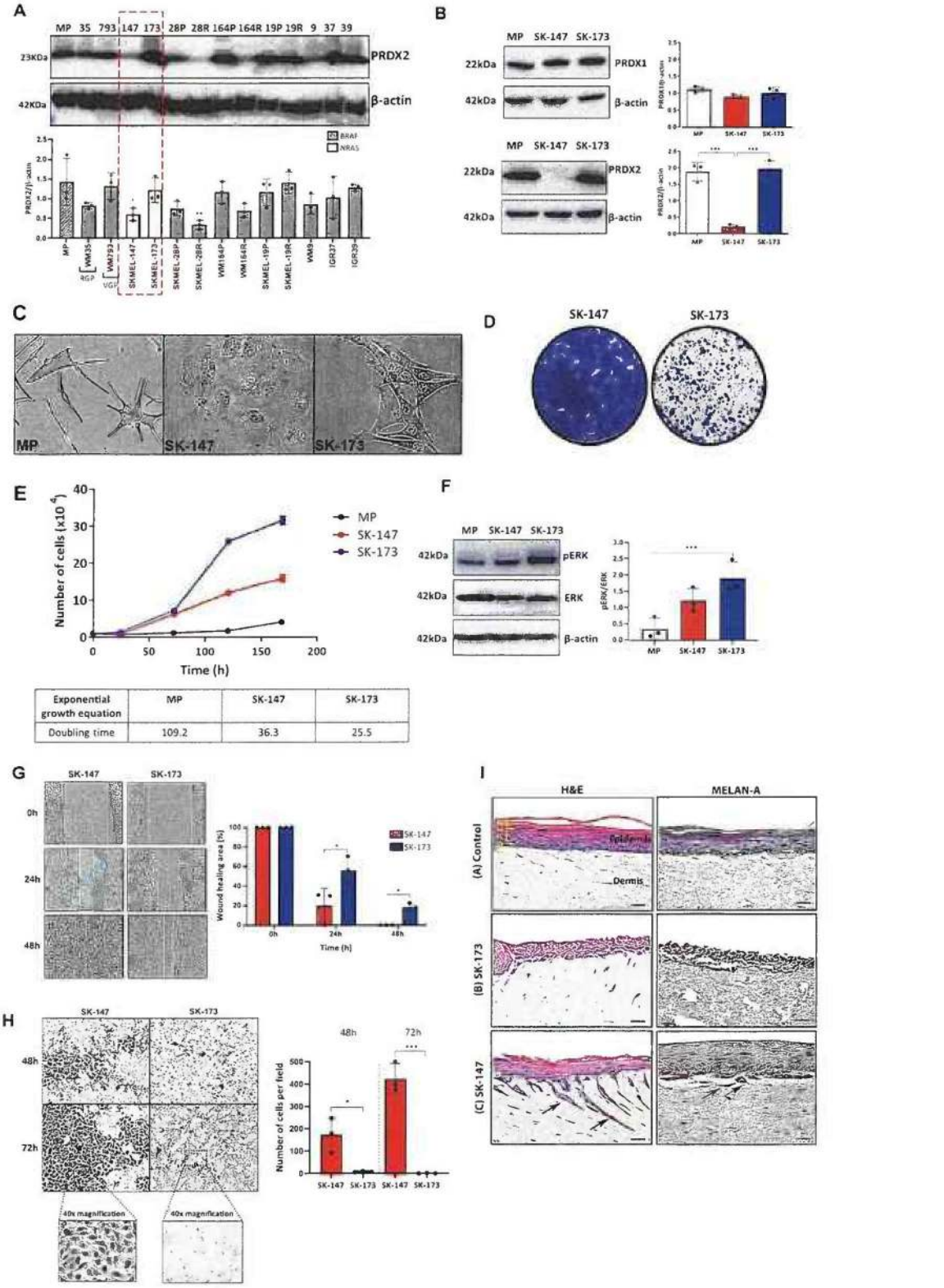
The silencing of the PRDX2 gene in cell lines was performed using short-hairpin RNA (shRNA) sequences to inhibit the messenger RNA (mRNA) of this gene. This process involved using bacterial cultures transfected with the Mission shRNA plasmid sequence (Sigma, USA). Three different shRNA sequences for PRDX2 were used to inhibit distinct regions of the mRNA. Bacterial stocks were cultivated for subsequent extraction and purification of plasmid DNA, following the Sigma Mission cell cloning protocol. Lentiviral vectors with a random sequence of nucleotides (scramble) were used to generate controls, which underwent the same manipulation conditions as the shRNA sequences. The shRNA vectors were co-transfected with lentiviral packaging plasmids into HEK293T cells, according to the manufacturer's instructions. These cells were selected using puromycin antibiotic (Sigma-Aldrich, USA). Afterward, the manipulated PRDX2 gene was validated by performing Western blotting and RT-PCR experiments. The knockdown cells (inhibited) were identified as scramble for the control (CN), PRDX2#0 for the cell line infected with shRNA sequence 0 PRDX2, PRDX2#1 for the cell line infected with shRNA PRDX2 sequence 1, and PRDX2#2 for the cell line infected with shRNA PRDX2 sequence 2 (Table 2).

2.10. Reconstruction of human skin (RHS) with melanoma

Primary human cells were isolated from skin samples extracted obtained from breast plastic surgery and/or postectomy in University Hospital of USP (CEP/HU-USP 943/09, SISNEP CAAE 0062.0.198.000–9). Approved was granted by Ethics Committee (CEP/FCF-USP 534). After extraction, the cells were tested for the presence of microorganisms (Human *Papillomavirus*, *Herpes Simplex* Virus, Cytomegalovirus, Hepatitis B, Hepatitis C, HIV-1, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Treponema pallidum* and *Trichomonas vaginalis*) by PCR. The reconstructed human skin model (RHS) followed the protocol described by Brohem *et al.* (2011) [18], with the following changes regarding the number of cells 50×10^4 melanoma cells/skin. The RHS were cultured and incubated submerged in DMEM:HAM F-12 1x (Gibco#21700–026) medium (1:3) + supplements (cholera toxin (1 μM), insulin (5 mg/mL), apo-transferrin (5 mg/mL), hydrocortisone-21 (0,1 mg/mL) and EGF (1 mg/mL)) for 24 hours, followed by 11 days of exposure to air-liquid interface to promote epithelial differentiation. After this period, the skins were collected, and fixed with 4 % paraformaldehyde and processed for histological analysis.

2.11. Histology and immunohistochemistry

Samples were processed according to Camarena et al., 2020 [19]. The immunostaining assay was performed using an anti-melan A antibody (ab51061) at a 1:50 dilution. A commercial kit with a goat secondary antibody against rabbit and mouse immunoglobulins (EnVision Flex/HRP, Dako Omnis, Santa Clara, CA, USA) was used in combination with 3,3′-diaminobenzidine (DAB; EnVision Flex DAB + Chromogen, Dako Omnis, Santa Clara, CA, USA) according to the manufacturer's instructions. All images were observed and photographed with an



Exponential growth equation	MP	SK-147	SK-173
Doubling time	109.2	36.3	25.5

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Fig. 1. PRDX2 expression in NRAS-mutated melanoma with different phenotypic profile. A Screening of the PRDX2 protein expression in different mutations and stages of melanoma cell lines (melanoma cell lines, including radial growth phase (RGP), vertical growth phase (VGP), NRAS-mutant melanoma, and BRAF-mutant melanoma). B Western blot was used to detect the protein expression of PRDX1 and PRDX2. C Cell morphology in 40x magnification. D Colony formation assay after ~15 days. E Growth curve at times 24, 72, 120 and 168 hours by trypan blue exclusion. F Western blot was used to detect the protein expression of ERK-phosphorylated. G Wound healing assay after 24 and 48 hours with wound area measurement. H Invasion assay after 48 and 72 hours and quantification of number of cells per field below of the chamber Matrigel. I Control skin without melanoma, skin with NRAS-mutant melanoma stained with Hematoxylin & Eosin and immunohistochemical staining of Melan-A, 20x magnification. Arrows indicate the sites of melanoma invasion in the dermis. Epidermis included SB: *stratum basale*, SS: *stratum spinosum*, SG: *stratum granulosum*, SC: *stratum corneum*. **A-H** All values are indicated as mean ± standard deviation (SD) of three independent experiments. P values are based on a one-way analysis of variance (ANOVA) followed by Tukey's test, *p<0.05 **p<0.01 and ***p<0.001. Images are representative of three independent biological experiments. Abbreviations: Primary melanocyte (MP), SK-MEL-147 (SK-147), and SK-MEL-173 (SK-173).

Eclipse i80 Nikon camera using the NIS-Elements Viewer (Nikon) program for image acquisition [19].

2.12. Statistical analysis

GraphPad Prism 7.0 was used for all in vitro data analysis. An independent Student's t-test was applied to two-group comparisons, while ANOVA with Tukey correction was applied to multiple groups/multiple predictors. Doubling times were obtained by non-linear regression analysis. Pairwise gene expression correlations were performed after normalizing the data, and the Pearson correlation was represented graphically. Analyses were considered significant when p-values were ≤ 0.05. Values were indicated as mean ± standard error of triplicate results from three independent experiments.

2.13. Patient data analysis

The Leeds melanoma cohort is a prospective cohort of patients diagnosed with primary melanoma in the North of England. These patients consented to be followed up starting from 3 to 6 months after diagnosis to monitor the risk of recurrence following the initial surgery. Death records were retrieved from the UK Office of National Statistics and confirmed by checking death certificates. Transcriptomic data were generated from primary tumor formalin-fixed paraffin-embedded (FFPE) blocks. Samples were independent and non-matched. Any duplicates included for quality control purposes were removed after data cleaning [20]. Transcriptomic data were generated using the Illumina HT12.4 DASL array. Illumina's software GenomeStudio was used to extract raw data from image files before they were exported into R Bioconductor for further processing. This processing included background correction and quantile normalization using Lumi [21], followed by batch correction by Singular Value Decomposition (SVD) in Swamp [22].

To explore the utility of in vitro results in patients, we performed the following analyses on this cohort using STATA 18 (StataCorp. 2023). Transcripts and clinical data from the Leeds Melanoma cohort are accessible in the European Genome-phenome Archive under accession number EGAS00001002922 [20]. This is one of the largest transcriptomic datasets on primary melanoma worldwide, with a sample size of 703 patients and long follow-up (>10 years).

Associations between transcripts of PRDX2 and candidate targets related to tumor progression were performed by linear regression in the entire cohort and stratified by oncogenic mutations BRAF and NRAS. Regression coefficients of these analyses were represented in a heatmap. We tested the association between PRDX2 expression and the presence/absence of BRAF and NRAS mutations using analysis of variance (ANOVA). Pearson's correlation was calculated between the expression of PRDX2 and the log-transformed Breslow thickness (the transformation allowed for an approximate normality distribution). A similar analysis was performed to test for association with tumor staging (AJCC, American Joint Committee on Cancer), and the results were plotted as violin plots or box plots.

We tested the effect of PRDX2 on melanoma-specific survival (using only deaths confirmed to be caused by melanoma) using Cox proportional hazards regression. Survival profiles were plotted with Kaplan-Meier curves. Survival analysis was performed after dividing PRDX2 expression into 5 groups (lower 20 % = 1st quintile, upper 20 % = 5th quintile). The number of 5 was arbitrarily chosen, but it is possible to merge similar groups. Finally, the top 4 quintiles (80 % of tumors with the highest PRDX2 expression) were grouped together because their effect on prognosis appeared identical.

3. Results

3.1. Investigating the role of PRDX2 in proliferation, colony formation, migration, and invasion in NRAS-mutated melanoma

Initially, we tested several cell lines with different mutations (BRAF and NRAS) and tumor stages (vertical or horizontal growth, and metastatic melanoma) to investigate the protein expression of PRDX2. Our data highlighted that, in NRAS-mutated cells, PRDX2 exhibited a distinct expression pattern (Fig. 1A). This observation led us to further investigate PRDX2 in N-RAS mutated cell as SK-MEL-147, derived from metastatic melanoma, and SK-MEL- 173, from primary melanoma.

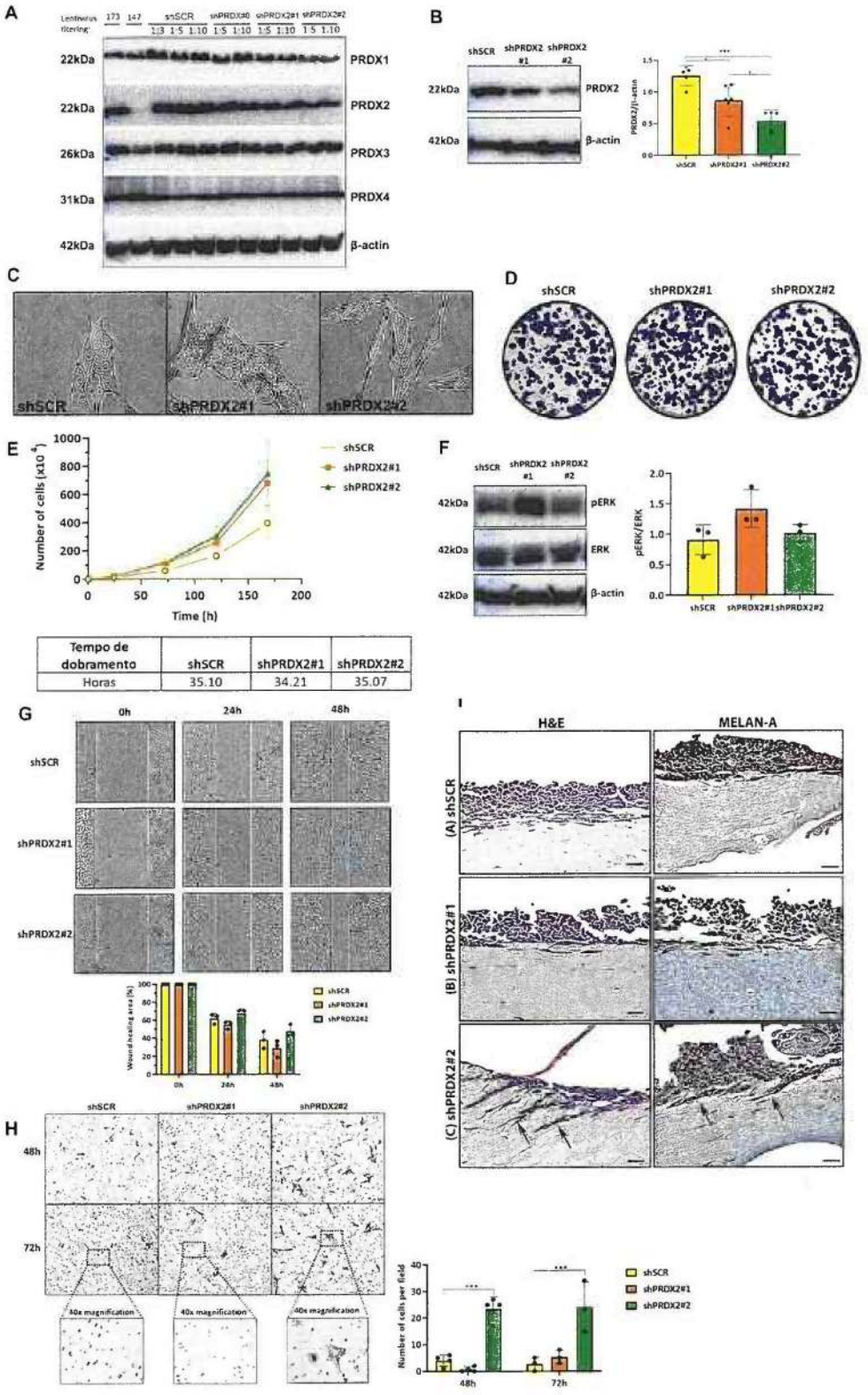
We assessed PRDX2 protein expression and observed high levels in our control melanocyte (MP) and in SK-MEL-173 compared to SK-MEL-147. Interestingly, PRDX1 expression did not vary among the cells tested (Fig. 1B). These cells with different mutations in NRAS, exhibited different morphologies and colony formations (Fig. 1C - D). SK-MEL-173 displayed a shorter doubling time and higher phosphorylated ERK levels (Fig. 1E – F), indicating a more proliferative profile than SK-MEL-147. Conversely, SK-MEL-147 showed greater potential for migration and invasion, observed by wound healing and invasion assay (Fig. 1G - H).

Using reconstructed human skin (RHS) models, we compared the profiles of these cell lines. We observed the presence of skin layers (corneal layer of the epidermis, dermis) and the melanoma cells which were stained with Melan-A. RHS-SK-MEL-173 melanoma cells demonstrated higher proliferation, growing over the stratum corneum, whereas RHS-SK-MEL-147 melanoma cells showed more pronounced invasion, infiltrating into the dermis (Fig. 1I).

3.2. Altering the phenotypic profile of SK-MEL-173 by knocking down PRDX2

We employed shRNAs to modulate PRDX2 expression and conducted knockdown (KD) experiments. The specificity of the shRNA was validated through Western Blot analysis, which showed a reduction specifically in the PRDX2 isoform. With shRNA PRDX2 sequence 1 (shPRDX2#1), we observed partial inhibition, and with shRNA PRDX2 sequence 2 (shPRDX2#2), there was more than a 60 % inhibition compared to the control – scramble (shSCR). We discarded shPRDX2#0 due to its lack of inhibitory effect (Fig. 2A - B).

Throughout our study, no differences were noted in the cell lines' morphology or colony formation characteristics (Fig. 2C - D). Post-KD experiments, the cells maintained their doubling time and phosphorylated ERK levels without statistically significant changes (Fig. 2E – F). The cells with shPRDX2#2 demonstrated more invasiveness capacity in transwell assay and in the reconstructed human skin model. However, we did not observe significative changes in the migration assay (Fig. 2G – H).



Tempo de dobramento	shSCR	shPRDX2#1	shPRDX2#2
Horas	35.10	34.21	35.07

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Fig. 2. Knockdown (KD) of PRDX2 by shRNA changed phenotypic profile in melanoma SK-MEL-173. A Western blotting analysis of 2-cys PRDX group proteins (PRDX1, PRDX2, PRDX3 and PRDX4). B Western blot was used to detect the protein expression of PRDX2 in cells KD PRDX2. C Cell morphology in 40x magnification. D Colony formation assay after ~15 days. E Growth curve at times 24, 72, 120 and 168 hours by trypan blue exclusion. F Western blot was used to detect the protein expression of ERK-phosphorylated. G Wound healing assay after 24 and 48 hours with wound area measurement. H Invasion assay after 48 and 72 hours and quantification of number of cells per field below of the chamber Matrigel. I Control skin without melanoma, skin with melanoma KD PRDX2 (shPRDX2#1 and shPRDX2#2 compared to shSCR- scramble) stained with Hematoxylin & Eosin and immunohistochemical staining of Melan-A, 20x magnification. Arrows indicate the sites of melanoma invasion in the dermis. A-H All values are indicated as mean ± standard deviation (SD) of three independent experiments. P values are based on a one-way analysis of variance (ANOVA) followed by Tukey's test, *p<0.05 **p<0.01 and ***p<0.001. Images are representative of three independent biological experiments.

In the RHS model, the control (shSCR) exhibited the same pattern as naive SK-MEL-173 (Fig. 1I). KD with shPRDX2#1 showed no difference, but KD with shPRDX2#2 revealed a notable increase in skin invasion (Fig. 2I). The skins were also subjected to immunohistochemistry (IHC) staining for Melan-A to confirm the invasion of melanoma cells. These results corroborate the observed differences in invasion in the 2D model following PRDX2 knockdown.

3.3. Expression of PRDX2 alters several genes and proteins related to invasion markers: a comparison between melanoma cell lines SK-MEL-147 and SK-MEL-173

Following the observation of phenotypic changes associated with PRDX2 downregulation, we investigated the potential proteins and genes that might be influenced by PRDX2. Our data revealed a higher expression of MITF in SK-MEL-173 (where PRDX2 levels are higher), contrasting with the expression of AXL (Fig. 3A-B). However, there was no significant difference in the expression of SOX10 (Fig. 3C). Only SK-MEL-147 (where PRDX2 levels are lower) showed expression of N-cadherin (Fig. 3D). β-catenin exhibited a pattern similar to MITF, being more highly expressed in SK-MEL-173 (Fig. 3E). E-cadherin was exclusively expressed in melanocytes (MP) and not in the melanoma cells (Fig. 3F). Additionally, real-time PCR analysis of gene expression confirmed higher expression of PRDX2 and MITF, and lower expression of AXL, FN1, SNAI1, and SMAD3 in SK-MEL-173 compared to SK-MEL-147 (Fig. 3G).

*3.4. **Comparing PRDX2 knockdown in melanoma SK-MEL-173: control (shSCR) vs. shPRDX2#1 and shPRDX2#2***

We observed that PRDX2 KD decreased the expression of MITF compared to control (scramble). The PRDX2 KD did not affect SOX10 expression, but it increased Snail expression at the protein level (Fig. 3H-K). In SK-MEL-173, AXL, N-cadherin, and E-cadherin were not expressed; therefor, the knockdown of PRDX2 in Sk-MEL-173 did not impact its expression (data not shown). Real-time PCR analysis for gene expression revealed that PRDX2 KD led to decreased levels of PRDX2, MITF, and SMAD2, and an increase in AXL and SNAI1 (Fig. 3L).

3.5. Gliotoxin (GT) treatment in NRAS-mutated melanoma

We evaluated gliotoxin (GT) as PRDX mimetic with antioxidant properties. Our viability tests revealed that melanoma cells were more sensitive to GT compared to fibroblast (FB) cells. However, there was no significant difference in sensitivity between melanocytes and the tested melanoma cell lines (Fig. 4A). The ability to form colonies was significantly reduced across all cell lines (Fig. 4B). Additionally, SK-MEL-173 cells showed no change in migration after GT treatment, whereas SK-MEL-147 cells exhibited a decrease in migration following GT treatment (Fig. 4C-D). Although GT did not affect cell proliferation, it altered the migration capability in SK-MEL-147 cells, which have lower PRDX2 levels.

3.6. Patient analysis in the Leeds Melanoma Cohort (LMC) of primary melanoma tumors

3.6.1. Gene correlation with PRDX2 in the total cohort and specifically in tumors exhibiting NRAS mutations

We validated our *in vitro* findings through bioinformatic analysis of patient data from the Leeds Melanoma Cohort (LMC, ethical approval MREC 1/3/57, PIAG 3–09(d)/2003). This analysis showed a positive correlation of PRDX2 with STAT3, CTNNB1, and CDH1, consistent with our *in vitro* assays, and a negative correlation with ZEB2, MAPK1, HIF1A, and PDGFB, which are genes implicated in tumor progression (Fig. 5A, all correlation coefficients are highly significant with $P<0.0001$). The cohort was subdivided into subgroups based on mutation type (BRAF, NRAS, and double wild type WT). It was observed that melanomas with NRAS mutations exhibited lower PRDX2 expression compared to those with BRAF mutations (Fig. 5B, $p=10^{-5}$). Despite that, PRDX2 did not have a difference in patient survival from the NRAS melanoma cohort (Sup. Fig. 1), suggesting PRDX2 is melanoma mutation-independent. Within the NRAS-mutated melanomas, PRDX2 showed varying correlations with different genes. It was negatively correlated with CSNK1A1, NRAS, AXL, and FN1, and positively correlated with ROS1, KRT18, AXIN1, and others, corroborating some of our *in vitro* results (Fig. 5C).

*3.6.2. **PRDX2 as a positive prognostic factor in the Leeds Melanoma Cohort (LMC)***

Primary melanomas from LMC patients were analyzed to corroborate our *in vitro* data. The results indicated that patients in the top 4 quintiles for PRDX2 expression (80 % high expressors) had a better survival profile compared to those with lower tumor PRDX2 expression (i.e. low expression has a double of hazard of high expression) (Fig. 5D-E). Consistent with this, lower expression of PRDX2 was associated with a more advanced stage of melanoma as indicated by AJCC stage (Fig. 5F) (mean values are 7.84 for AJCC stage I, 7.57 for AJCC stage 2 and 3) or Breslow thickness (Fig. 5G). Put together, our findings in *in vitro* and clinical datasets (especially in a large unbiased population-based cohort) suggest that PRDX2 expression in tumors is a favorable prognostic factor in primary melanomas, regardless of mutation status.

4. Discussion

PRDX2 is an antioxidant enzyme that plays an important role in redox homeostasis during carcinogenesis and in several cellular processes. While some studies have investigated the role of PRDX2 in melanomas, they were limited to BRAF-mutated melanomas, and the mechanism by which this protein contributes to melanoma progression has not been completely explored. Therefore, our aim was to understand the role of PRDX2 in NRAS-mutated melanoma and how it may influence cellular phenotypic pathways.

Our results showed that cell lines with higher PRDX2 expression have a more proliferative profile, whereas those with lower PRDX2 expression exhibit a more invasive profile. This aligns with other studies that linked PRDX2 silencing to increased migration and invasion in BRAF-mutated melanomas and gastric tumors [12,23]. Several studies have noted that MITF expression promotes proliferation and suppresses invasiveness [6,24]. Additionally, β-catenin, involved in adherens

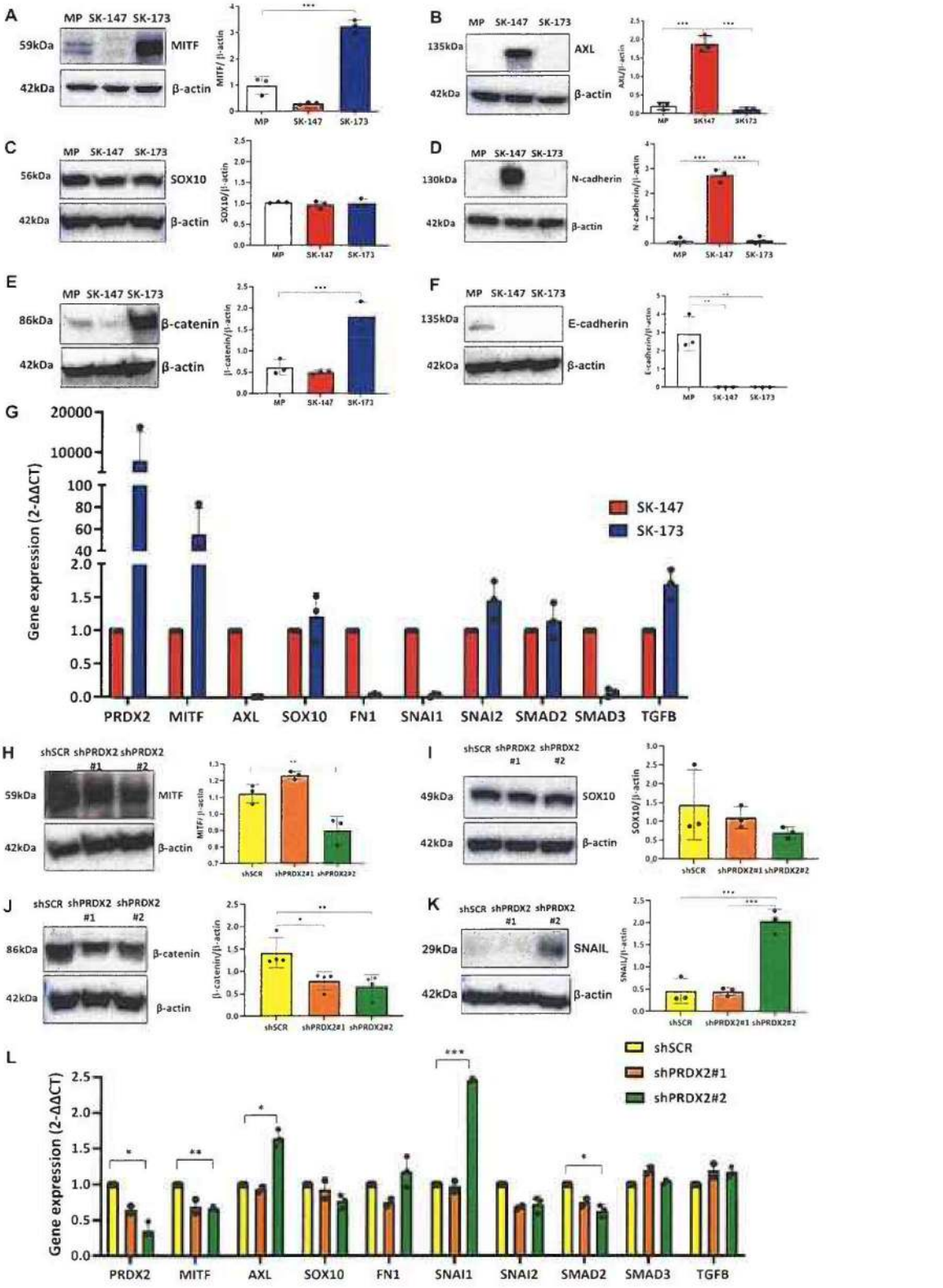

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Fig. 3. Lack of PRDX2 expression promotes invasive properties by regulation of genes and proteins related MITF and EMT markers in NRAS-mutated melanoma. A Western blot was used to detect the protein expression of MITF, (B) AXL, (C) SOX10, (D) N-cadherin, (E) β-catenin and (F) E-cadherin in NRAS-mutant melanoma cell lines (SK-MEL-147 and SK-MEL-173). G Genes expressions in NRAS-mutant melanoma cell lines based in delta delta CT. H Western blot was used to detect the protein expression of MITF, (I) SOX10, (J) β-catenin and (K) Snail in melanoma KD PRDX2. L Genes expressions in melanoma KD PRDX2 based in delta delta CT. **A-L** All values are indicated as mean ± standard deviation (SD) of three independent experiments. P values are based on a one-way analysis of variance (ANOVA) followed by Tukey's test, *p<0.05 **p<0.01 and ***p<0.001. Images are representative of three independent biological experiments. Abbreviations: Primary melanocyte (MP), SK-MEL-147 (SK-147), SK-MEL-173 (SK-173), PRDX2 knockdown using shRNA: scramble sequence (shSCR), sequence 1 (shPRDX2#1), and sequence 2 (shPRDX2#2).

junctions complexes [12], cooperates with the canonical Wnt pathway [25]. This pathway is important for MAP kinase signaling, regulating MITF expression and activity, and promoting melanoma proliferation [25]. Our data demonstrated that silencing PRDX2 correlates with decreased β-catenin and MITF levels, leading to increased cell migration and invasion.

Alterations in MITF expression levels are linked to phenotypic plasticity, and the acquisition of migration and invasion capabilities. Extremely low MITF expression is characteristic of invasive melanoma cells, while high MITF expression characterizes non-invasive melanoma cells. Phenotypic plasticity refers to the ability of malignant melanoma cells to undergo transient and reversible morphological and functional changes, similar to the embryonic neural crest invasion program. Although this phenotype switching resembles epithelial-mesenchymal transition (EMT), this term is not entirely appropriate for melanoma, as melanocytes are not epithelial cells. Instead, EMT-like processes, which play a key role during the formation and migration of neural crest cells, are considered [26,27]. Epithelial-mesenchymal transition-regulating transcription factors can induce EMT-like phenotype switching in melanoma, involving proteins like SNAIL, TWIST, and ZEB [28]. Melanocyte proliferation and differentiation from neural crest precursors heavily depend on the canonical WNT signaling pathway, mediated through β-catenin, which regulates MITF [26].

In the non-canonical pathway, blocking β-catenin expression and increasing Snail expression leads to metastasis. Snail (encoded by the SNAI1 gene) and Slug (encoded by the SNAI2 gene) are transcriptional repressors. Snail is expressed in mesenchymal cells and is upregulated during EMT, while Slug is expressed in epithelial cells. This is in line with our findings, where PRDX2 knockdown increased Snail expression and invasion. This coincides with an increase in N-cadherin, a major component of the cytoskeleton in mesenchymal cells and a marker of EMT [29,30]. FN1, the gene encoding fibronectin, shows a negative correlation with PRDX2 expression. Fibronectin is an extracellular matrix component facilitating tumor progression, expressed in the metastatic niches of lung melanoma cells and identified as a regulator of the Wnt/β-catenin pathway [31]. SMAD4 mediates canonical TGF-β signaling, essential for melanoma cell proliferation *in vivo*. A TGF-β family member, BMP7, stimulates melanoma cell proliferation [26].

We validated our 2D results in three-dimensional reconstructed human skin (RHS) models. This model replicates human skin histology and is used for functional analysis of melanoma development. The radial growth phase of melanoma, occurs proliferation of cancer cells in the dermal reconstruct, and the vertical growth phase, the cells invaded deep into the dermis, similar to *in vivo* scenarios [18,32]. The RHS using SK-MEL-173, the cell line containing higher levels of PRDX2, exhibited higher rates of cell proliferation, leading to an increased proportion of melanoma in the skin, surpassing the epidermis. Conversely, the SK-MEL-147 cell line, that showed lower levels of PRDX2, displayed invasion sites in the dermis due to its high invasive capacity observed in transwell assays (2D model). In the RHS using the melanoma cell line SK-MEL-173 KD for PRDX2 (shPRDX2#2), the reduction of PRDX2 in the more proliferative cell line, resulted in an increased invasion sites, evidencing the phenotypic modulatory potential of PRDX2. Our data suggest a metastatic potential in NRAS-mutated melanoma with lower PRDX2 expression, which is consistent with several studies that demonstrated the negative regulation of PRDX2 gene in metastatic melanomas [9,12,33].

Gliotoxin, an antioxidant compound described as a 2-Cys-PRDX mimetic, can reduces H_2O_2 and be restored by accepting electrons from NADPH via the TR-Trx redox system [34]. Viability assays conducted post-GT treatment revealed no differences between lineages. However, continuous GT exposure for over three weeks decreased colony formation in all tested cell lines. Notably, only the SK-MEL-147 cell line, lacking PRDX2, exhibited a difference in migration in response to GT treatment. This suggests that GT may have a compensatory effect in the absence of PRDX in SK-MEL-147 cells, changing their profile after mimetic use. This observation aligns with another study which observed a reduction in migration using GT in BRAF-melanoma [12].

In the analysis of patient samples, several genes exhibited distinct correlations with PRDX2, with notable differences observed in ZEB2 and STAT3. ZEB2 (Zinc finger E-box binding homeobox 2) negatively correlated with PRDX2 and is known to be associated with the E-cadherin promoter, thus activating the EMT pathway and contributing to metastatic invasions [35,36]. PRDX2 also regulates STAT3-mediated transcriptional activity through redox mechanisms, transferring oxidative equivalents to STAT3, hence their positive correlation [37].

Interestingly, melanomas with NRAS mutations had lower PRDX2 expression than those with BRAF mutations. Which may explain in part the more aggressive NRAS-mutated melanoma profile. When analyzed by mutation type, the NRAS group showed different gene correlations. Within the NRAS-mutant melanoma cohort, there were increases in ROS1 and KRT18 expression, and a decrease in CSNK1A1, all correlated with PRDX2. ROS1, a receptor tyrosine kinase involved in various cancers, regulates cell proliferation, differentiation, and growth [38]. KRT18 contributes to cytoskeletal filaments that influence cell architecture [39]. Additionally, CSNK1A1 plays a role in cytoskeletal dynamics and cell motility, with its increased expression associated with metastasis [40]. An inverse relationship between PRDX2 and EMT gene signatures was observed.

We analyzed primary tumors from the Leeds' cohort *in silico*. PRDX2 had a significant impact on survival across the entire cohort of primary tumor patients (n=703, 204 deaths); very low expression of PRDX2 was detrimental (only deaths from melanoma were analyzed). Additionally, these data revealed that tumors with lower PRDX2 expression exhibited higher Breslow thickness and staging, indicating higher malignancy levels [41,42]. These clinical findings suggest the potential of PRDX2 as a biomarker in metastatic melanoma. In an osteosarcoma study, PRDX2 has already been used as a biomarker to predict treatment response [43].

Although we have not conducted *in vivo* analyses, our data aligns with findings already observed in studies using mouse models with BRAF melanoma. Furthermore, we established an *in silico* relationship using patient data, which is highly relevant for prospecting this target in a clinical context. Nevertheless, our PRDX2 baseline results with high and low PRDX2 expression strongly indicate these relationships. Despite these limitations, this study has contributed to understanding the role of PRDX2 as a target and biomarker for metastatic melanoma, particularly in NRAS-mutated.

In conclusion, our data demonstrated that PRDX2 modulates EMT-like proteins such as MITF, β-catenin, N-cadherin, and Snail, which participate in the migration/invasion process. Also, PRDX2 is associated with favorable clinical features, and Gliotoxin could be used as a potential treatment. Furthermore, we established an *in silico* relationship using patient data, which is highly relevant for prospecting this target in a clinical context. This study contributes to our knowledge about genes

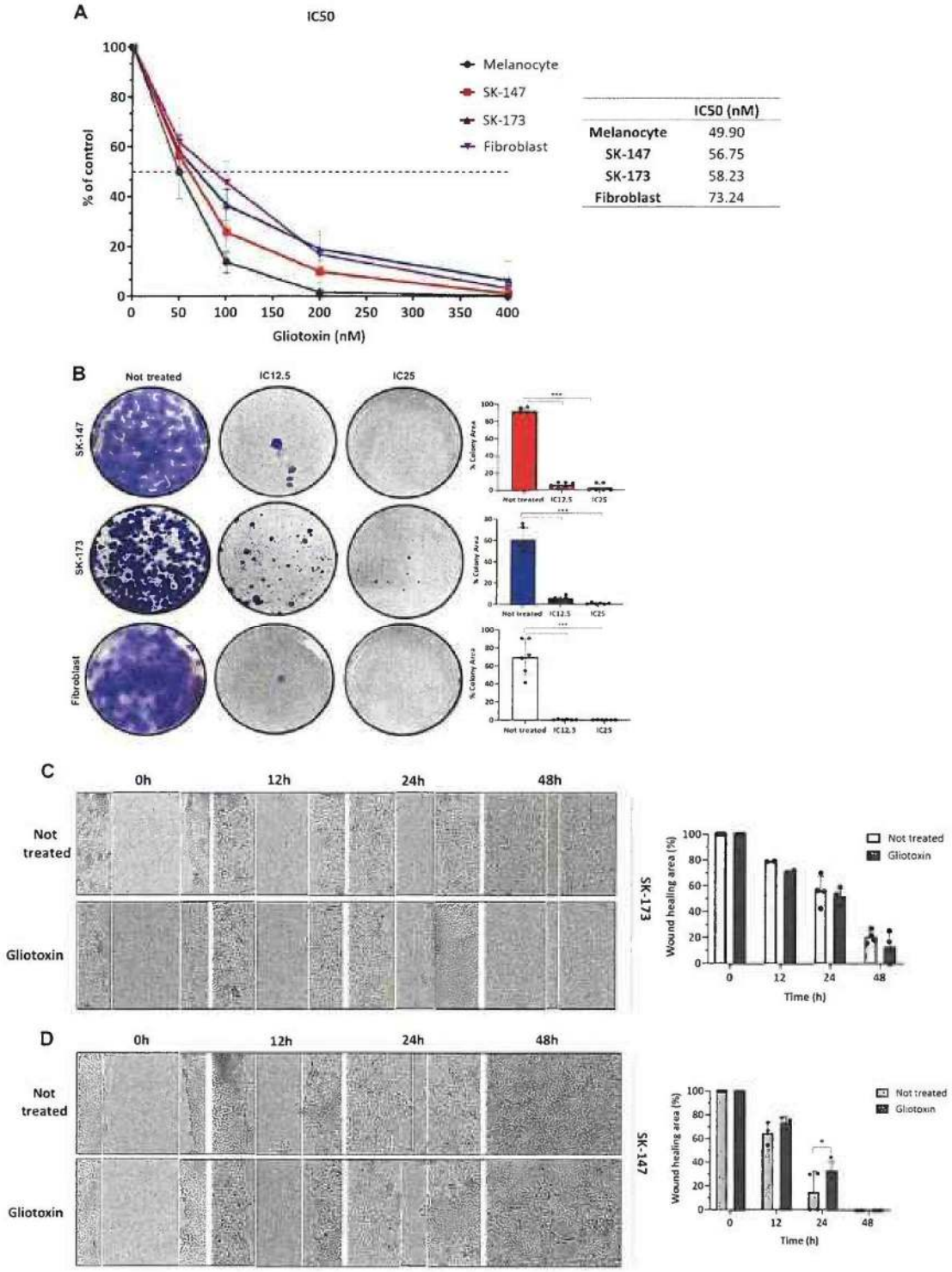

Fig. 4. Mimetic of PRDX2 inhibits cell proliferation, colony formation and migration ability in NRAS-melanoma cells. A Viability curve and table with calculated IC50 values. B Glyotoxin sensitivity test on NRAS-mutated melanoma and fibroblast (FP) cell lines for colony formation compared to untreated control using IC25 and IC12.5 concentration. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's test, ***p<0.001, **p<0.01 and *p<0.05. C-D Wound healing test for SK-MEL-173 and SK-MEL-147 with IC75 (99.7 nM for SK-MEL-147 and 114.1 nM for SK-MEL-173). Statistical analysis was performed by two-way analysis of variance (ANOVA) followed by Tukey's test, ***p<0.001, **p<0.01 and *p<0.05. A-D Values are indicated as mean ± SD of triplicate results from three independent experiments. Abbreviations: Primary melanocyte (MP), SK-MEL-147 (SK-147), and SK-MEL-173 (SK-173).

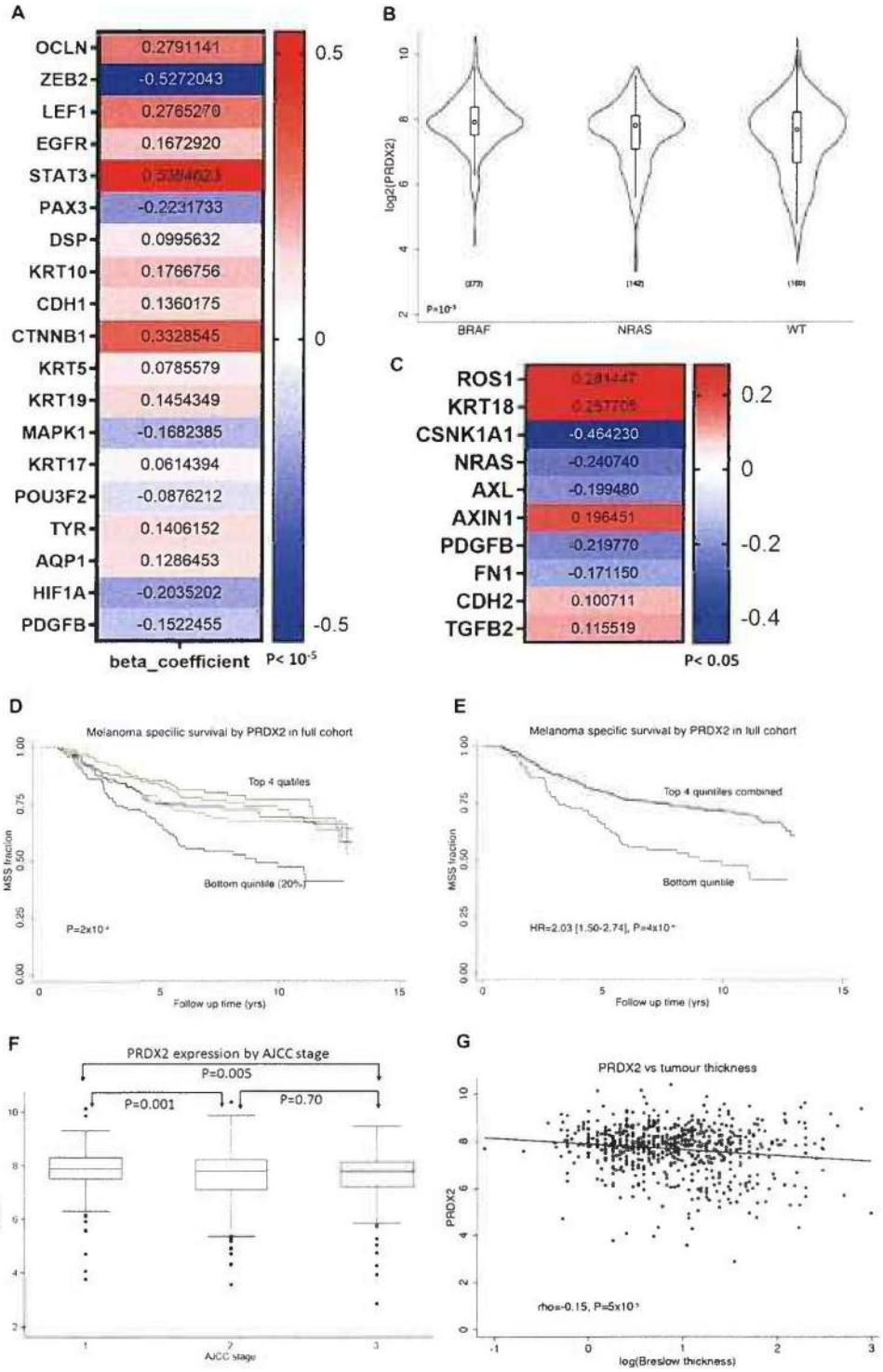

Fig. 5. Bioinformatic analysis in LMC from melanoma primary tumors: PRDX2 correlation with other genes and clinic features. A and C Regression coefficients between the expression of PRDX2 and other genes in primary melanomas in the whole LMC (n=703). Negative coefficients indicate a negative correlation while positive coefficients indicate a positive correlation. B PRDX2 tumor expression by mutation status (WT=double wild type, Pvalue=10^{-5}). C Regression coefficients between expressions of PRDX2 and other genes in NRAS-mutated primary melanoma subset of LMC (all Pvalues<0.001). D-E Melanoma specific survival (MSS) by PRDX2 in full cohort: the first 4 quintiles (80 % with high PRDX2 expression) were grouped after they appeared to have identical prognosis profiles. F Correlation of PRDX2 expression with primary tumor staging (AJCC). G Correlation of PRDX2 expression with the Breslow thickness of primary melanomas.

involved in phenotype switching and opens a new perspective for PRDX2 as a biomarker and target in NRAS-mutated melanomas.

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CRediT authorship contribution statement

Jérémie Nsengimana: Formal analysis, Data curation. **Julia-Newton Bishop:** Formal analysis, Data curation. **Sophia Tavares de Souza:** Methodology. **Manoel Oliveira de Moraes Junior:** Methodology, Formal analysis. **Renaira Oliveira Silva:** Methodology, Formal analysis. **Denisse Esther Mallaupoma Camarena:** Methodology, Formal analysis. **Larissa Anastacio da Costa Carvalho:** Writing – review & editing, Methodology, Data curation, Conceptualization, Formal analysis. **Isabella Harumi Yonehara Noma:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Silvya Stuchi Maria-Engler:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2024.116953.

References

[1] R.L. Siegel Mph, K.D. Miller, N. Sandeep, W. Mbbs, | Ahmedin, J. Dvm, R.L. Siegel, Cancer statistics, 2023, CA, Cancer J. Clin. 73 (2023) 17–48, https://doi.org/10.3322/CAAC.21763.

[2] F. Huang, F. Santinon, R.E. Flores González, S.V. del Rincón, Melanoma Plasticity: Promoter of Metastasis and Resistance to Therapy, Front. Oncol. 11 (2021), https://doi.org/10.3389/FONC.2021.756001.

[3] T. Randic, I. Kozar, C. Margue, J. Utikal, S. Kreis, NRAS mutant melanoma: Towards better therapies, Cancer Treat. Rev. 99 (2021), https://doi.org/10.1016/J.CTRV.2021.102238.

[4] D.B. Johnson, I. Puzanov, Treatment of NRAS-mutant melanoma, Curr. Treat. Options Oncol. 16 (2015) 15, https://doi.org/10.1007/s11864-015-0330-z.

[5] M. Berger, G. Richtig, K. Kashofer, A. Aigelsreiter, E. Richtig, The window of opportunities for targeted therapy in BRAFwt/NRASwt/KITwt melanoma: biology and clinical implications of fusion proteins and other mutations, G. Ital. Di Dermatol. e Venereol. Organo Uff. Soc. Ital. Di Dermatol. e Sifilogr. 153 (2018) 349–360, https://doi.org/10.23736/S0392-0488.18.05970-9.

[6] F. Rambow, J.C. Marine, C.R. Goding, Melanoma plasticity and phenotypic diversity: therapeutic barriers and opportunities, Genes Dev. 33 (2019) 1295–1318, https://doi.org/10.1101/GAD.329771.119.

[7] J. Wang, D. Sun, L. Huang, S. Wang, Y. Jin, Targeting Reactive Oxygen Species Capacity of Tumor Cells with Repurposed Drug as an Anticancer Therapy, Oxid. Med. Cell. Longev. 2021 (2021), https://doi.org/10.1155/2021/8532940.

[8] S.W. Kang, S. Lee, J.H.S. Lee, Cancer-Associated Function of 2-Cys Peroxiredoxin Subtypes as a Survival Gatekeeper, Antioxid. (Basel, Switz.) 7 (2018), https://doi.org/10.3390/ANTIOX7110161.

[9] L.A.C. Carvalho, R.G. Queijo, A.L.B. Baccaro, Á.D.D. Siena, W.A. Silva, T. Rodrigues, S.S. Maria-Engler, Redox-Related Proteins in Melanoma Progression, Antioxid. (Basel, Switz.) 11 (2022), https://doi.org/10.3390/ANTIOX11030438.

[10] L.A.C. Carvalho, I.H.Y. Noma, A.H. Uehara, Á.D.D. Siena, L.H. Osaki, M.P. Mori, N. C. Souza-Pinto, V.M. Freitas, W.A.S. Junior, K.S.M. Smalley, S.S. Maria-Engler, Modeling Melanoma Heterogeneity In Vitro: Redox, Resistance and Pigmentation Profiles, Antioxid. (Basel, Switz.) 13 (2024), https://doi.org/10.3390/antiox13050555.

[11] H.R. Hintsala, Y. Soini, K.M. Haapasaari, P. Karihtala, Dysregulation of redox-state-regulating enzymes in melanocytic skin tumours and the surrounding microenvironment, Histopathology 67 (3) (2015) 348–357, https://doi.org/10.1111/his.12659. PMID: 25627040.

[12] D.J. Lee, D.H. Kang, M. Choi, Y.J. Choi, J.Y. Lee, J.H. Park, Y.J. Park, K.W. Lee, S. W. Kang, Peroxiredoxin-2 represses melanoma metastasis by increasing E-cadherin/β-catenin complexes in adherens junctions, Cancer Res 73 (2013) 4744–4757, https://doi.org/10.1158/0008-5472.CAN-12-4226/650915/AM/PEROXIREDOXIN-2-REPRESSES-MELANOMA-METASTASIS-BY.

[13] S.W. Kang, S.G. Rhee, T.S. Chang, W. Jeong, M.H. Choi, 2-Cys peroxiredoxin function in intracellular signal transduction: therapeutic implications, Trends Mol. Med. 11 (2005) 571, https://doi.org/10.1016/J.MOLMED.2005.10.006.

[14] N. Chandimali, D.K. Jeong, T. Kwon, Peroxiredoxin II Regulates Cancer Stem Cells and Stemness-Associated Properties of Cancers, Cancers (Basel) 10 (2018), https://doi.org/10.3390/CANCERS10090305.

[15] F. Liu, Y. Fu, F.L. Meyskens, MiTF regulates cellular response to reactive oxygen species through transcriptional regulation of APE-1/Ref-1, J. Invest. Dermatol. 129 (2009) 422–431, https://doi.org/10.1038/JID.2008.255.

[16] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods 25 (2001) 402–408, https://doi.org/10.1006/METH.2001.1262.

[17] C. Guzmán, M. Bagga, A. Kaur, J. Westermarck, D. Abankwa, ColonyArea: an ImageJ plugin to automatically quantify colony formation in clonogenic assays, PLoS One 9 (2014) e92444, https://doi.org/10.1371/journal.pone.0092444.

[18] C.A. Brohem, L.B. Da Silva Cardeal, M. Tiago, M.S. Soengas, S.B. De Moraes Barros, S.S. Maria-Engler, Artificial skin in perspective: concepts and applications, Pigment Cell Melanoma Res 24 (2011) 35–50, https://doi.org/10.1111/J.1755-148X.2010.00786.X.

[19] D.E.M. Camarena, L.S.A.S. Matsuyama, S.S. Maria-Engler, L.H. Catalani, Development of Epidermal Equivalent from Electrospun Synthetic Polymers for In Vitro Irritation/Corrosion Testing, Nanomater. 2020, Vol. 10, Page 2528 10 (2020) 2528, https://doi.org/10.3390/NANO10122528.

[20] J. Nsengimana, J. Laye, A. Filia, S. O'Shea, S. Muralidhar, J. Pożniak, A. Droop, M. Chan, C. Walker, L. Parkinson, J. Gascoyne, T. Mell, M. Polso, R. Jewell, J. Randerson-Moor, G.P. Cook, D.Timothy Bishop, J. Newton-Bishop, β-Catenin-mediated immune evasion pathway frequently operates in primary cutaneous melanomas, J. Clin. Invest. 128 (2018) 2048–2063, https://doi.org/10.1172/JCI95351.

[21] P. Du, W.A. Kibbe, S.M. Lin, lumi: a pipeline for processing Illumina microarray, Bioinformatics 24 (2008) 1547–1548, https://doi.org/10.1093/BIOINFORMATICS/BTN224.

[22] M. Lauss, I. Visne, A. Kriegner, M. Ringnér, G. Jönsson, M. Höglund, Monitoring of technical variation in quantitative high-throughput datasets, Cancer Inf. 12 (2013) 193–201, https://doi.org/10.4137/CIN.S12862.

[23] S.H. Hong, C. Min, Y. Jun, D.J. Lee, S.H. Kim, J.H. Park, J.H. Cheong, Y.J. Park, S. Y. Kim, S. Lee, S.W. Kang, Silencing of peroxiredoxin II by promoter methylation is necessary for the survival and migration of gastric cancer cells, Exp. Mol. Med. 50 (2018), https://doi.org/10.1038/EMM.2017.267.

[24] J. Müller, O. Krijgsman, J. Tsoi, L. Robert, W. Hugo, C. Song, X. Kong, P.A. Possik, P.D.M. Cornelissen-Steijger, M.H.G. Foppen, K. Kemper, C.R. Goding, U. McDermott, C. Blank, J. Haanen, T.G. Graeber, A. Ribas, R.S. Lo, D.S. Peeper, Low MITF/AXL ratio predicts early resistance to multiple targeted drugs in melanoma, Nat. Commun. 5 (2014), https://doi.org/10.1038/NCOMMS6712.

[25] M.R. Webster, C.H. Kugel, A.T. Weeraratna, The Wnts of change: How Wnts regulate phenotype switching in melanoma, Biochim. Biophys. Acta 1856 (2015) 244–251, https://doi.org/10.1016/J.BBCAN.2015.10.002.

[26] S.M. Hossain, M.R. Eccles, Phenotype Switching and the Melanoma Microenvironment; Impact on Immunotherapy and Drug Resistance, Int. J. Mol. Sci. 24 (2023), https://doi.org/10.3390/ijms24021601.

[27] E. Tuncer, R.R. Calçada, D. Zingg, S. Varum, P. Cheng, S.N. Freiberger, C.-X. Deng, I. Kleiter, M.P. Levesque, R. Dummer, L. Sommer, SMAD signaling promotes melanoma metastasis independently of phenotype switching, J. Clin. Invest. 129 (2019) 2702–2716, https://doi.org/10.1172/JCI94295.

[28] B.De Craene, G. Berx, Regulatory networks defining EMT during cancer initiation and progression, Nat. Rev. Cancer 13 (2013) 97–110, https://doi.org/10.1038/nrc3447.

[29] L. Hao, J.R. Ha, P. Kuzel, E. Garcia, S. Persad, Cadherin switch from E- to N-cadherin in melanoma progression is regulated by the PI3K/PTEN pathway

through Twist and Snail, Br. J. Dermatol. 166 (2012) 1184–1197, https://doi.org/10.1111/J.1365-2133.2012.10824.X.
[30] D. Ribatti, R. Tamma, T. Annese, Epithelial-Mesenchymal Transition in Cancer: A Historical Overview, Transl. Oncol. 13 (2020), https://doi.org/10.1016/J.TRANON.2020.100773.
[31] S. Spada, A. Tocci, F. Di Modugno, P. Nisticò, Fibronectin as a multiregulatory molecule crucial in tumor matrisome: from structural and functional features to clinical practice in oncology, J. Exp. Clin. Cancer Res. 40 (2021), https://doi.org/10.1186/S13046-021-01908-8.
[32] A. Leikeim, M. Wußmann, F.F. Schmidt, N.G.B. Neto, F. Benz, K. Tiltmann, C. Junger, M.G. Monaghan, B. Schilling, F.K. Groeber-Becker, A preclinical model of cutaneous melanoma based on reconstructed human epidermis, Sci. Rep. 12 (2022), https://doi.org/10.1038/S41598-022-19307-0.
[33] F. Carta, P.R. Demuro, C. Zanini, A. Santona, D. Castiglia, S. D'Atri, P.A. Ascierto, M. Napolitano, A. Cossu, B. Tadolini, F. Turrini, A. Manca, M.C. Sini, G. Palmieri, C. Rozzo, Analysis of candidate genes through a proteomics-based approach in primary cell lines from malignant melanomas and their metastases, Melanoma Res 15 (2005) 235–244, https://doi.org/10.1097/00008390-200508000-00002.
[34] H.S. Choi, J.S. Shim, J.A. Kim, S.W. Kang, H.J. Kwon, Discovery of gliotoxin as a new small molecule targeting thioredoxin redox system, Biochem. Biophys. Res. Commun. 359 (2007) 523–528, https://doi.org/10.1016/J.BBRC.2007.05.139.
[35] M. Fardi, M. Alivand, B. Baradaran, M. Farshdousti Hagh, S. Solali, The crucial role of ZEB2: From development to epithelial-to-mesenchymal transition and cancer complexity, J. Cell. Physiol. 234 (2019) 14783–14799, https://doi.org/10.1002/JCP.28277.
[36] A. Sathyanarayanan, K.S. Chandrasekaran, D. Karunagaran, microRNA-145 modulates epithelial-mesenchymal transition and suppresses proliferation, migration and invasion by targeting SIP1 in human cervical cancer cells, Cell. Oncol. (Dordr.). 40 (2017) 119–131, https://doi.org/10.1007/S13402-016-0307-3.
[37] M.C. Sobotta, W. Liou, S. Stöcker, D. Talwar, M. Oehler, T. Ruppert, A.N.D. Scharf, T.P. Dick, Peroxiredoxin-2 and STAT3 form a redox relay for H2O2 signaling, Nat. Chem. Biol. 11 (2015) 64–70, https://doi.org/10.1038/NCHEMBIO.1695.
[38] A.T. Shaw, S.-H.I. Ou, Y.-J. Bang, D.R. Camidge, B.J. Solomon, R. Salgia, G.J. Riely, M. Varella-Garcia, G.I. Shapiro, D.B. Costa, R.C. Doebele, L.P. Le, Z. Zheng, W. Tan, P. Stephenson, S.M. Shreeve, L.M. Tye, J.G. Christensen, K.D. Wilner, J.W. Clark, A. J. Iafrate, Crizotinib in ROS1-rearranged non-small-cell lung cancer, N. Engl. J. Med. 371 (2014) 1963–1971, https://doi.org/10.1056/NEJMOA1406766.
[39] A.M. Fortier, E. Asselin, M. Cadrin, Keratin 8 and 18 loss in epithelial cancer cells increases collective cell migration and cisplatin sensitivity through Claudin1 up-regulation, J. Biol. Chem. 288 (2013) 11555, https://doi.org/10.1074/JBC.M112.428920.
[40] D. Sun, M. Zhou, C.M. Kowolik, V. Trisal, Q. Huang, K.H. Kernstine, F. Lian, B. Shen, Differential expression patterns of capping protein, protein phosphatase 1 and casein kinase 1 may serve as diagnostic markers for malignant melanoma, Melanoma Res 21 (2011) 335, https://doi.org/10.1097/CMR.0B013E328346B715.
[41] M.A. El Sharouni, R.V. Rawson, V. Sigurdsson, A.J. Witkamp, C.H. van Gils, R. A. Scolyer, J.F. Thompson, P.J. van Diest, S.N. Lo, The progressive relationship between increasing Breslow thickness and decreasing survival is lost in patients with ultrathick melanomas (≥15 mm in thickness), J. Am. Acad. Dermatol. 87 (2022) 298–305, https://doi.org/10.1016/J.JAAD.2022.01.040.
[42] R.A. Scolyer, R.V. Rawson, J.E. Gershenwald, P.M. Ferguson, V.G. Prieto, Melanoma pathology reporting and staging, Mod. Pathol. 33 (2020) 15–24, https://doi.org/10.1038/S41379-019-0402-X.
[43] D. Kubota, K. Mukaihara, A. Yoshida, H. Tsuda, A. Kawai, T. Kondo, Proteomics study of open biopsy samples identifies peroxiredoxin 2 as a predictive biomarker of response to induction chemotherapy in osteosarcoma, J. Proteom. 91 (2013) 393–404, https://doi.org/10.1016/j.jprot.2013.07.022.

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Newcastle upon Tyne, 27 October 2025

United States Department of Homeland Security
U.S. Citizenship and Immigration Services

Re: Letter of support to Dr. Isabella Harumi Yonehara Noma

To whom it may concern

I am pleased to write this letter of support to Dr Isabella Harumi Yonehara Noma's petition for classification as an individual of extraordinary ability under the EB-1 category. I do not personally know Dr Noma; however, as an academic working in the same field, I am familiar with her work through her publications and scientific presentations. During conduct of her PhD, I advised on one of her projects after being contacted by her supervisor, resulting in a joint publication in 2024 (Noma et al., 2024). Having interacted with Dr Noma, I am able to evaluate her scientific aptitudes.

I am a Senior Lecturer in Biostatistics at Newcastle University (UK), with a PhD in Statistical Genetics. I conduct original research and co-authored multiple high impact publications. I lecture in postgraduate programs and supervise dissertations. An important portfolio of my research is melanoma biomarking aimed at patient stratification and new drug target discovery. Dr Noma's work has similar objectives, which is how our paths crossed.

Dr Noma is undeniably skilled and has already made considerable achievements in melanoma research at this early stage of scientific career. To date, she has published eleven peer-reviewed articles in respected journals, including six in 2024 alone, which are attracting the attention of peers with a growing number of citations (Google Scholar H-index 6). In the publication that we co-authored (Noma et al., 2024), the original

experiments that she skillfully and tenaciously conducted provided for the first time the evidence that a gene called PRDX2 modulates the epithelial–mesenchymal transition (EMT) in NRAS-mutated melanomas. This was a truly transformative finding in the puzzling mechanism of cancer phenotype switching (theory of "grow or go", i.e. growing locally or reprogramming to metastasize). This mechanism induces drug resistance and is currently a major bottleneck in cancer research. Dr Noma's finding was therefore significant, it suggested potential new diagnostic testing and new treatment options, based on antioxidants. My own contribution to this exciting work was to confirm this hypothesis in a databank of 700 primary melanoma patients collected in the UK.

Since that work, Dr. Noma has developed an innovative post-doctoral research project to test the hypothesis that UVR-induced activation of another gene called HDAC8 allows some melanocytes to avoid cell death and escape the immune attack. She aims to define how UVR induced epigenetic reprogramming leads to the persistence of melanocytes with pathogenic mutations which later develop into melanoma. Understanding these complex mechanisms can be harnessed into improved melanoma diagnosis and more efficient patient care. I have no doubt that Dr Noma will continue to use her skills and dedication in this project with a huge translational potential. Currently, Dr. Noma is working as a Postdoctoral Fellow in the Tumor Microenvironment and Metastasis Department at the Moffitt Cancer Center, under an O-1A visa, which is granted to individuals of extraordinary ability in their field. This prestigious position in one of the leading cancer research institutions further reinforces her recognition as a scientist of exceptional merit.

In summary, it is abundantly clear that Dr Noma is a highly capable researcher full of skills, energy and tenacity, all of which are key ingredients for academic success. Her extraordinary abilities have allowed her to make impactful achievements. Her advanced scientific knowledge and dedication are demonstrated by an impressive and steadily growing number of original publications that very few of her peers can match at this career stage. She has the potential to make discoveries that could be vital for the public health in the United States and the World. I strongly support Dr Noma's EB-1 visa application.

Sincerely,

Dr Jeremie Nsengimana

Co-authored article:
Noma IHY, Carvalho LADC, Camarena DEM, Silva RO, Moraes Junior MO, de Souza ST, Newton-Bishop J, **Nsengimana J**, Maria-Engler SS (2024). *Peroxiredoxin-2 represses NRAS-mutated melanoma cells invasion by modulating EMT markers*. Biomedicine & Pharmacotherapy 177:116953 doi:10.1016/j.biopha.2024.116953.

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Resume

Senior Lecturer (Associate Professor) of Biostatistics at Newcastle University Faculty of Medical Sciences (UK). Previously worked at the University of Leeds School of Medicine (UK) and the International Agency for Research on Cancer (Lyon, France). PhD in Statistical Genetics (UCLouvain, Belgium, 2003). Lead research in efficient design and analysis of preclinical translational studies with a particular focus on biomarker and drug target discovery to inform Stratified Medicine. Member of international consortia in melanoma, testicular and liver cancers. Organiser of scientific conferences and workshops, member of the International Society of Clinical Biostatisics (ISCB), the International Genetic Epidemiology Society (IGES) and the American Association for Cancer Research (AACR).

Research portfolio funded by the US NIH (Testicular cancer consortium), UK NIHR (Parkinson disease consortium), Horizon Europe (Liver cancer consortium), and charities (Diabetes UK, Motor Neuron Disease Scotland, Welcome Trust, JGW Patterson Foundation...). Successfully supervised 3 PhDs, currently supervise 2 with an additional 2 to start next month (09/2024). Published 70 original articles (Google scholar H=32, i10=44). Below are selected publications (full list on my website).

List of publications

1. Noma IHY, Carvalho LADC, Camarena DEM, Silva RO, Moraes Junior MO, de Souza ST, Newton-Bishop J, **Nsengimana** J, Maria-Engler SS (2024). Peroxiredonixin-2 represses NRAS-mutated melanoma cells invasion by modulating EMT markers. *Biomedicine & Pharmacotherapy*, 177:116953.
2. Godson L, Alemi N, **Nsengimana J**, Cook GP, Clarke EL, Treanor D, Bishop DT, Newton-Bishop J, Gooya A, Magee D (2024). Immune subtyping of melanoma whole slide images using multiple instance learning. *Medical Image Analysis* 93:103097
3. Tagore M, Hergenreder E, Perlee SC, Cruz NM, Menocal L, Suresh S, Chan E, Baron M, Melendez S, Dave A, Chatila WK, **Nsengimana J,** Koche RP, Hollmann TJ, Ideker T, Studer L, Schietinger A, White RM (2023). GABA regulates electrical activity and tumour initiation in melanoma. *Cancer Discovery* 13:2270-2291.
4. Suresh S, Rabbie R, Garg M, Lumaquin D, Huang TH, Montal E, Ma Y, Cruz NM, Tang X, **Nsengimana J**, Newton-Bishop J, Hunter MV, Zhu Y, Chen K, de Stanchina E, Adams DJ, White RM (2023). Identifying the transcriptional drivers of metastasis embedded within localized melanoma. *Cancer Discovery* 13:194-215.
5. Castaneda-García C, Iyer V, **Nsengimana** J, Trower A, Droop A, Brown KM, Choi J, Zhang T, Harland M, Newton-Bishop JA, Bishop DT, Adams DJ, Iles MM, Robles-Espinoza CD (2022). Defining novel causal SNPs and linked phenotypes at melanoma-associated loci. *Human Molecular Genetics* 31: 2845-2856.
6. Papaiz DD, Rius FE, Ayub ALP, Oligassa CS, Gujar H, Pessoa DO, Reis EM, **Nsengimana J**, Newton-Bishop J, Mason CE, Weisenberger DJ, Liang G, Jasiulionis MG (2022). Genes regulated by DNA methylation are involved in distinct phenotypes during melanoma progression and are prognostic factors for patients. *Molecular Oncology* 16:1913-1930.
7. Davies J, Muralidhar S, Randerson-Moor J, Harland M, O'Shea S, Diaz J, Walker C, **Nsengimana J**, Laye J, Mell T, Chan M, Appleton L, Birkeälv S, Adams DJ, Cook GP, Ball G, Bishop DT, Newton-Bishop JA (2022). Ulcerated melanoma: Systems biology evidence of inflammatory imbalance towards pro-tumourigenicity. *Pigment Cell and Melanoma Research* 35:252-267.

8. Bi, O, Anene CA, **Nsengimana J**, Shelton M, Roberts W, Newton-Bishop J, Boyne JR (2021). SFPQ promotes an oncogenic transcriptomic state in melanoma, *Oncogene* 40(33):5192-5203.

9. Garg M, Couturier DL, **Nsengimana J**, Fonseca NA, Wongchenko M, Yan Y, Lauss M, Jönsson GB, Newton-Bishop J, Parkinson C, Middleton MR, Bishop DT, McDonald S, Stefanos N, Tadross J, Vergara IA, Lo S, Newell F, Wilmott JS, Thompson JF, Long GV, Scolyer RA, Corrie P, Adams DJ, Brazma A, Rabbie R (2021). Tumour gene expression signature in primary melanoma predicts long-term outcomes. *Nature Communications* 12: 1137.

10. Pessoa DO, Rius FE, Papaiz DD, Ayub ALP, Morais AS, de Souza CF, da Paixão VF, Setubal JC; Newton-Bishop J, **Nsengimana J**, Azevedo H, Reis EM, Jasiulionis MG (2021). Transcriptional signatures underlying dynamic phenotypic switching and novel disease biomarkers in a linear cellular model of melanoma progression. *Neoplasia* 23: 439-455.

11. Juraleviciute M, **Nsengimana** J, Newton-Bishop Julia, Hendriks GJ, Slipicevic A (2021). MX2 mediates establishment of interferon response profile, regulates XAF1 and can sensitize melanoma cells to targeted therapy. *Cancer Medicine* 10:2840–2854

12. Emran AA, **Nsengimana J**, Punnia-Moorthy G, Schmitz U, Gallagher SJ, Newton-Bishop J, Tiffen JC, Hersey P (2020). Study of the female sex survival advantage in melanoma – A focus on X-linked epigenetic regulators and immune responses in two cohorts. *Cancers (Basel)*: 12: 2082.

13. Maurichi A, Miceli R, Eriksson H, Newton-Bishop J, **Nsengimana J**, Chan M, Hayes AJ, Heelan K, Adams D, Patuzzo R, Barretta F, Gallino G, Harwood C, Bergamaschi D, Bennett D, Lasithiotakis K, Ghiorzo P, Dalmasso B, Manganoni A, Consoli F, Mattavelli I, Barbieri C, Leva A, Cortinovis U, Espeli V, Mangas C, Quaglino P, Ribero S, Broganelli P, Pellacani G, Longo C, Del Forno C, Borgognoni L, Sestini S, Pimpinelli N, Fortunato S, Chiarugi A, Nardini P, Morittu E, Florita A, Cossa M, Valeri B, Milione M, Pruneri G, Zoras O, Anichini A, Mortarini R, Santinami M J (2020). Factors affecting sentinel node metastasis in thin cutaneous melanomas: development and external validation of a predictive nomogram. *Journal of Clinical Oncology* 38:1591-1601

14. Close HJ, Stead LF, **Nsengimana J**, Reilly KA, Droop A, Wurdak H, Mathew RK, Corns R, Newton-Bishop J, Melcher AA, Short SC, Cook GP, Wilson EB (2020). Expression profiling of single cells and patient cohorts identifies multiple immunosuppressive pathways and altered NK cell phenotype in glioblastoma. *Clinical and Experimental Immunology* 200:33-44.

15. Muralidhar S, Filia A, **Nsengimana J**, Poźniak J, O'Shea SJ, Diaz JM, Harland M, Randerson-Moor JA, Reichrath J, Laye JP, van der Weyden L, Adams DJ, Bishop DT, Newton-Bishop J (2019). Vitamin D-VDR signalling inhibits Wnt/beta-catenin signalling-mediated melanoma progression and promotes antitumor immunity. *Cancer Research* 79:5986-5998.

16. Thakur R, Laye JP, Lauss M, Diaz JMS, O'Shea SJ, Poźniak J, Filia A, Harland M, Gascoyne J, Randerson-Moor JA, Chan M, Mell T, Jönsson G, Bishop DT, Newton-Bishop J, Barrett JH, **Nsengimana J** (2019). Transcriptomic analysis reveals prognostic molecular signatures of stage I melanoma. *Clinical Cancer Research* 25:7424-7435.

17. Poźniak J, **Nsengimana J**, Laye J, O'Shea S, Harland M, Diaz JMS, Filia A, Muralidhar S, Mell T, Polso M, Droop A, Schindler S, Levesque M, Randerson-Moor J, Cook GP, Bishop DT, Newton-Bishop J (2019). Genetic and environmental factors modulating immune response to melanoma. *Cancer Research* 79:2684-96.

18. **Nsengimana J**, Laye J, Filia A, Droop A, Pozniak J, Chan M, O'Shea S, Walker C, Parkinson L, Polso M, Jewell R, Randerson-Moor J, Cook GP, Bishop DT, Newton-Bishop J (2018). Beta-catenin-mediated immune evation pathway frequently operates in primary cutaneous melanoma. *Journal of Clinical Investigations* 128:2048-2063.

19. Litchfield K, Levy M, Orlando G, Law P, Migliorini G, Holroyd A, Broderick P, **Nsengimana J**, Fenwick K, Assiotis I, Muir K, Peto J, Eeles R, Easton DF, Dudakia D, Orr N, Bishop DT, Reid A, Huddart RA, Shipley J, Grotmol T, Wiklund F, Houlston RS, Turnbull C (2017). Identification of 19 new risk loci and potential regulatory mechanisms influencing susceptibility to testicular germ cell tumour. *Nature Genetics* 49:1133-40.

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Modeling Melanoma Heterogeneity In Vitro: Redox, Resistance and Pigmentation Profiles

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Abstract: Microenvironment and transcriptional plasticity generate subpopulations within the tumor, and the use of BRAF inhibitors (BRAFis) contributes to the rise and selection of resistant clones. We stochastically isolated subpopulations (C1, C2, and C3) from naïve melanoma and found that the clones demonstrated distinct morphology, phenotypic, and functional profiles: C1 was less proliferative, more migratory and invasive, less sensitive to BRAFis, less dependent on OXPHOS, more sensitive to oxidative stress, and less pigmented; C2 was more proliferative, less migratory and invasive, more sensitive to BRAFis, less sensitive to oxidative stress, and more pigmented; and C3 was less proliferative, more migratory and invasive, less sensitive to BRAFis, more dependent on OXPHOS, more sensitive to oxidative stress, and more pigmented. Hydrogen peroxide plays a central role in oxidative stress and cell signaling, and PRDXs are one of its main consumers. The intrinsically resistant C1 and C3 clones had lower MITF, PGC-1α, and PRDX1 expression, while C1 had higher AXL and decreased pigmentation markers, linking PRDX1 to clonal heterogeneity and resistance. PRDX2 is depleted in acquired BRAFi-resistant cells and acts as a redox sensor. Our results illustrate that decreased pigmentation markers are related to therapy resistance and decreased antioxidant defense.

Keywords: melanoma; heterogeneity; pigmentation; peroxiredoxin 1; peroxiredoxin 2; BRAFi resistance

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1. Introduction

Melanoma is the most aggressive type of skin cancer [1,2]. Although BRAF inhibitors (BRAFi) have revolutionized therapy, residual disease persists and relapse is very common [3–6]. Melanoma is a heterogeneous tumor and BRAFi treatment contributes to the selection of intrinsic and acquired resistant subpopulations [7–9]. The key biomarker for this phenotype switching is the melanocyte lineage-specific transcription factor MITF (microphthalmia-associated transcription factor) [10,11]. It has been demonstrated that the $MITF^{high}/AXL^{low}$ phenotype is linked to BRAFi sensitivity while $MITF^{low}/AXL^{high}$ predicts resistance [12]. MITF is also linked to oxidative metabolism by controlling the transcriptional cofactor PGC-1α (peroxisome proliferator-activated receptor γ coactivator 1), which activates mitochondrial biogenesis and oxidative phosphorylation (OXPHOS) [13,14]. Increased expression of PGC-1α can restore OXPHOS in melanoma and, consequently, lead to higher ROS production by the mitochondria [14,15]. Acquired mutations in BRAF

suppresses OXPHOS, MITF, and PGC-1α expression, while BRAFi increases OXPHOS, MITF, PGC-1α, and ROS production. Increased ROS levels makes the tumor more sensitive to oxidative stress [16,17], rendering cells highly dependent on antioxidant defense. BRAFi-resistant cells frequently have adaptive antioxidant mechanisms to tolerate ROS [18]. PGC-1 coactivators are regulators of melanin production via regulation of MITF and melanin production itself is an oxidative process that generates ROS [19,20]. It is known that PGC-1s regulates broad protective programs against ROS [21] and MITF has also been implicated in controlling ROS [22]. Coregulation of melanogenesis and an anti-ROS program by PGC-1s may thus be critical for protecting activated melanocytes from ROS damage [13]. However, the role of melanin on metastasis and resistance remains poorly explored at the heterogeneity level [23].

Transient production of ROS is necessary for cell signaling, but persistent production could be cytotoxic. In this sense, cancer cells require increased levels of ROS for continuous proliferation, but overexpress cellular peroxidases such as peroxiredoxins (PRDXs) to maintain homeostasis [24]. PRDXs are antioxidant enzymes that react with ROS and RNS, and they play a central role in redox signaling acting as redox sensors [25–27]; also, the oxidation state of PRDXs, provides information about redox homeostasis [28,29]. Moreover, PRDXs are differentially expressed in patients during melanoma progression [30] and decreased expression of PRDX1 and PRDX2 is a biomarker in melanoma compared to nevus [31]. Additionally, the role of PRDX2 in preventing invasion in BRAF-mutant melanoma has been described in a heterogenous population [32], but its roles in heterogeneity, resistance, and pigmentation are not known [31]. In this study, we investigated clonal heterogeneity and demonstrated a link among pigmentation, resistance, and oxidative stress that could help better understand therapy failure.

2. Materials and Methods

2.1. Human Melanoma Cell Culture

The human melanoma cell line (SK-MEL-28) was kindly donated by Dr. Maria S. Soengas (Melanoma Group, CNIO, Spain). The SK-MEL-28 BRAFi-resistant cells (SK-MEL-28R) were generated using vemurafenib (PLX4032) as previously described [33]. STR profiling was performed in naïve cells. Cells were cultured in DMEM supplemented with 10% FBS and antibiotics at 37 °C, 5% CO_2 and humidified atmosphere. All cells were tested for mycoplasma.

2.2. Clonal Subpopulations Isolation

Stochastic isolation was performed by seeding naïve SK-MEL-28 one cell per well. Cells were cultivated until confluence was reached and three distinct clones were isolated, called C1, C2, and C3.

2.3. Cell Proliferation

Cells (1×10^4 cells/well) were harvested at times 24, 72, 120, and 168 h, and the viable cells were counted using trypan blue in a Neubauer chamber. Doubling times were obtained by non-linear regression analysis with GraphPad Prism 5 (La Jolla, CA, USA).

2.4. Wound Healing Assay

The cell (5×10^4 cells/well) monolayer was scratched using a pipette tip after reaching more than 90% confluence and maintained in DMEM containing 1% FBS. Images were taken at 0, 12, and 24 h until the control wound was fully closed. Images were processed and quantified in ImageJ 8 (Bethesda, MD, USA). Results represent the measurements of each wounded area in three independent experiments.

2.5. Evaluation of Intrinsic BRAFi Resistance

Cells (2×10^4 cells/well) were treated with 0, 1, or 6 μM of BRAFi. At times 24, 48, and 72 h, viable cells were counted using trypan blue in a Neubauer chamber.

2.6. Colony Formation Assay

Cells (1000 cells/well) were treated with 0, 1, and 6 μM BRAFi every 2–3 days for 4 weeks. They were then washed with PBS, fixed, and stained with violet crystal solution for 10 min at RT.

2.7. Protein Levels by Western Blot

Cells were lysed with RIPA buffer (Tris 10 mM pH 7.5, NaCl 150 mM, NP 40 1%, SDS 0.1%, Sodium Deoxycolate 1%, EDTA 5 mM, EGTA 5 mM, NaF 25 mM and Na_2VO_4 1 mM) and a mixture of protease and phosphatase inhibitors. For non-reducing Western blot, N-ethylmaleimide 30 mM was added. Proteins were quantified by Bradford, separated by SDS-PAGE under reducing or non-reducing buffer, transferred to a PVDF membrane, and incubated for 1 h at RT with blocking solution (BSA 5%). Then, the membrane was incubated overnight at 4 °C with the primary antibody (1:1000). Next day, the membrane was incubated for 1 h with the secondary antibody (1:2500) and protein bands detected by an ECL system (Luminata™ Forte Western HRP Substrate) (Millipore Corporation, FL, USA) in an Amersham Imager 680 device. Relative densitometry was semi-quantified by ImageJ.

2.8. mRNA Levels by RT-qPCR

RNA was extracted with the RNeasy Plus Mini Kit (Qiagen, Hilden, Germany). Purity was evaluated by absorbance at 260/280 and 260/230 nm. The cDNA was obtained by ThermoScript™ RT-PCR System kit from Invitrogen and amplified with the Taqman Mix® Master and Real Time PCR Assays (Thermo Fischer, Waltham, MA, USA). Transcriptions were normalized with β-actin levels in the samples using the Real-Time PCR System from Applied Biosystems™ StepOne™. Relative expression was calculated using the ΔΔCt method [34].

2.9. Oxygen Consumption Rate (OCR)—Seahorse XF24 Flux Analyzer

OCR was measured in intact cells using the XF24 Extracellular Flow Analyzer (Seahorse Bioscience, North Billerica, MA, USA). Cells (3×10^4 cells/well) were plated in XF24 V7 cell culture microplates. After 12–16 h, XF test medium supplemented with D-glucose 10 mM, sodium pyruvate 1 mM, and GlutaMAX™ (Gibco™) (Thermo Fischer, Waltham MA, USA)2 mM was added and incubated in a free-of-CO_2 atmosphere at 37 °C for 1 h. Respiratory parameters were analyzed based on the protocol for the Seahorse XF cell stress test kit with minor modifications. Three background measurements were performed before the addition of mitochondrial inhibitors. Sequential additions of 1.5 μM oligomycin, 30 μM dinitrophenol (DNP), and 1 μM rotenone plus antimycin A were made. The results are shown relative to the background.

2.10. Redox State Assessment by Dihydrorhodamine 123 (DHR) Probe

Cells (5×10^4 cells/well) were incubated in PBS containing 1 g/L of glucose and the DHR fluorescent probe (ThermoFischer, D23806, 10 μM) was added. After 3 h, fluorescence was read (Synergy, Biotek, VT, USA) with excitation at 485 nm and emission at 528 nm.

2.11. Evaluation of Hydrogen Peroxide Cytotoxicity

Cells (2×10^4 cells/well) were treated with hydrogen peroxide (0, 50, 100, 200, 300, and 400 μM) for 3 h. Hydrogen peroxide was quantified at 240 nm ($\varepsilon_{240nm} = 43.6\ M^{-1}cm^{-1}$). MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 1 mg/mL) was added and cells incubated for 3 h. Reduced formazan was read at 570 nm (BioTek Instruments, Winooski, VT, USA). IC50 values were obtained by non-linear regression analysis with GraphPad Prism 5.

2.12. Evaluation of Gliotoxin (GT) Cytotoxicity

Cells (2×10^4 cells/well) were treated with 0, 100, 200, 350, and 500 nM GT (Sigma-Aldrich G9893) (Sigma-Aldrich, Sao Paulo, Brail). Viable cells were counted using trypan

blue in a Neubauer chamber. IC50 values were obtained by non-linear regression analysis with GraphPad Prism 5.

2.13. Reconstructed Human Skin

Normal human skin cells were obtained from donated foreskin samples from the University of São Paulo Hospital (HU/USP) (CAAE:76737917.5.1001.0067). Cells were isolated as described before [33]. Fibroblasts were cultured in DMEM with antibiotics and 10% FBS; melanocytes were cultured in medium 254; and keratinocytes were cultured in KBM medium. The reconstructed human skin containing melanoma cells was prepared as described previously [33]. Briefly, fibroblasts (1.5×10^4 cells/skin) were mixed in type I collagen and incubated at 37 °C for polymerization of the dermal matrix. Then, keratinocytes (2.5×10^4 cells/skin), melanocytes (0.8×10^4 cells/skin), and melanoma cells (50×10^4 cells/skin) were added. In the next day, skins were transferred to an air–liquid interface for epithelial differentiation for 10 days.

2.14. Hematoxylin and Eosin

Reconstructed skins were paraffin included, sectioned (5 μm), and stained with H&E for morphological analysis. All images were obtained by optical microscopy and analyzed by the NIS Elements software 5.02 (Melville, NY, USA).

2.15. Immunofluorescence

Slides were deparaffinized, submitted to antigen retrieval with 10 mM sodium citrate, and washed with PBS. A quantity of 10% goat serum was used to prevent unspecific binding of antibodies, followed by incubation with primary antibodies. Sections were incubated with secondary antibodies for 1 h. Slides were mounted in SlowFade antifade mounting with DAPI (Thermo Scientific) for nuclear counterstaining. All images were acquired with a fluorescence microscope (Axio observer, Zeiss, Oberkochen, Germany) using ZEN software 3.4.

2.16. In Silico Analysis

Public data were used from 472 samples of primary and metastatic melanoma of the TCGA (*The Cancer Genome Atlas*), which were downloaded from the repository of the University of Carolina Santa Cruz—UCSC (https://xena.ucsc.edu/) (accessed on 1 February 2020). Samples were separated in reference to their expression values normalized by the first and last quartile as "low" and "high", respectively. The expression values of the gene of interest were verified for these groups and then *boxplots* were generated with the results. The statistical analysis corresponds to the *two-tail* T test among the samples presented.

2.17. cBioPortal Database Analysis

To analyze the integrative relationships of the genes and their clinical characteristics in skin cutaneous melanoma, the cBioPortal for Cancer Genomics was used. The cBioPortal for Cancer genomics is an open access resource (http://www.cbioportal.org/) (accessed on 5 February 2020) [35,36]. The terms "MITF", "PPARGC1A", "SOX10", "PRDX1", "PRDX2", and "AXL" were searched for in the cBioPortal database for skin cutaneous melanoma (TCGA, Firehose Legacy), n = 479 samples. The selected genomic profiles were mRNA expression and protein expression with a z-score threshold of 1.5.

2.18. scRNAseq Analysis

The analysis of single-cell datasets was performed using Interactive Single-Cell Visual Analytics (ISCVA), a computational tool consisting of two major components. The first is a collection of Bash and R scripts that uses many of the widely used algorithms in single cells, such as Seurat for general processing [37], SingleR for cell-type recognition [38], and single-cell signature explorer for gene set signature scoring [39], which processed the scRNA-Seq data offline. The second is a web-based component including react.js from Facebook,

tensorflow.js from Google, and Plotly.js. These components allow real-time interactive exploration and ad hoc analysis. As a part of the analytical modules, the heterogeneity analyses implemented in SinCHet [40] were also performed. A node.js backend was created to serve the on-demand queries of the web application, allowing for real-time interactive investigation of genes expressed in selected samples or subsets of cells. Data analyzed are from previous published work (GSE174401 and GSE77940) [41,42].

2.19. Statistical Analysis

Results are expressed as mean ± standard error of three independent experiments. One-way analysis of variance (ANOVA) was used followed by the Newman–Keuls post-test to test for multiple comparisons with a given significance level of $p < 0.05$. Alternatively, the *t*-test was used when indicated. Significant differences between the control and treated groups are indicated by * $p > 0.05$, ** $p > 0.01$, and *** $p > 0.001$. All experiments were performed in independent triplicates. All data analyzed and images generated were obtained using the R platform and Prism GraphPad 5.

3. Results

3.1. Clones Exhibited Distinct Morphology, Proliferation Rate, Intrinsic Resistance, Migration, and Invasion Capacity

Clone C1 is more spherical than C2 and C3, while clone C3 showed a more elongated shape. A mixture of these morphologies can be seen in the parental cell line (Figure 1A). Clone C2 (24.3 h) and SK-MEL-28P (24.5 h) are more proliferative than C1 (45.5 h) and C3 (48.6 h, Figure 1B). Confirming our results, the phosphorylation of ERK is decreased in C1 and C3 (Figure 1C). Clone C2 seems to be the less migratory than the others (Figure 1D). After a long-term treatment (3 weeks) with BRAFi (acquired resistance), C1 and C3 were capable of surviving and forming viable colonies, unlike C2 (Figure 1E). We tested the intrinsic resistance by short-term treatment with 24, 48, and 72 h of BRAFi exposure and demonstrated that the SK-MEL-28P and C2 were sensitive at 24 h, while C1 and C3 needed 48h to respond (Figure 1F).

In a 3D skin model, SK-MEL-28P invaded the dermis and proliferated on the corneal layer (Figure 2A). The SK-MEL-28R also invaded the dermis even in the presence of BRAFi. Among the clones, C1 showed the most invasive profile, followed by C3 and C2. We confirmed the melanoma invasion within the dermis with S100 and HMB45 staining in Figure 2B. Further studies demonstrated that clone C1 and the SK-MEL-28R cells showed higher levels of AXL and beta-catenin (Figure 2C,D).

3.2. Clones Exhibited Distinct Oxidative Metabolism

PGC-1α levels were decreased in all clones, with C1 and C3 showing the lowest levels (Figure 3A). C1 was less dependent on OXPHOS, C3 was more dependent, and C2 resembled the profile of SK-MEL-28P (Figure 3B). Clones C1 and C3 showed higher ROS production compared to C2. The SK-MEL-28P exhibited higher levels of ROS, probably because of the presence of other clones (Figure 3C). The antioxidant system in general was decreased in C1 (Figure 3D). C1 and C3 expressed less PRDX1 when compared to SK-MEL-28P. In addition, SK-MEL-28P and the clones C1 and C3 exhibited decreased expression of PRDX1 when compared to primary human melanocytes, while clone C2 and SK-MEL-28R showed comparable levels to melanocytes (Figure 3E). No differences in PRDX2 levels were seen among the clones (Figure 3E), but PRDX2 was depleted in the SK-MEL-28R while PRDX1 was increased in SK-MEL-28R compared to SK-MEL28P (Figure 3E). Once PRDX dimers were formed after oxidation, non-reducing Western blot was performed to probe oxidative stress. As expected, cells that showed increased levels of PRDX1 also had increased expression of monomers and dimers. We calculated the dimer/monomer ratio to evaluate the redox status [43] (Figure 3F, upper figure) and found no differences among the cells. In contrast, levels of PRDX2 monomers were lower in C1 and C3 while the dimers were higher, providing evidence that these had a more oxidative environment (Figure 3F, lower figure), in agreement

with the data obtained with DHR (Figure 3C). Once PRDX1 levels are variable among the clones and PRDX2 levels are maintained, this result suggests that PRDX2, but not PRDX1, could be used as an oxidative stress probe. We then tested the sensitivity of the clones to hydrogen peroxide (Figure 3G) and gliotoxin (GT), an antioxidant and PRDX mimetic (Figure 3H). C1 was the most sensitive to hydrogen peroxide (IC50 = 93.95 μM), followed by C3 (IC50 = 103.1 μM), SK-MEL28R (106.5 μM), SK-MEL-28P (113.6 μM), and C2 (134.6 μM). By contrast, clone C2 was the most sensitive to GT (IC50 = 85.01 nM), followed by SK-MEL-28P (IC50 = 131.2 nM), SK-MEL-28R (IC50 = 151.2 nM), clone C3 (IC50 = 151.8 nM), and clone C1 (IC50 = 160.3 nM). In summary, C1 and C3, which were the most sensitive to oxidative stress, were also the most resistant to GT. So, it seems that C1 and C3 produce more ROS and/or exhibit a weaker antioxidant system compared to clone C2.

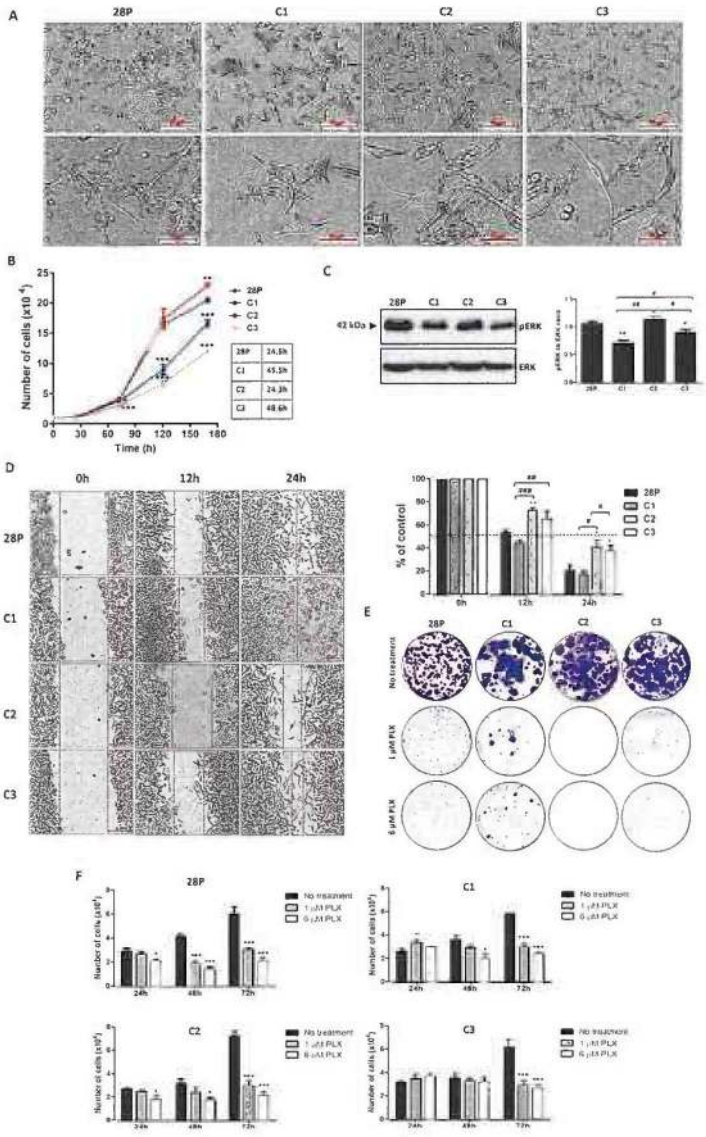


Figure 1. The isolated clones exhibited distinct morphology, proliferation rate, migration capacity, and intrinsic resistance. (**A**) Cell morphology. Scale 100 μm, magnification 10× (above) and 40× (below).

(**B**) Proliferation rate. The cells (1×10^4 cells/well) were plated and the viable ones were counted with trypan blue exclusion after 24, 72, 120, and 168 h. (**C**) Evaluation of proliferation by expression of pERK and ERK. (**D**) Wound healing assay. The cells (2×10^5 cells/well) were plated, and after the scratch, photos were taken at 0, 12, and 24h. Scale 50 μm, magnification 10×. (**E**) Colony formation assay. The cells (1000 cells/well) were treated with PLX (0, 1, and 6 μM) for 3 weeks and stained with crystal violet. (**F**) Intrinsic resistance to BRAFi. The cells (2×10^4 cells/well) were plated and treated with PLX (0, 1 and 6 μM), and the viable ones were counted with trypan blue exclusion after 24, 48, and 72 h. All the images are representative of three independent experiments. The values are indicated by mean ± standard error of three independent experiments. The statistical analysis was performed by one-way analysis of variance (ANOVA) followed by a Newmann–Keuls test; *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ when compared to control and # when compared among the groups. 28P: SK-MEL-28 parental cell line. PLX: vemurafenib (BRAFi) (### $p < 0.001$, ## $p < 0.01$, and # $p < 0.05$).

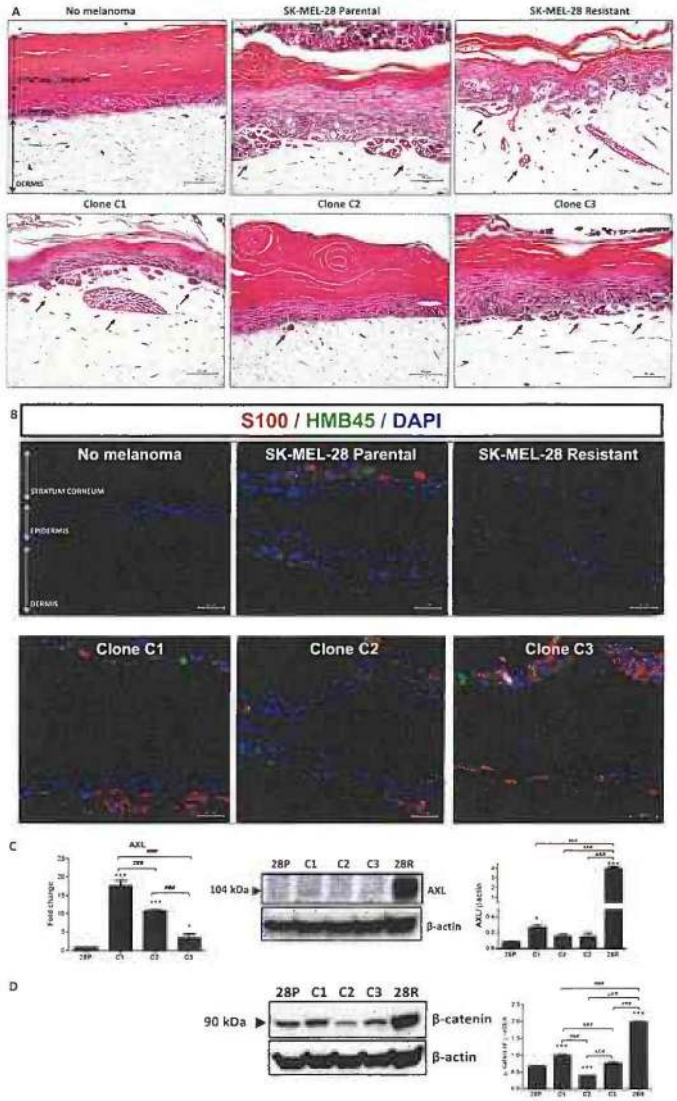
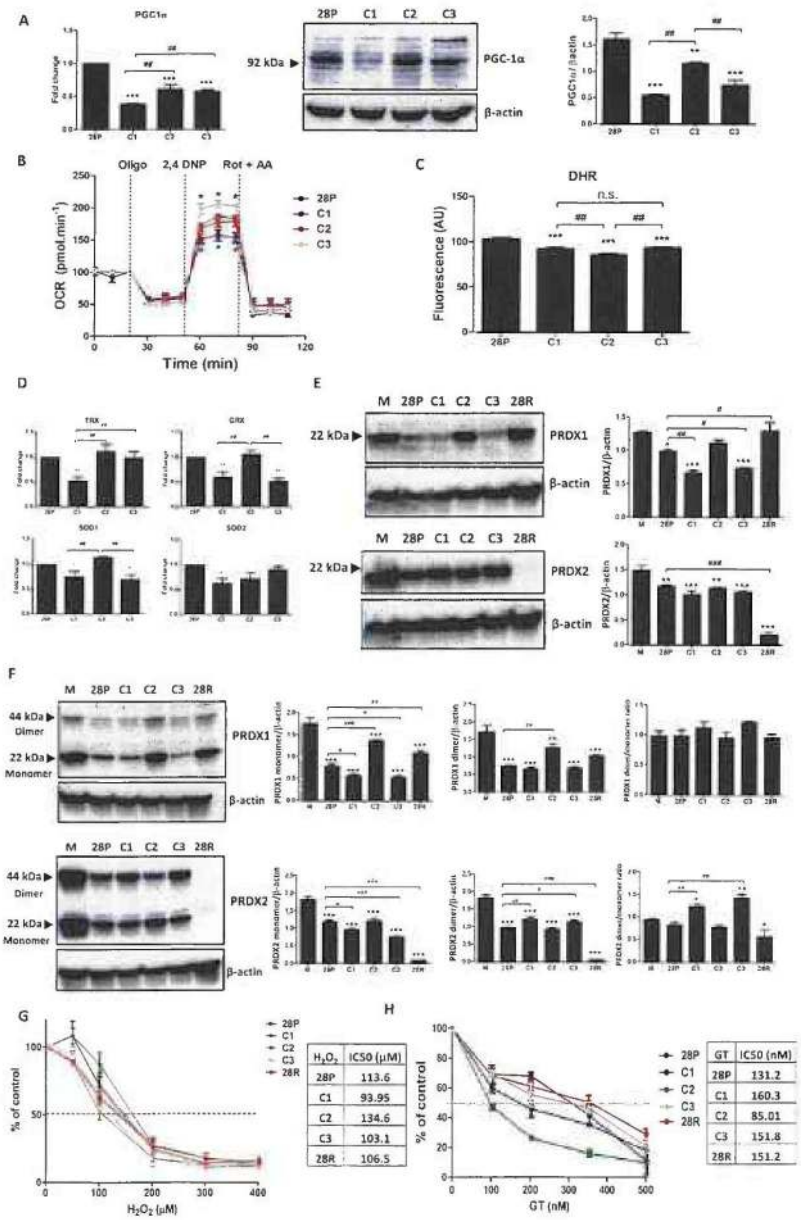


Figure 2. Clones exhibited distinct invasion profiles. (**A**) Human reconstructed skin containing no melanoma, SK-MEL-28 parental, clone C1, clone C2, clone C3, or SK-MEL-28 resistant stained for

H&E. Scale 50 µm, magnification 40×. (**B**) Immunofluorescence for human reconstructed skin containing no melanoma, SK-MEL-28 parental, clone C1, clone C2, clone C3, or SK-MEL-28 resistant stained for S100 and HMB45. Scale 50 µm, magnification 40×. (**C**) mRNA and protein levels of AXL in SK-MEL-28 parental, clones, and SK-MEL-28R. (**D**) Protein levels of beta-catenin in SK-MEL-28 parental, clones and SK-MEL-28R. 28P: SK-MEL-28 parental cell line. 28R: SK-MEL-28-resistant cell line. The statistical analysis was performed by one-way analysis of variance (ANOVA) followed by a Newmann–Keuls test; *** $p < 0.001$ and * $p < 0.05$ when compared to control and # when compared among the groups (### $p < 0.001$).



H_2O_2	IC50 (µM)
28P	113.6
C1	93.95
C2	134.6
C3	103.1
28R	106.5

GT	IC50 (nM)
28P	131.2
C1	160.3
C2	85.01
C3	151.8
28R	151.2

Figure 3. Clones exhibited distinct oxidative metabolism. (**A**) Expression of mRNA and protein of PGC−1α. (**B**) Oxygen consumption rate (OCR). The cells (3×10^4 cells/well) were treated with indicated

drugs (oligomycin 1.5 μM, 2,4-dinitrophenol 30 mM, rotenone 1 μM, and antimycin A 1 μM) at the determined times. The values are indicated by mean ± standard error of three independent experiments. Statistical analysis was performed by *t*-test comparing with the parental SK-MEL-28P control, * $p < 0.05$. (**C**) Detection of total reactive oxygen species using DHR probe. The cells (5×10^4 cells/well) were seeded in a black plate, treated with DHR (10 μM) for 3 h and then fluorescence was read (λexc 485 nm and λem 528 nm). The values are indicated by mean ± standard error of three independent experiments. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Newmann–Keuls test, *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$ compared to parental control (# when compared among groups). (**D**) TRX, GPX, SOD1, and SOD2 mRNA levels in clones. (**E**) Protein levels of PRDX1 and PRDX2 in melanocytes (M), SK-MEL-28 parental, clones, and SKM-MEL-28R. (**F**) Western blot redox for PRDX1 and PRDX2. (**G**) Determination of the sensitivity of the cells to hydrogen peroxide by calculating the IC50. (**H**) Determination of the sensitivity of the cells to gliotoxin by calculating the IC50. The cells (2×10^4 cells/well) were treated with increasing concentrations of hydrogen peroxide (0, 100, 200, 300, and 400 nM). After 24 h and 5 h, viable cells were determined. 28P: SK-MEL-28 parental cell line. 28R: SK-MEL-28-resistant cell line (### $p < 0.001$, ## $p < 0.01$, and # $p < 0.05$).

3.3. Clones Showed Different Profiles of Pigmentation

An analysis of melanocyte lineage/pigmentation markers demonstrated that NRF2 expression was significantly increased in clone C1, while SOX10 and MITF were significantly decreased (Figure 4A–C). It was further noted that Melan-A levels were decreased in the skin containing C1 or SK-MEL-28R (Figure 4D). Skins exhibited different patterns of pigmentation with the SK-MEL-28P and the clones C2 and C3 exhibiting the most pigmented, while C1 and SK-MEL28R showed no pigmentation (Figure 4E). We confirmed these results showing that the pigmentation markers Melan-A, TYR, TRP1, and TRP2 were decreased in C1 and SK-MEL-28R (Figure 4F). We also demonstrated that RAB7 and RAB27 were decreased in C1 and SK-MEL-28R (Figure 4G).

3.4. In Silico Analysis of PRDX1 and PRDX2 Expression in Melanoma Patients

PRDX1 shows significant co-expression with MITF in melanoma patients, with PRDX2 showing a tendency towards mutual exclusivity (Figure 5A). There was also a tendency for co-occurrence between PRDX2 and AXL, whereas PRDX1 and AXL were mutually exclusive (Figure 5A). Patients with low levels of MITF have significantly lower levels of PRDX1 and higher levels of PRDX2, while no differences were found on AXL and PGC1α compared to PRDX1 and PRDX2 (Figure 5B). Additionally, BRAF-mutated patients with decreased expression of PRDX1 demonstrated poor survival rates compared to those with higher expression of PRDX1 (Figure 5C). Using single-cell data from metastatic melanoma patients published by [42], we observed that MITF, SOX10, MLANA, PGC1a, TYR, TYRP1, and TYRP2/DCT are present only or enriched in melanoma cells. Additionally, NRF2 expression was noted in all cell types at a lower expression than that of MITF in melanoma cells. The expression of PRDX1 was higher than that of PRDX2 in melanoma cells and more similar to that of MITF than that of PRDX2. Furthermore, the expressions of TYR, TYRP1, and TYRP2/DCT were similar to those of MITF and MLANA, corroborating our data obtained using the isolated clones, where C1 expresses less MITF, MLANA, TYR, TYRP1, TYPR2/DCT, and PRDX1 (Figure 5D). We also analyzed another dataset published in [41], containing melanoma patients with skin, brain, and leptomeningeal metastasis. As expected, PRDX1 and PRDX2 were distributed across all cell types. However, it was interesting to note that the expressions of PRDX1 and PRDX2 were different among the cells. We found PRDX1 enriched in neurons more than PRDX2, while some populations of melanoma cells express more PRDX2 than PRDX1. Then, PRDX1 expression is similar to MITF in patient melanoma cells, which corroborates our in vitro data using the clones (Figure 5E).

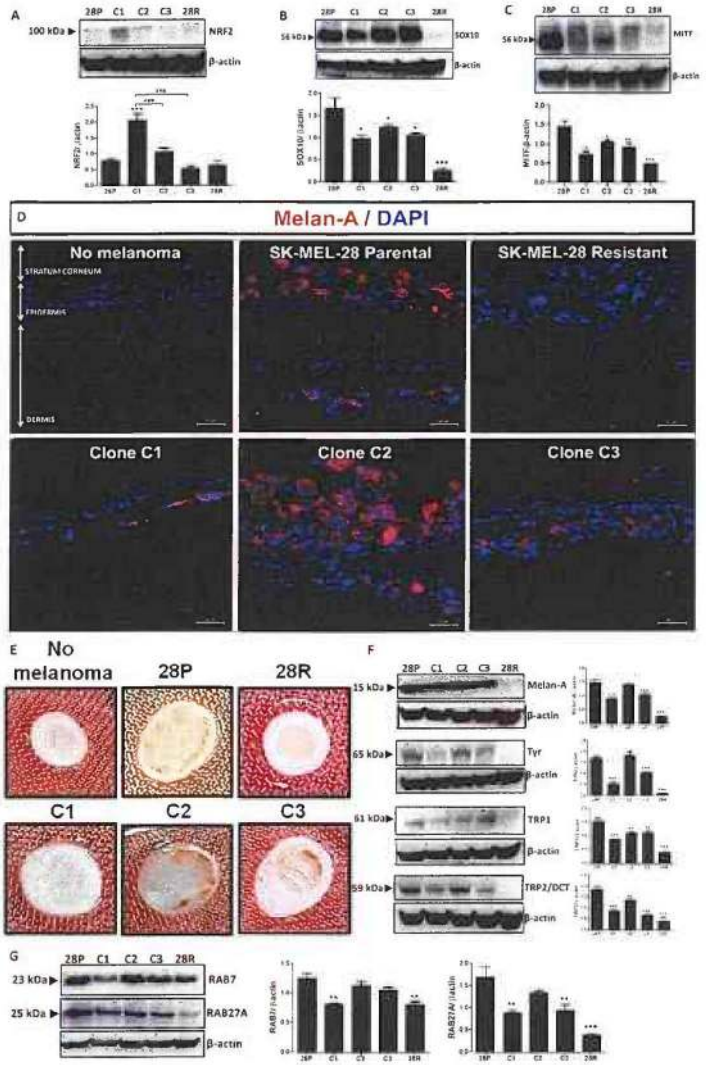


Figure 4. Clones exhibited different patterns of pigmentation. (**A**) Protein levels of NRF2. (**B**) Protein levels of SOX10. (**C**) Protein levels of MITF. (**D**) Immunofluorescence of skin reconstructed containing no melanoma, SK-MEL28 parental, clones, or SK-MEL-28R stained for Melan-A. Scale 50 μm, magnification 40×. (**E**) Images of the reconstructed skins showing the differences in pigmentation. (**F**) Protein levels of Melan-A, TYR, TRP1, and TRP2. (**G**) Protein levels of RAB7 and RAB27. The values are indicated by mean ± standard error of three independent experiments. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Newmann–Keuls test, *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$ when compared to parental control (# when compared among groups). 28P: SK-MEL-28 parental cell line. 28R: SK-MEL-28-resistant cell line (### $p < 0.001$).

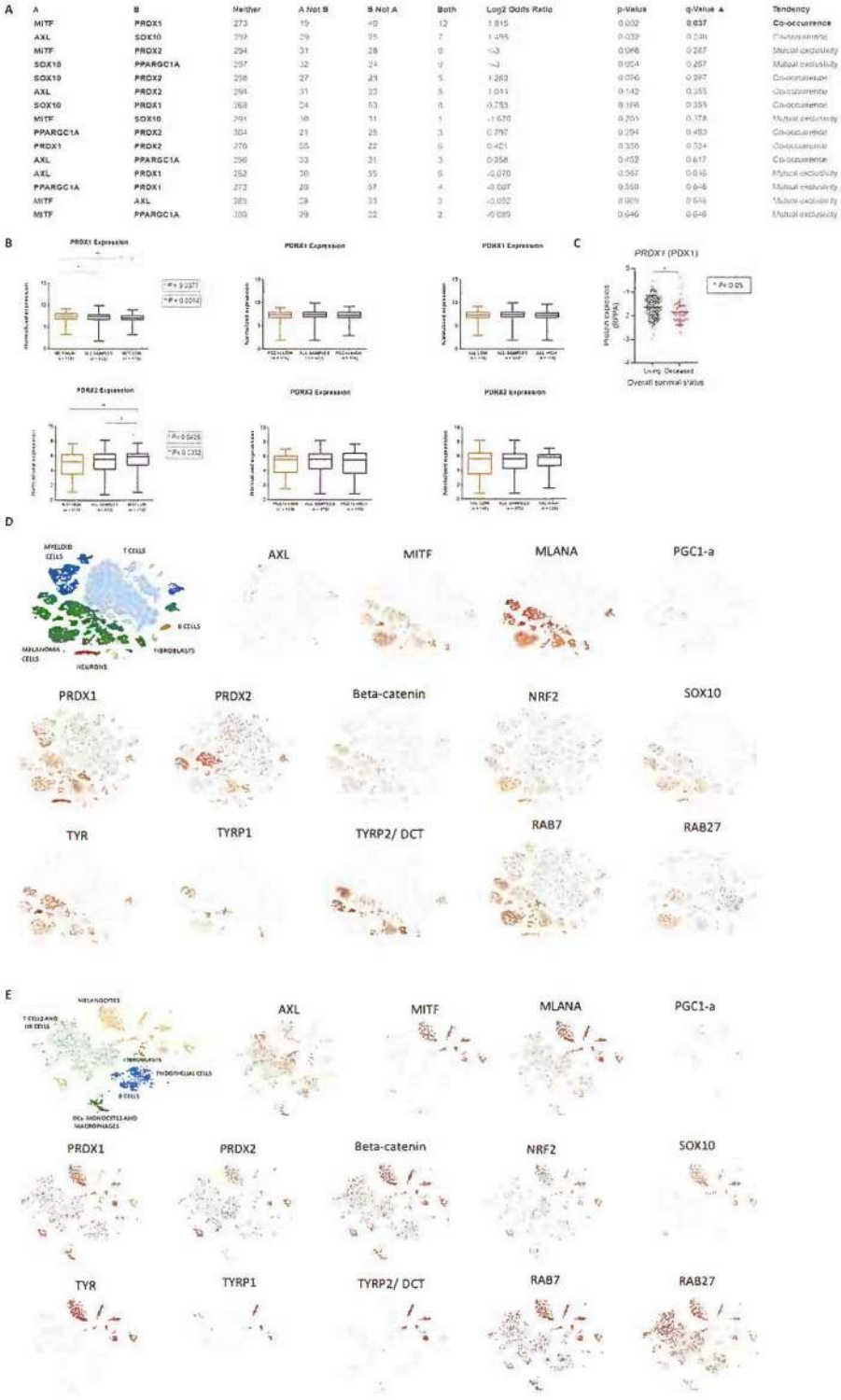


Figure 5. In silico analysis of the expression of PRDX1 and PRDX2 in melanoma patients. (**A**) Correlation between AXL, SOX10, MITF, PGC1−α, PRDX1, and PRDX2 in melanoma patients

according to cBioPortal. (**B**) Expression of PRDX1 and PRDX2 in cells expressing high or lower levels of MITF, PGC1-α and AXL. (**C**) Correlation of overall survival and levels of PRDX1. (**D**,**E**) scRNAseq data of proteins involved in proliferation, invasion, metabolism, redox processes, BRAFi, resistance, and pigmentation.

4. Discussion

Tumor heterogeneity is one of the causes of treatment resistance and failure [44,45]. It arises from complex genetic, epigenetic, and proteomic/transcriptomic modifications that drive phenotypic selection and alterations in response to microenvironment pressures [46]. In fact, a recent finding showed the potential to predict initial BRAFi sensitivity at the single-cell level [47]. The understanding of driver agents, the functional consequences of heterogeneity, and the transcriptional profiles of the clonal subpopulations may provide new insights that can optimize therapy. MITF regulates phenotype switching since $MITF^{high}$ cells exhibit a proliferative profile, while $MITF^{low}$ cells exhibit a more invasive one [10,11]. These data are in agreement with low MITF expression in C1 and C3. Considering that MITF controls the expression of PGC-1α, clones C1 and C3 also exhibited significant decreased expression of PGC-1α. SOX10 contributes to melanoma development by regulating the SOX10-MITF pathway, but also contributes to melanoma cell survival, proliferation, and metastasis formation [48,49]. AXL is linked to the mesenchymal state in the epithelium–mesenchymal transition, while melanoma cells with low AXL expression resemble the epithelial state [50]. The $MITF^{high}/AXL^{low}$ phenotype is linked to BRAFi sensitivity and the $MITF^{low}/AXL^{high}$ phenotype is linked to resistance [12], and we confirmed that C1 had the $MITF^{low}/AXL^{high}$ profile along with intrinsic resistance to BRAFi. Of relevance, cells that are $MITF^{low}/AXL^{high}$ can co-exist with $MITF^{high}/AXL^{low}$ cells and $MITF^{high}$ cells, and can cluster with distinct transcriptional states and be drug-sensitive or resistant [47,51].

BRAFi resistance is characterized by high mitochondrial activity that can occur regardless of PGC-1α expression [18]. Our results corroborate previous data and provide new evidence that naïve and intrinsically BRAFi-resistant cells like C1 could also display low mitochondrial activity. The presence of a BRAF mutation is associated with suppression of OXPHOS, MITF, and PGC-1α expression, while BRAFi can increase OXPHOS, MITF, PGC-1α, and ROS production. Additionally, ROS can be increased through a dependent or independent PGC1α mechanism [13,18]. MITF is redox-sensitive, since oxidative injury induced by hydrogen peroxide drives downregulation of MITF and its downstream targets [52]. Additionally, MITF dysfunction may lead to oxidative damage once MITF deficiency in mice leads to higher levels of ROS. Overexpression of MITF upregulates the antioxidant response and mitochondrial biogenesis by regulating PGC-1α [53]. Therefore, MITF is possibly involved in redox signaling by controlling the PGC1α/mitochondria/OXPHOS/antioxidant axis [53]. MITF also controls the cellular response to ROS by regulating APE-1 [22]. Then, $MITF^{high}$ cells were found to be more resistant to hydrogen peroxide-induced cell death [22], which corroborates our data obtained for C2 treated with hydrogen peroxide. It was demonstrated that APE-1/PRDX1 could act as an anti-inflammatory agent avoiding cancer invasion and metastasis [54]. Additionally, the transcription factor NRF2, which is the major mediator of oxidative stress responses and linked to therapy resistance, suppresses MITF activity and reduces the expression of pigmentation markers in melanoma [55].

MITF targets the pigment genes that encode enzymes necessary for melanin synthesis, TYR, TYRP1, and TYRP2, together with Melan-A [56], and PGC-1s are involved in this regulation [13,19]. The role of melanin in melanoma is contradictory, and a clinical study showed that amelanotic melanoma was associated with poorer patient survival than pigmented melanoma [57]; however, pigmentation is also associated with a worse outcome in metastatic melanoma patients and increased resistance to therapy [58]. Some studies demonstrated that melanin is associated with invasion suppression [59,60] and inhibition of metastasis [24,58,61]. Inhibiting melanoma growth and invasion at early stages, when melanogenesis is deregulated at advanced stages, and its intermediates have an immunosuppressive effect [62], can also induce mutations and DNA damage, and can

switch energy yielding metabolism [63]. RAB7 expression is induced in early-stage tumors and downregulated in invading melanomas [64,65]. RAB27 was identified as a melanoma driver; its levels increase during melanoma progression and its depletion decreases tumor progression and metastasis [66], and it is also linked with decreased survival [67]. In contrast to RAB27, RAB7 expression is not regulated by MITF and high RAB7 expression was linked to a decrease in invasiveness and an increase in patient survival [67]. C1 had decreased expression of pigmentation markers and RAB7 and RAB27, suggesting that the presence of melanin could inhibit invasion and resistance.

Interestingly, depletion of PGC-1α caused a significant decrease in antioxidant protein expression [14]. We confirmed that C1 and C3 had lower PRDX1, so it is likely that the imbalance in antioxidant defense could lead to increased ROS production, supporting our observation that C1 and C3 exhibit high levels of ROS. ROS can play a dual role in cancer: a tumorigenic role by promoting proliferation and genomic instability [68], or a toxic role by inducing cellular damage and death [69]. Redox signaling can be regulated by redox-sensitive proteins such as thiol-containing proteins, which guarantee the high specificity of signal transduction, in addition to avoiding the accumulation of ROS [70]. PRDXs are thiol-containing enzymes that play an important role in redox signaling associated with tumorigenesis [70]. PRDXs can directly react with transcription factors and are involved in regulation of apoptosis, differentiation, and proliferation [71]. We have previously demonstrated that PRDX1 was increased in melanoma patients from the vertical growth phase compared to common nevus, and was decreased in metastatic melanoma compared to dysplastic nevus, whereas PRDX2 is decreased in the vertical growth phase and in melanoma metastasis when compared to dysplastic nevus [30]. We observed that PRDX1 and PRDX2 levels were decreased in SK-MEL-28P compared to normal melanocytes and PRDX1 is increased in resistance, while PRDX2 is depleted in acquired resistant SK-MEL-28R. Using data from melanoma patients, we observed that low levels of MITF expression are associated with decreased PRDX1 expression. Our results suggested that PRDX1 and PRDX2 may play distinct roles in melanoma heterogeneity.

It was demonstrated that PRDXs can contribute to resistance to therapy [72,73] and act as an oncogene or tumor suppressor, depending on the tumor [74] and the melanoma stage [30]. In melanoma it is accepted that they are downregulated in metastasis [30,31,75]. PRDXs are known to be involved in receptor tyrosine kinase-mediated signal transduction [76] and modulation of phosphokinase signaling cascades [25,77,78], and may play an important role as tumor suppressor [77,79]. On the other hand, increased expression of PRDX1 by ROS can prevent cell death and PRDX1 is involved in melanoma resistance [80–82]. Downregulation of PRDX2 in melanoma cells is related to more production of ROS, proliferation, and migration [32]. In fact, it was demonstrated that PRDX2 prevents metastasis by increasing E-cadherin/β-catenin complexes [32], and that PRDX2 is downregulated in melanomas and could be restored by a demethylating agent [83]. GT is a mimetic of PRDX by reducing hydrogen peroxide to water and accelerating NADPH oxidation by the thioredoxin system [84]. Its potent activity has already been demonstrated in different types of cancer [85–89]. In melanoma, its IC50 is 100–200 nM [32,86]. Interestingly, we found an inverse correlation among sensitivity to oxidative stress and antioxidant treatment.

Taken together, our results demonstrated that clones in the same tumor can naturally express less PRDX1, MITF, SOX10, PGC-1α, and pigmentation markers. We linked this genetic profile to a phenotype that is more invasive, less pigmented, and intrinsically resistant. These results provide evidence for the influence of redox-sensitive reactions in melanoma heterogeneity and the role of pigmentation in this context, highlighting the importance of antioxidant response in treatment resistance.

Author Contributions: Conceptualization, L.A.d.C.C. and S.S.M.-E.; methodology, L.A.d.C.C., I.H.Y.N., A.H.U., Á.D.D.S., L.H.O. and M.P.M.; formal analysis, L.A.d.C.C., A.H.U., Á.D.D.S., L.H.O. and M.P.M.; investigation, L.A.d.C.C. and S.S.M.-E.; resources, S.S.M.-E.; writing—original draft preparation, L.A.d.C.C. and S.S.M.-E.; writing—review and editing, L.A.d.C.C., I.H.Y.N., A.H.U.,

Á.D.D.S., L.H.O., M.P.M., N.C.d.S.P., V.M.F., W.A.S.J., K.S.M.S. and S.S.M.-E.; supervision, S.S.M.-E.; project administration, S.S.M.-E.; funding acquisition, S.S.M.-E. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of SCHOOL OF PHARMACEUTICAL SCIENCES, UNIVERSITY OF SÃO PAULO (FCF/USP) (CAAE:76737917.5.1001.0067, 15 January 2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are contained within the article.

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Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Gray-Schopfer, V.; Wellbrock, C.; Marais, R. Melanoma biology and new targeted therapy. *Nature* **2007**, *445*, 851–857. [CrossRef] [PubMed]
2. Helfrich, I.; Scheffrahn, I.; Bartling, S.; Weis, J.; Von Felbert, V.; Middleton, M.; Kato, M.; Ergün, S.; Augustin, H.G.; Schadendorf, D. Resistance to antiangiogenic therapy is directed by vascular phenotype, vessel stabilization, and maturation in malignant melanoma. *J. Exp. Med.* **2010**, *207*, 491–503. [CrossRef] [PubMed]
3. Chapman, A.; del Ama, L.F.; Ferguson, J.; Kamarashev, J.; Wellbrock, C.; Hurlstone, A. Heterogeneous tumor subpopulations cooperate to drive invasion. *Cell Rep.* **2014**, *8*, 688–695. [CrossRef] [PubMed]
4. Flaherty, K.T.; Robert, C.; Hersey, P.; Nathan, P.; Garbe, C.; Milhem, M.; Demidov, L.V.; Hassel, J.C.; Rutkowski, P.; Mohr, P.; et al. Improved survival with MEK inhibition in BRAF-mutated melanoma. *N. Engl. J. Med.* **2012**, *367*, 107–114. [CrossRef] [PubMed]
5. Smalley, K.S.; Sondak, V.K. Melanoma—An unlikely poster child for personalized cancer therapy. *N. Engl. J. Med.* **2010**, *363*, 876–878. [CrossRef] [PubMed]
6. Sosman, J.A.; Kim, K.B.; Schuchter, L.; Gonzalez, R.; Pavlick, A.C.; Weber, J.S.; McArthur, G.A.; Hutson, T.E.; Moschos, S.J.; Flaherty, K.T.; et al. Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. *N. Engl. J. Med.* **2012**, *366*, 707–714. [CrossRef] [PubMed]
7. Hoek, K.S.; Goding, C.R. Cancer stem cells versus phenotype-switching in melanoma. *Pigment Cell Melanoma Res.* **2010**, *23*, 746–759. [CrossRef] [PubMed]
8. Quail, D.F.; Joyce, J.A. Microenvironmental regulation of tumor progression and metastasis. *Nat. Med.* **2013**, *19*, 1423–1437. [CrossRef] [PubMed]
9. Tasdogan, A.; Faubert, B.; Ramesh, V.; Ubellacker, J.M.; Shen, B.; Solmonson, A.; Murphy, M.M.; Gu, Z.; Gu, W.; Martin, M.; et al. Metabolic heterogeneity confers differences in melanoma metastatic potential. *Nature* **2020**, *577*, 115–120. [CrossRef]
10. Falletta, P.; Sanchez-Del-Campo, L.; Chauhan, J.; Effern, M.; Kenyon, A.; Kershaw, C.J.; Siddaway, R.; Lisle, R.; Freter, R.; Daniels, M.J.; et al. Translation reprogramming is an evolutionarily conserved driver of phenotypic plasticity and therapeutic resistance in melanoma. *Genes Dev.* **2017**, *31*, 18–33. [CrossRef]
11. Rambow, F.; Marine, J.C.; Goding, C.R. Melanoma plasticity and phenotypic diversity: Therapeutic barriers and opportunities. *Genes Dev.* **2019**, *33*, 1295–1318. [CrossRef] [PubMed]
12. Müller, J.; Krijgsman, O.; Tsoi, J.; Robert, L.; Hugo, W.; Song, C.; Kong, X.; Possik, P.A.; Cornelissen-Steijger, P.D.; Geukes Foppen, M.H.; et al. Low MITF/AXL ratio predicts early resistance to multiple targeted drugs in melanoma. *Nat. Commun.* **2014**, *5*, 5712. [CrossRef] [PubMed]
13. Haq, R.; Shoag, J.; Andreu-Perez, P.; Yokoyama, S.; Edelman, H.; Rowe, G.C.; Frederick, D.T.; Hurley, A.D.; Nellore, A.; Kung, A.L.; et al. Oncogenic BRAF regulates oxidative metabolism via PGC1α and MITF. *Cancer Cell* **2013**, *23*, 302–315. [CrossRef] [PubMed]
14. Vazquez, F.; Lim, J.H.; Chim, H.; Bhalla, K.; Girnun, G.; Pierce, K.; Clish, C.B.; Granter, S.R.; Widlund, H.R.; Spiegelman, B.M.; et al. PGC1α expression defines a subset of human melanoma tumors with increased mitochondrial capacity and resistance to oxidative stress. *Cancer Cell* **2013**, *23*, 287–301. [CrossRef] [PubMed]
15. Kluza, J.; Corazao-Rozas, P.; Touil, Y.; Jendoubi, M.; Maire, C.; Guerreschi, P.; Jonneaux, A.; Ballot, C.; Balayssac, S.; Valable, S.; et al. Inactivation of the HIF-1α/PDK3 signaling axis drives melanoma toward mitochondrial oxidative metabolism and potentiates the therapeutic activity of pro-oxidants. *Cancer Res.* **2012**, *72*, 5035–5047. [CrossRef] [PubMed]
16. Kozar, I.; Margue, C.; Rothengatter, S.; Haan, C.; Kreis, S. Many ways to resistance: How melanoma cells evade targeted therapies. *Biochim. Biophys. Acta Rev. Cancer* **2019**, *1871*, 313–322. [CrossRef] [PubMed]

17. Smith, L.K.; Parmenter, T.; Kleinschmidt, M.; Kusnadi, E.P.; Kang, J.; Martin, C.A.; Lau, P.; Patel, R.; Lorent, J.; Papadopoli, D.; et al. Adaptive translational reprogramming of metabolism limits the response to targeted therapy in BRAFV600 melanoma. *Nat. Commun.* **2022**, *13*, 1100. [CrossRef]
18. Corazao-Rozas, P.; Guerreschi, P.; Jendoubi, M.; André, F.; Jonneaux, A.; Scalbert, C.; Garçon, G.; Malet-Martino, M.; Balayssac, S.; Rocchi, S.; et al. Mitochondrial oxidative stress is the Achille's heel of melanoma cells resistant to Braf-mutant inhibitor. *Oncotarget* **2013**, *4*, 1986–1998. [CrossRef] [PubMed]
19. Shoag, J.; Haq, R.; Zhang, M.; Liu, L.; Rowe, G.C.; Jiang, A.; Koulisis, N.; Farrel, C.; Amos, C.I.; Wei, Q.; et al. PGC-1 coactivators regulate MITF and the tanning response. *Mol. Cell* **2013**, *49*, 145–157. [CrossRef]
20. Wittgen, H.G.; van Kempen, L.C. Reactive oxygen species in melanoma and its therapeutic implications. *Melanoma Res.* **2007**, *17*, 400–409. [CrossRef]
21. St-Pierre, J.; Drori, S.; Uldry, M.; Silvaggi, J.M.; Rhee, J.; Jäger, S.; Handschin, C.; Zheng, K.; Lin, J.; Yang, W.; et al. Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell* **2006**, *127*, 397–408. [CrossRef] [PubMed]
22. Liu, F.; Fu, Y.; Meyskens, F.L., Jr. MiTF regulates cellular response to reactive oxygen species through transcriptional regulation of APE-1/Ref-1. *J. Investig. Dermatol.* **2009**, *129*, 422–431. [CrossRef]
23. Slominski, R.M.; Zmijewski, M.A.; Slominski, A.T. The role of melanin pigment in melanoma. *Exp. Dermatol.* **2015**, *24*, 258–259. [CrossRef] [PubMed]
24. Kang, S.W.; Lee, S.; Lee, J.H.S. Cancer-Associated Function of 2-Cys Peroxiredoxin Subtypes as a Survival Gatekeeper. *Antioxidants* **2018**, *7*, 161. [CrossRef] [PubMed]
25. Jarvis, R.M.; Hughes, S.M.; Ledgerwood, E.C. Peroxiredoxin 1 functions as a signal peroxidase to receive, transduce, and transmit peroxide signals in mammalian cells. Free Radic. *Biol. Med.* **2012**, *53*, 522–530. [CrossRef] [PubMed]
26. Sobotta, M.C.; Liou, W.; Stöcker, S.; Talwar, D.; Oehler, M.; Ruppert, T.; Scharf, A.N.; Dick, T.P. Peroxiredoxin-2 and STAT3 form a redox relay for H2O2 signaling. *Nat. Chem. Biol.* **2015**, *11*, 64–70. [CrossRef] [PubMed]
27. Winterbourn, C.C.; Hampton, M.B. Redox biology: Signaling via a peroxiredoxin sensor. *Nat. Chem. Biol.* **2015**, *11*, 5–6. [CrossRef] [PubMed]
28. Poynton, R.A.; Hampton, M.B. Peroxiredoxins as biomarkers of oxidative stress. *Biochim. Biophys. Acta* **2014**, *1840*, 906–912. [CrossRef]
29. Rhee, S.G.; Woo, H.A. Multiple functions of 2-Cys peroxiredoxins, I and II, and their regulations via post-translational modifications. Free Radic. *Biol. Med.* **2020**, *152*, 107–115. [CrossRef]
30. Carvalho, L.A.C.; Queijo, R.G.; Baccaro, A.L.B.; Siena, Á.D.D.; Silva, W.A., Jr.; Rodrigues, T.; Maria-Engler, S.S. Redox-Related Proteins in Melanoma Progression. *Antioxidants* **2022**, *11*, 438. [CrossRef]
31. Hintsala, H.R.; Soini, Y.; Haapasaari, K.M.; Karihtala, P. Dysregulation of redox-state-regulating enzymes in melanocytic skin tumours and the surrounding microenvironment. *Histopathology* **2015**, *67*, 348–357. [CrossRef] [PubMed]
32. Lee, D.J.; Kang, D.H.; Choi, M.; Choi, Y.J.; Lee, J.Y.; Park, J.H.; Park, Y.J.; Lee, K.W.; Kang, S.W. Peroxiredoxin-2 Represses Melanoma Metastasis by Increasing E-Cadherin/β-Catenin Complexes in Adherens Junctions. *Cancer Res.* **2013**, *73*, 4744–4757. [CrossRef] [PubMed]
33. Sandri, S.; Faião-Flores, F.; Tiago, M.; Pennacchi, P.C.; Massaro, R.R.; Alves-Fernandes, D.K.; Berardinelli, G.N.; Evangelista, A.F.; de Lima Vazquez, V.; Reis, R.M.; et al. Vemurafenib resistance increases melanoma invasiveness and modulates the tumor microenvironment by MMP-2 upregulation. *Pharmacol. Res.* **2016**, *111*, 523–533. [CrossRef] [PubMed]
34. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **2001**, *25*, 402–408. [CrossRef]
35. Cerami, E.; Gao, J.; Dogrusoz, U.; Gross, B.E.; Sumer, S.O.; Aksoy, B.A.; Jacobsen, A.; Byrne, C.J.; Heuer, M.L.; Larsson, E.; et al. The cBio cancer genomics portal: An open platform for exploring multidimensional cancer genomics data. *Cancer Discov.* **2012**, *2*, 401–404. [CrossRef] [PubMed]
36. Gao, J.; Aksoy, B.A.; Dogrusoz, U.; Dresdner, G.; Gross, B.; Sumer, S.O.; Sun, Y.; Jacobsen, A.; Sinha, R.; Larsson, E.; et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci. Signal.* **2013**, *6*, pl1. [CrossRef]
37. Butler, A.; Hoffman, P.; Smibert, P.; Papalexi, E.; Satija, R. Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nat. Biotechnol.* **2018**, *36*, 411–420. [CrossRef]
38. Aran, D.; Looney, A.P.; Liu, L.; Wu, E.; Fong, V.; Hsu, A.; Chak, S.; Naikawadi, R.P.; Wolters, P.J.; Abate, A.R.; et al. Reference-based analysis of lung single-cell sequencing reveals a transitional profibrotic macrophage. *Nat. Immunol.* **2019**, *20*, 163–172. [CrossRef]
39. Pont, F.; Tosolini, M.; Fournié, J.J. Single-Cell Signature Explorer for comprehensive visualization of single cell signatures across scRNA-seq datasets. *Nucleic Acids Res.* **2019**, *47*, e133. [CrossRef]
40. Li, J.; Smalley, I.; Schell, M.J.; Smalley, K.S.M.; Chen, Y.A. SinCHet: A MATLAB toolbox for single cell heterogeneity analysis in cancer. *Bioinformatics* **2017**, *33*, 2951–2953. [CrossRef]
41. Smalley, I.; Chen, Z.; Phadke, M.; Li, J.; Yu, X.; Wyatt, C.; Evernden, B.; Messina, J.L.; Sarnaik, A.; Sondak, V.K.; et al. Single-Cell Characterization of the Immune Microenvironment of Melanoma Brain and Leptomeningeal Metastases. *Clin. Cancer Res.* **2021**, *27*, 4109–4125. [CrossRef]
42. Tirosh, I.; Izar, B.; Prakadan, S.M.; Wadsworth, M.H.; Treacy, D.; Trombetta, J.J.; Rotem, A.; Rodman, C.; Lian, C.; Murphy, G.; et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science* **2016**, *352*, 189–196. [CrossRef]

43. McGinnis, A.; Klichko, V.I.; Orr, W.C.; Radyuk, S.N. Hyperoxidation of Peroxiredoxins and Effects on Physiology of Drosophila. *Antioxidants* **2021**, *10*, 606. [CrossRef]
44. Jamal-Hanjani, M.; Quezada, S.A.; Larkin, J.; Swanton, C. Translational implications of tumor heterogeneity. *Clin. Cancer Res.* **2015**, *21*, 1258–1266. [CrossRef] [PubMed]
45. Ramón, Y.; Cajal, S.; Sesé, M.; Capdevila, C.; Aasen, T.; De Mattos-Arruda, L.; Diaz-Cano, S.J.; Hernández-Losa, J.; Castellví, J. Clinical implications of intratumor heterogeneity: Challenges and opportunities. *J. Mol. Med.* **2020**, *98*, 161–177. [CrossRef]
46. Assenov, Y.; Brocks, D.; Gerhäuser, C. Intratumor heterogeneity in epigenetic patterns. *Semin. Cancer Biol.* **2018**, *51*, 12–21. [CrossRef] [PubMed]
47. Smalley, I.; Kim, E.; Li, J.; Spence, P.; Wyatt, C.J.; Eroglu, Z.; Sondak, V.K.; Messina, J.L.; Babacan, N.A.; Maria-Engler, S.S.; et al. Leveraging transcriptional dynamics to improve BRAF inhibitor responses in melanoma. *EBioMedicine* **2019**, *48*, 178–190. [CrossRef]
48. Goding, C.R. Melanocyte development and malignant melanoma. *Forum* **2000**, *10*, 176–187. [PubMed]
49. Tudrej, K.B.; Czepielewska, E.; Kozłowska-Wojciechowska, M. SOX10-MITF pathway activity in melanoma cells. *Arch. Med. Sci.* **2017**, *13*, 1493–1503. [CrossRef]
50. Boshuizen, J.; Koopman, L.A.; Krijgsman, O.; Shahrabi, A.; van den Heuvel, E.G.; Ligtenberg, M.A.; Vredevoogd, D.W.; Kemper, K.; Kuilman, T.; Song, J.Y.; et al. Cooperative targeting of melanoma heterogeneity with an AXL antibody-drug conjugate and BRAF/MEK inhibitors. *Nat. Med.* **2018**, *24*, 203–212. [CrossRef]
51. Rambow, F.; Rogiers, A.; Marin-Bejar, O.; Aibar, S.; Femel, J.; Dewaele, M.; Karras, P.; Brown, D.; Chang, Y.H.; Debiec-Rychter, M.; et al. Toward Minimal Residual Disease-Directed Therapy in Melanoma. *Cell* **2018**, *174*, 843–855.e19. [CrossRef]
52. Jiang, W.; Li, S.; Chen, X.; Zhang, W.; Chang, Y.; He, Y.; Zhang, S.; Su, X.; Gao, T.; Li, C.; et al. Berberine protects immortalized line of human melanocytes from H2O2-induced oxidative stress via activation of Nrf2 and Mitf signaling pathway. *J. Dermatol. Sci.* **2019**, *94*, 236–243. [CrossRef]
53. Hua, J.; Chen, H.; Chen, Y.; Zheng, G.; Li, F.; Qu, J.; Ma, X.; Hou, L. MITF acts as an anti-oxidant transcription factor to regulate mitochondrial biogenesis and redox signaling in retinal pigment epithelial cells. *Exp. Eye Res.* **2018**, *170*, 138–147. [CrossRef]
54. Nassour, H.; Wang, Z.; Saad, A.; Papaluca, A.; Brosseau, N.; Affar, E.B.; Alaoui-Jamali, M.A.; Ramotar, D. Peroxiredoxin 1 interacts with and blocks the redox factor APE1 from activating interleukin-8 expression. *Sci. Rep.* **2016**, *6*, 29389. [CrossRef]
55. Jessen, C.; Kreß, J.K.C.; Baluapuri, A.; Hufnagel, A.; Schmitz, W.; Kneitz, S.; Roth, S.; Marquardt, A.; Appenzeller, S.; Ade, C.P.; et al. The transcription factor NRF2 enhances melanoma malignancy by blocking differentiation and inducing COX2 expression. *Oncogene* **2020**, *39*, 6841–6855. [CrossRef]
56. Hsiao, J.J.; Fisher, D.E. The roles of microphthalmia-associated transcription factor and pigmentation in melanoma. *Arch. Biochem. Biophys.* **2014**, *563*, 28–34. [CrossRef]
57. Thomas, N.E.; Kricker, A.; Waxweiler, W.T.; Dillon, P.M.; Busman, K.J.; From, L.; Groben, P.A.; Armstrong, B.K.; Anton-Culver, H.; Gruber, S.B.; et al. Comparison of clinicopathologic features and survival of histopathologically amelanotic and pigmented melanomas: A population-based study. *JAMA Dermatol.* **2014**, *150*, 1306–1314. [CrossRef]
58. Brożyna, A.A.; Poliseno, L.; Slominski, A.T. Editorial: Role of pigmentation in melanoma. *Front. Oncol.* **2022**, *12*, 1084717. [CrossRef] [PubMed]
59. Carreira, S.; Goodall, J.; Denat, L.; Rodriguez, M.; Nuciforo, P.; Hoek, K.S.; Testori, A.; Larue, L.; Goding, C.R. Mitf regulation of Dia1 controls melanoma proliferation and invasiveness. *Genes Dev.* **2006**, *20*, 3426–3439. [CrossRef] [PubMed]
60. Hoek, K.S.; Schlegel, N.C.; Brafford, P.; Sucker, A.; Ugurel, S.; Kumar, R.; Weber, B.L.; Nathanson, K.L.; Phillips, D.J.; Herlyn, M.; et al. Metastatic potential of melanomas defined by specific gene expression profiles with no BRAF signature. *Pigment Cell Res.* **2006**, *19*, 290–302. [CrossRef] [PubMed]
61. Sarna, M.; Zadlo, A.; Hermanowicz, P.; Madeja, Z.; Burda, K.; Sarna, T. Cell elasticity is an important indicator of the metastatic phenotype of melanoma cells. *Exp. Dermatol.* **2014**, *23*, 813–818. [CrossRef] [PubMed]
62. Slominski, A.; Zbytek, B.; Slominski, R. Inhibitors of melanogenesis increase toxicity of cyclophosphamide and lymphocytes against melanoma cells. *Int. J. Cancer* **2009**, *124*, 1470–1477. [CrossRef] [PubMed]
63. Slominski, A.; Kim, T.-K.; Brożyna, A.A.; Janjetovic, Z.; Brooks, D.L.P.; Schwab, L.P.; Skobowiat, C.; Jóźwicki, W.; Seagroves, T.N. The role of melanogenesis in regulation of melanoma behavior: Melanogenesis leads to stimulation of HIF-1α expression and HIF-dependent attendant pathways. *Arch. Biochem. Biophys.* **2014**, *563*, 79–93. [CrossRef] [PubMed]
64. Alonso-Curbelo, D.; Riveiro-Falkenbach, E.; Pérez-Guijarro, E.; Cifdaloz, M.; Karras, P.; Osterloh, L.; Megías, D.; Cañón, E.; Calvo, T.G.; Olmeda, D.; et al. RAB7 controls melanoma progression by exploiting a lineage-specific wiring of the endolysosomal pathway. *Cancer Cell* **2014**, *26*, 61–76. [CrossRef] [PubMed]
65. Williams, K.C.; Coppolino, M.G. Phosphorylation of membrane type 1-matrix metalloproteinase (MT1-MMP) and its vesicle-associated membrane protein 7 (VAMP7)-dependent trafficking facilitate cell invasion and migration. *J. Biol. Chem.* **2011**, *286*, 43405–43416. [CrossRef] [PubMed]
66. Peinado, H.; Alečković, M.; Lavotshkin, S.; Matei, I.; Costa-Silva, B.; Moreno-Bueno, G.; Hergueta-Redondo, M.; Williams, C.; García-Santos, G.; Ghajar, C.; et al. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat. Med.* **2012**, *18*, 883–891. [CrossRef] [PubMed]

67. Guo, D.; Lui, G.Y.L.; Lai, S.L.; Wilmott, J.S.; Tikoo, S.; Jackett, L.A.; Quek, C.; Brown, D.L.; Sharp, D.M.; Kwan, R.Y.Q.; et al. RAB27A promotes melanoma cell invasion and metastasis via regulation of pro-invasive exosomes. *Int. J. Cancer* **2019**, *144*, 3070–3085. [CrossRef] [PubMed]
68. Weinberg, F.; Chandel, N.S. Mitochondrial metabolism and cancer. *Ann. N. Y. Acad. Sci.* **2009**, *1177*, 66–73. [CrossRef] [PubMed]
69. Trachootham, D.; Alexandre, J.; Huang, P. Targeting cancer cells by ROS-mediated mechanisms: A radical therapeutic approach? *Nat. Rev. Drug Discov.* **2009**, *8*, 579–591. [CrossRef]
70. Netto, L.E.; Antunes, F. The Roles of Peroxiredoxin and Thioredoxin in Hydrogen Peroxide Sensing and in Signal Transduction. *Mol. Cells* **2016**, *39*, 65–71. [CrossRef]
71. Benfeitas, R.; Uhlen, M.; Nielsen, J.; Mardinoglu, A. New Challenges to Study Heterogeneity in Cancer Redox Metabolism. *Front. Cell Dev. Biol.* **2017**, *5*, 65. [CrossRef] [PubMed]
72. Chang, X.Z.; Li, D.Q.; Hou, Y.F.; Wu, J.; Lu, J.S.; Di, G.H.; Jin, W.; Ou, Z.L.; Shen, Z.Z.; Shao, Z.M. Identification of the functional role of peroxiredoxin 6 in the progression of breast cancer. *Breast Cancer Res.* **2007**, *9*, R76. [CrossRef] [PubMed]
73. Lu, W.; Fu, Z.; Wang, H.; Feng, J.; Wei, J.; Guo, J. Peroxiredoxin 2 is upregulated in colorectal cancer and contributes to colorectal cancer cells' survival by protecting cells from oxidative stress. *Mol. Cell. Biochem.* **2014**, *387*, 261–270. [CrossRef] [PubMed]
74. Egler, R.A.; Fernandes, E.; Rothermund, K.; Sereika, S.; de Souza-Pinto, N.; Jaruga, P.; Dizdaroglu, M.; Prochownik, E.V. Regulation of reactive oxygen species, DNA damage, and c-Myc function by peroxiredoxin 1. *Oncogene* **2005**, *24*, 8038–8050. [CrossRef] [PubMed]
75. Ding, C.; Fan, X.; Wu, G. Peroxiredoxin 1—An antioxidant enzyme in cancer. *J. Cell. Mol. Med.* **2017**, *21*, 193–202. [CrossRef] [PubMed]
76. Kang, S.W.; Rhee, S.G.; Chang, T.S.; Jeong, W.; Choi, M.H. 2-Cys peroxiredoxin function in intracellular signal transduction: Therapeutic implications. *Trends Mol. Med.* **2005**, *11*, 571–578. [CrossRef] [PubMed]
77. Neumann, C.A.; Fang, Q. Are peroxiredoxins tumor suppressors? *Curr. Opin. Pharmacol.* **2007**, *7*, 375–380. [CrossRef]
78. Wei, Q.; Jiang, H.; Xiao, Z.; Baker, A.; Young, M.R.; Veenstra, T.D.; Colburn, N.H. Sulfiredoxin-Peroxiredoxin IV axis promotes human lung cancer progression through modulation of specific phosphokinase signaling. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 7004–7009. [CrossRef]
79. Ito, T.; Kimura, S.; Seto, K.; Warabi, E.; Kawachi, Y.; Shoda, J.; Tabuchi, K.; Yamagata, K.; Hasegawa, S.; Bukawa, H.; et al. Peroxiredoxin I plays a protective role against UVA irradiation through reduction of oxidative stress. *J. Dermatol. Sci.* **2014**, *74*, 9–17. [CrossRef]
80. Nawarak, J.; Huang-Liu, R.; Kao, S.H.; Liao, H.H.; Sinchaikul, S.; Chen, S.T.; Cheng, S.L. Proteomics analysis of A375 human malignant melanoma cells in response to arbutin treatment. *Biochim. Biophys. Acta* **2009**, *1794*, 159–167. [CrossRef]
81. Sinha, P.; Poland, J.; Kohl, S.; Schnölzer, M.; Helmbach, H.; Hütter, G.; Lage, H.; Schadendorf, D. Study of the development of chemoresistance in melanoma cell lines using proteome analysis. *Electrophoresis* **2003**, *24*, 2386–2404. [CrossRef] [PubMed]
82. Stone, W.L.; Krishnan, K.; Palau, V.E.; Lightner, J.W.; Brannon, M.F. AKT1 Activation Up Regulates Peroxiredoxin 1 in Human Melanoma Cells. *Free. Radic. Biol. Med.* **2016**, *100*, S130. [CrossRef]
83. Furuta, J.; Nobeyama, Y.; Umebayashi, Y.; Otsuka, F.; Kikuchi, K.; Ushijima, T. Silencing of Peroxiredoxin 2 and aberrant methylation of 33 CpG islands in putative promoter regions in human malignant melanomas. *Cancer Res.* **2006**, *66*, 6080–6086. [CrossRef] [PubMed]
84. Choi, H.S.; Shim, J.S.; Kim, J.A.; Kang, S.W.; Kwon, H.J. Discovery of gliotoxin as a new small molecule targeting thioredoxin redox system. *Biochem. Biophys. Res. Commun.* **2007**, *359*, 523–528. [CrossRef]
85. Chen, J.; Wang, C.; Lan, W.; Huang, C.; Lin, M.; Wang, Z.; Liang, W.; Iwamoto, A.; Yang, X.; Liu, H. Gliotoxin Inhibits Proliferation and Induces Apoptosis in Colorectal Cancer Cells. *Mar. Drugs* **2015**, *13*, 6259–6273. [CrossRef]
86. Hubmann, R.; Sieghart, W.; Schnabl, S.; Araghi, M.; Hilgarth, M.; Reiter, M.; Demirtas, D.; Valent, P.; Zielinski, C.; Jäger, U.; et al. Gliotoxin Targets Nuclear NOTCH2 in Human Solid Tumor Derived Cell Lines In Vitro and Inhibits Melanoma Growth in Xenograft Mouse Model. *Front. Pharmacol.* **2017**, *8*, 319. [CrossRef]
87. Hung, M.L.V.; Song, Y.W.; Cho, S.K. Effects of the Combination of Gliotoxin and Adriamycin on the Adriamycin-Resistant Non-Small-Cell Lung Cancer A549 Cell Line. *Mar. Drugs* **2018**, *16*, 105. [CrossRef] [PubMed]
88. Kweon, Y.O.; Paik, Y.H.; Schnabl, B.; Qian, T.; Lemasters, J.J.; Brenner, D.A. Gliotoxin-mediated apoptosis of activated human hepatic stellate cells. *J. Hepatol.* **2003**, *39*, 38–46. [CrossRef]
89. Vigushin, D.M.; Mirsaidi, N.; Brooke, G.; Sun, C.; Pace, P.; Inman, L.; Moody, C.J.; Coombes, R.C. Gliotoxin is a dual inhibitor of farnesyltransferase and geranylgeranyltransferase I with antitumor activity against breast cancer in vivo. *Med. Oncol.* **2004**, *21*, 21–30. [CrossRef]

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2-Methoxyestradiol-3,17-*O*,*O*-bis-sulfamate (STX140) Inhibits Proliferation and Invasion via Senescence Pathway Induction in Human BRAFi-Resistant Melanoma Cells

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Abstract: The endogenous estradiol derivative 2-Methoxyestradiol (2-ME) has shown good and wide anticancer activity but suffers from poor oral bioavailability and extensive metabolic conjugation. However, its sulfamoylated derivative, 2-methoxyestradiol-3,17-*O*,*O*-bis-sulfamate (STX140), has superior potential as a therapeutic agent, acts by disrupting microtubule polymerization, leading to cell cycle arrest and apoptosis in cancer cells and possesses much better pharmaceutical properties. This study investigated the antiproliferative and anti-invasive activities of STX140 in both SKMEL-28 naïve melanoma (SKMEL28-P) cells and resistant melanoma cells (SKMEL-28R). STX140 inhibited cell proliferation in the nanomolar range while having a less pronounced effect on human melanocytes. Additionally, STX140 induced cell cycle arrest in the G2/M phase and sub-G1, reduced migration, and clonogenic potential in monolayer models, and inhibited invasion in a 3D human skin model with melanoma cells. Furthermore, STX140 induced senescence features in melanoma and activated the senescence machinery by upregulating the expression of senescence genes and proteins related to senescence signaling. These findings suggest that STX140 may hold potential as a therapeutic agent for melanoma treatment.

Keywords: STX140; estradiol analogue; melanoma; senescence; B-raf-inhibitor resistance

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1. Introduction

Melanoma is one of the most aggressive types of cancer that arises from melanin-producing cells, the melanocytes [1]. Although it has a low incidence rate compared to other types of skin cancers, it manifests a relatively high mortality number representing a huge public health problem throughout the world [2,3]. The worst prognosis given to patients with melanoma is mainly due to its typical characteristic of evasion of the immune system and the high rate of cell proliferation and metastasis [4,5].

Melanoma's high proliferative rate arises mainly from alteration in the MAPK (Mitogen-Activated Protein Kinase) pathway in around 90% of patients, and essentially, the $BRAF^{V600E}$ point mutation represents about 50% of cutaneous melanoma [6–8]. Thus, tumor cells with the $BRAF^{V600E}$ mutation usually maintain the active conformation of this intracellular receptor, constitutively activating MAPK signaling, which is responsible for stimulating cell survival, proliferation, migration, apoptosis evasion, and metastasis [9,10]. Therefore,

the MAPK pathway is currently the primary target in melanoma treatment, mainly with BRAF-inhibitors (BRAFi), such as vemurafenib [11,12].

Although the development of BRAFi has revolutionized melanoma treatment increasing overall survival (OS, 14–17 months) and response rate (RR, 48–53%) over dacarbazine (5.6–7.8 m; 5–20%, respectively), it is expected that in a 6- to 12-month period, patients experience a therapeutic relapse due heterogeneity that gives rise to intrinsic and acquired resistant cells that repopulate the tumor [13,14]. Furthermore, among the various resistance phenotypes, the hyperactivation of compensatory pathways such as PI3K-AKT-mTOR, overexpression of tyrosine kinase receptors, BRAF/CRAF isoform switch, and metabolic reprogramming are some challenges [15]. More recently, clinical trials demonstrated that polytherapy with BRAFi associated with MEK inhibitors and immune checkpoint modulators could increase OS (29.8 months) and response rate (63%), postponing resistance phenotype appearance [5,16]. Despite these recent efforts, new therapeutic alternatives aiming to overcome BRAFi resistance mechanisms that target metastatic melanoma are still an open public health demand [11].

In this context, a major endogenous metabolite of 17-beta-estradiol, 2-methoxyestradiol (2-ME), has been studied in vitro, in vivo, and in clinical trials against several types of cancer, including melanoma. Our previous study reported that 2-ME activity can induce cell death, it acts as an antiangiogenic compound and as a senescence inductor [17]. In addition, other studies showed that 2-ME also reversed metabolic-related pro-carcinogenic phenotype in osteosarcoma cells [18], inhibited skin injuries induced by radiation exposure [19], increased the infiltration of cytotoxic CD8+ T lymphocytes and the expression of PD-L1 in B16 tumors, in which authors suggest enhancing the efficacy of PD-1 blockade immunotherapy [20]. However, despite promising results, 2-ME has shown limited application due to its poor oral bioavailability (<2%) and extensive bioconjugation in vivo [21,22].

Therewith, the 2-ME derivative, 2-methoxyestradiol-3,17-*O*,*O*-bis-sulfamate (commonly referred to as STX140, Figure 1) has demonstrated excellent oral bioavailability (>85%) [23–26]. In addition, STX140 also demonstrated an estradiol-independent induction of angiogenesis, along with an in vitro and in vivo multitargeting anticancer activity, including breast and prostate cancer models [27–30]. Recently, STX140 was shown to have excellent in vivo activity in an animal model of multiple sclerosis [31]. However, STX140's potential action against melanoma model cells, especially those with the resistant BRAFi phenotype, still needs to be addressed. Therefore, the present work analyzes the senescence-inducing activity and antiproliferative and anti-invasive effects of STX140 against metastatic melanoma with BRAF mutation and BRAFi-resistant cells.

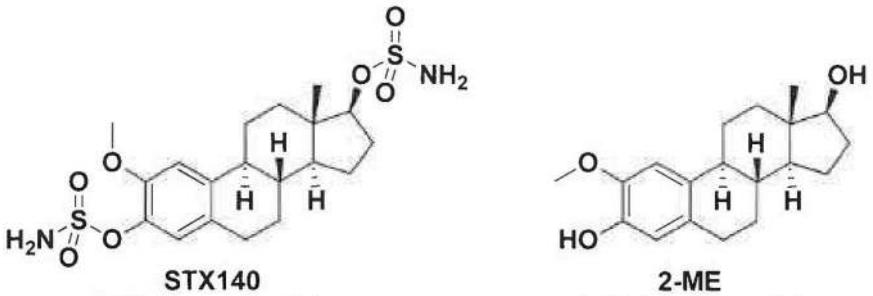

Figure 1. Chemical structures of STX140 and 2-ME.

2. Results

2.1. STX140 Showed Antiproliferative and Anti-Invasive Activity in Melanoma Cells

As evidenced in the curves of Figure 2a and Table 1, STX140 demonstrated cytotoxicity in the nanomolar range for all cell lines tested, being non-selective for resistant or parental melanomas and having a less pronounced effect on melanocytes (IC_{50} = 222.3 nM). The compound potency against melanoma cells increased time-dependently around 30–40%, but only in 72 h-incubation (61.9 and 68.2 nM for SKMEL-28P and -28R, respectively), with no pronounced variation in IC_{50} between 24 h (95.3 and 112.3 nM for SKMEL-28P and -28R, respectively) and 48 h (114.9 and 101.0 nM for SKMEL-28P and -28R, respectively). Considering prolonged and chronic exposure to STX140, the clonogenic evaluation showed inhibition of the clonogenicity potential of resistant melanoma from 50 nM up and for both melanoma (parental and resistant) cells from 100 nM, with total clonogenic inhibition at 200 nM.

Table 1. STX140 antiproliferative activity quantification.

	SKMEL-28P	SKMEL-28R	Melanocyte	Fibroblasts
24 h	95.3	112.3	222.3	133.5
48 h	114.9	101.0	ND	ND
72 h	61.9	68.2	ND	ND

Half-maximal inhibitory concentration (IC_{50}) values are expressed in nanomolar (nM). ND = not determined.

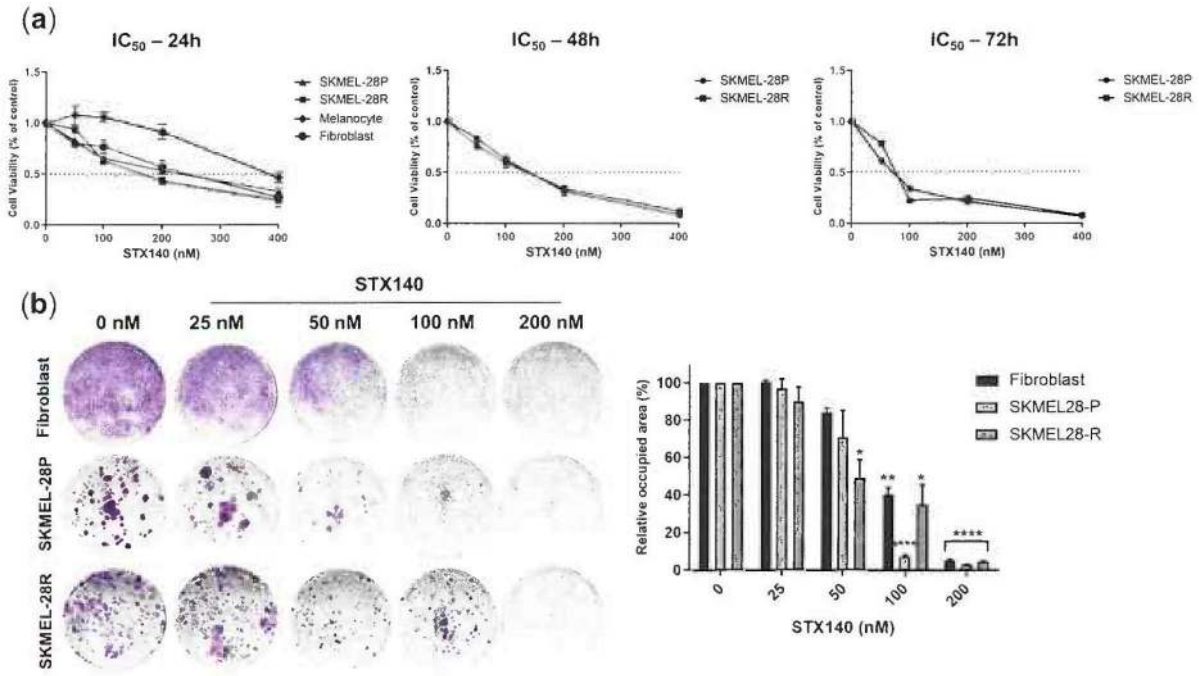

Figure 2. Antiproliferative activity of STX140 against human melanoma cells. (**a**) Cell viability evaluation of melanoma and primary cells for IC_{50} estimation; (**b**) clonogenic assay. Results are expressed as the mean of three independent experiments (n = 3) ± S.D.; significance is indicated by * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$. Representative micrographs from inverted microscope (4×).

In addition, STX140 (100 nM) also demonstrated a significant ability to inhibit cell migration after 24 and 48 h of incubation, with a more pronounced effect for SKMEL-28P in the monolayer model (Figure 3). In the three-dimensional skin model (Figure 4), STX140 (500 nM) demonstrated cytotoxicity with inhibition of SKMEL-28P proliferation when compared to the untreated control, in addition to inhibition of invasion of the dermal layer by SKMEL28-R compared to the untreated control. However, STX140 treatment did not appear to affect tissue formation when evaluating non-melanoma skin (Figure 4a). Furthermore, STX140 (100 nM) caused a significant decrease in IL-8 quantification only in SKMEL28-R, compared to the untreated control (Figure 4b). IL-6 was not affected by STX140 at the tested concentration.

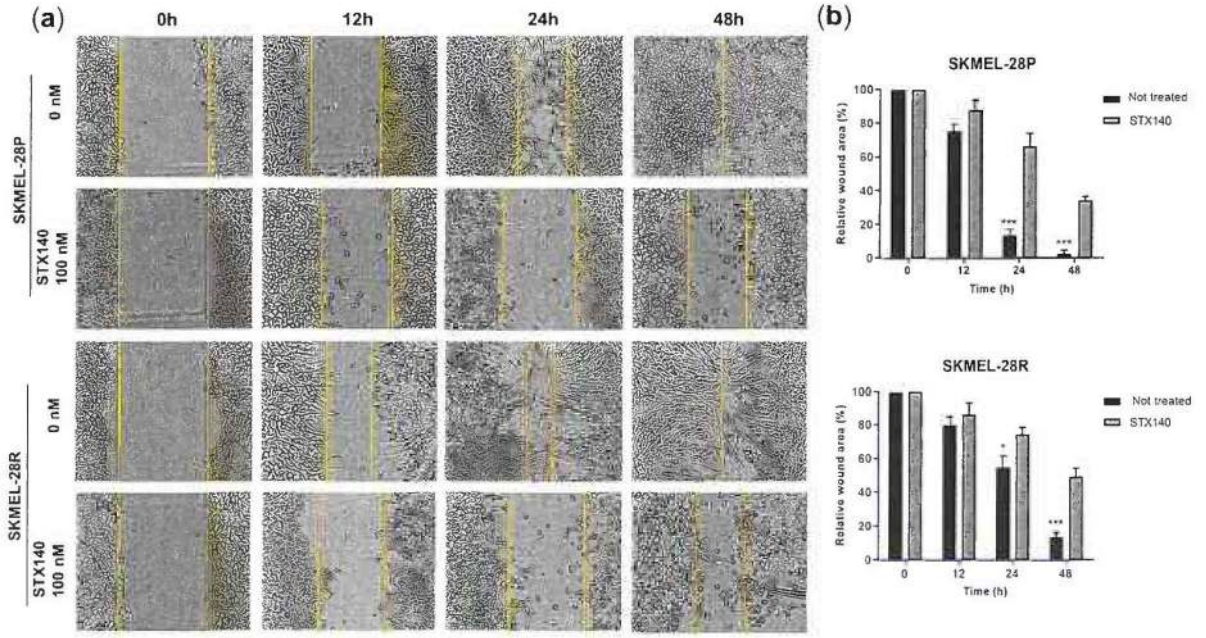

Figure 3. Antimigration activity of STX140 against human melanoma cells. (**a**) Representative micrographs from inverted microscope (20×); (**b**) Graphical representation, results are expressed as mean of three independent experiments ($n = 3$) ± S.D.; significance is indicated by * $p < 0.05$ and *** $p < 0.001$. Yellow lines represent the edge of the cell-wound.

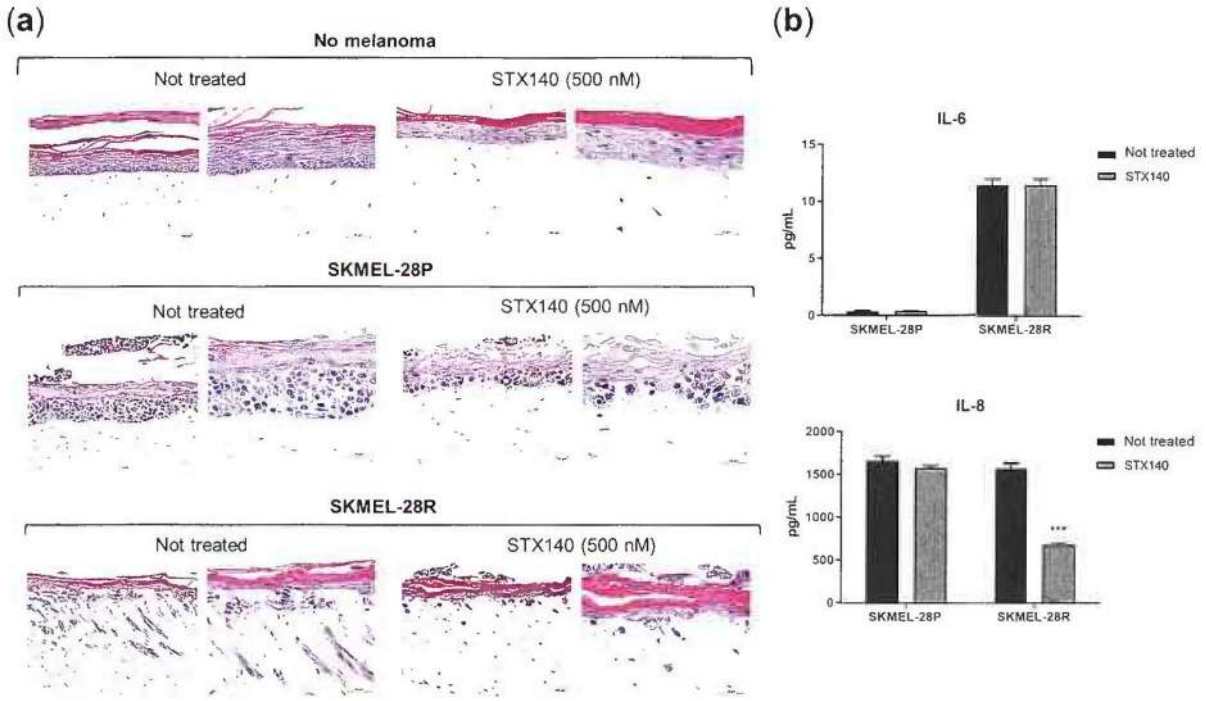

Figure 4. Anti-invasion activity of STX140 against human melanoma cells. (**a**) Reconstructed human skin with melanoma micrographs from inverted microscope (20× and 40×, respectively) dyed with hematoxylin/eosin (H&E); (**b**) Interleukin quantification in media from the 3D reconstructed human skin samples, STX140 (100 nM), results are expressed as the mean of three independent experiments ($n = 3$) ± S.D.; significance is indicated by *** $p < 0.001$.

2.2. *STX140 Treatment Led to Cell Cycle Arrest*

In order to study cell death induction caused by STX140 (Figure 5), we monitored the externalization of phosphatidylserine as a marker of early apoptosis which, combined with cell membrane fragmentation marked by positive nucleus fluorescence with propidium iodide (PI), indicated late apoptosis characteristics. Results showed that only 500 nM of STX140 was able to increase significantly ($p < 0.05$) the number of cells in early apoptosis in SKMEL-28P and -R, with no cells in late apoptosis.

Concerning cell cycle progression, although STX140 did not show remarkable apoptosis-triggering potential, this compound was able to cause cell cycle arrest at 200–500 nM concentrations with a significant increase ($p < 0.001$) in the number of cells in the G2/M phase compared to the untreated control in both parental and resistant melanoma cells (Figure 6). In addition, the significant increase ($p < 0.05$) in the percentage of cells in the sub-G1 population, commonly associated with DNA fragmentation, is also worth mentioning, caused by STX140 only at 500 nM and slightly more severe in SKMEL-28P.

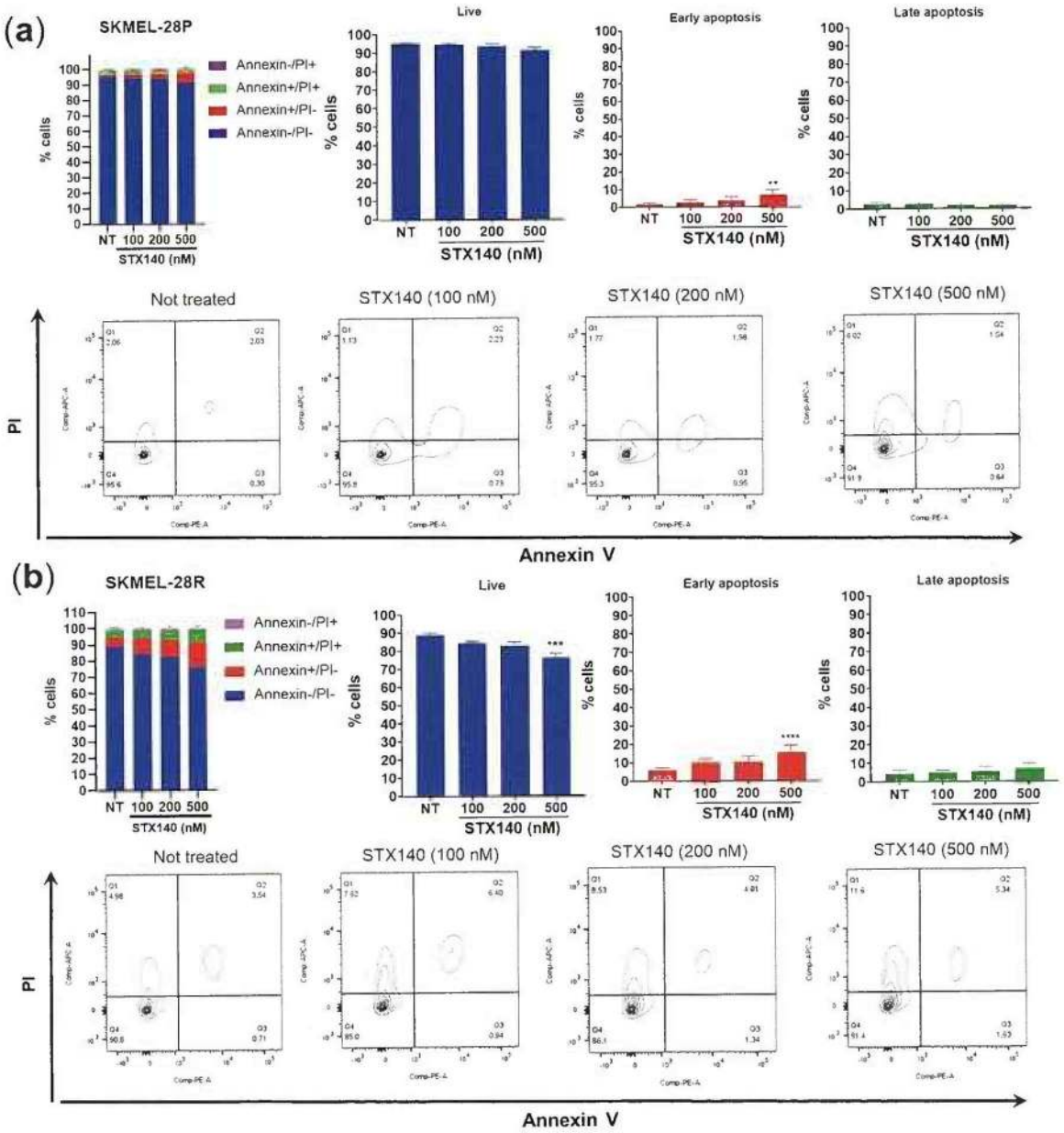

Figure 5. Cell death evaluation by STX140 activity against human melanoma cells. (**a**) SKMEL-28P; (**b**) SKMEL-28R. Graphical results are expressed as the mean of three independent experiments ($n = 3$) ± S.D.; significance is indicated by ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.001$. PI = propidium iodide; NT = not treated cells.

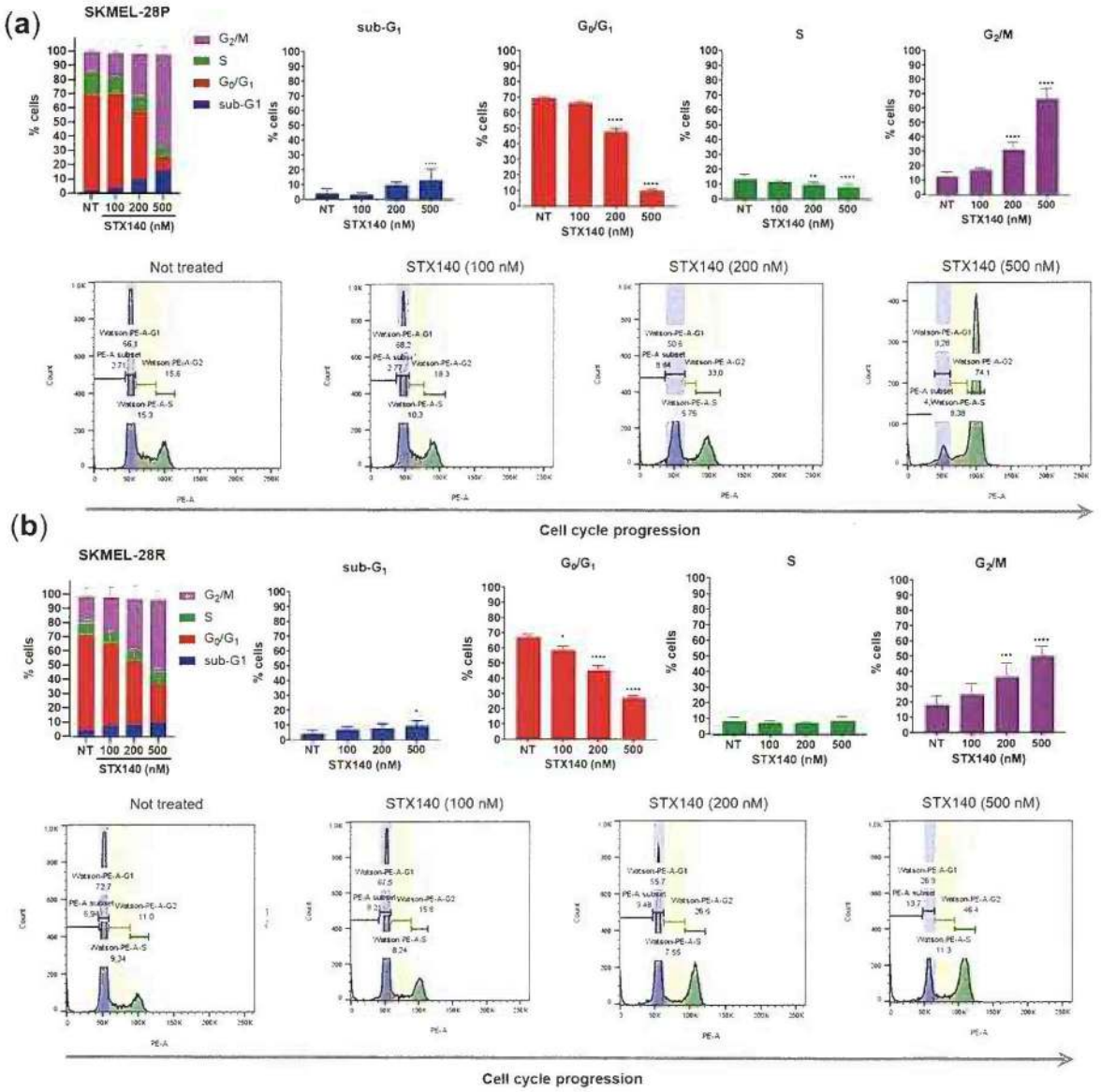

Figure 6. Cell cycle evaluation by STX140 activity against human melanoma cells. (**a**) SKMEL-28P; (**b**) SKMEL-28R. Graphical results are expressed as the mean of three independent experiments ($n = 3$) ± S.D.; significance is indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. NT = not treated cells.

2.3. STX140 Triggered Senescence Phenotype

Since parental and resistant melanoma cells suffered an antiproliferative activity with a cycle arrest in the G2/M phase and possible DNA damage with no apoptosis features, we evaluated the ability of STX140 to trigger senescence mechanisms. As evidenced in Figure 7a using the cell senescence dye, there was a significant increase ($p < 0.01$) concerning the untreated control by treatment with STX140 (100 nM), SA-β-gal stained in around 60% of SKMEL28-P and -R, by fluorescence microscopy. In addition, we observed melanoma cell morphology alterations when treated with STX140. These cells lost the characteristic stellate morphology into an acquired more spread-out shape.

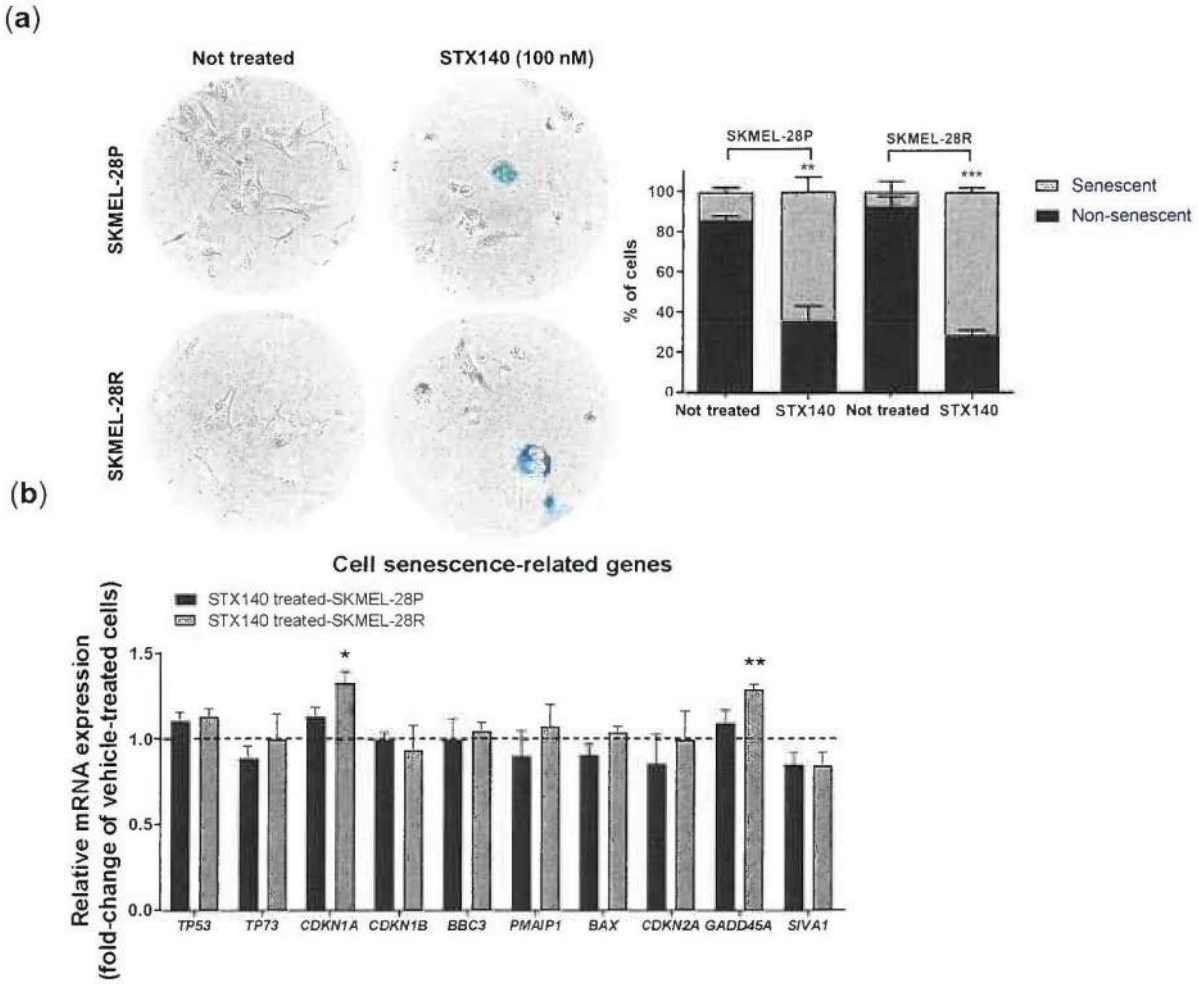

Figure 7. STX140 (100 nM) treatment induces senescence profile in human melanoma cells. (**a**) β-galactosidase staining with statistical quantification; (**b**) Cell senescence-related genes modulation; Results are expressed as the mean of three independent experiments ($n = 3$) ± S.D.; relative mRNA expression is compared to not treated cells (represented as the dash lines), significance is indicated by * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Representative micrographs from inverted microscope (10×).

Thus, associated with morphological and functional changes, the impact of STX140 on modulating the expression of genes related to senescence and apoptosis phenotypes was evaluated. As seen in Figure 7b, there was no alteration in the gene expression of SKMEL-28P. Also, *TP53*, *TP73*, *CDKN1B*, *CDKN2A*, *BBC3*, *PMAIP1*, *BAX*, and *SIVA1* genes remained unchanged in SKMEL-28R cell line. However, STX140 (100 nM) significantly ($p < 0.05$) increased *CDKN1A* and *GADD45A* expression in resistant melanoma cells.

Further, autophagy, apoptosis, and senescence signaling proteins were also quantified. STX140 could not significantly modulate the activity of PARP1 (apoptosis- and DNA damage-related) along with LC3B and SQSMT1/p62 (autophagy-related). However, compared to the negative control, STX140 induced significantly ($p < 0.05$) an elevated expression of p21 (only in SKMEL-28R) and γH2AX expression (in both SKMEL28-P and -R), as demonstrated in Figure 8.

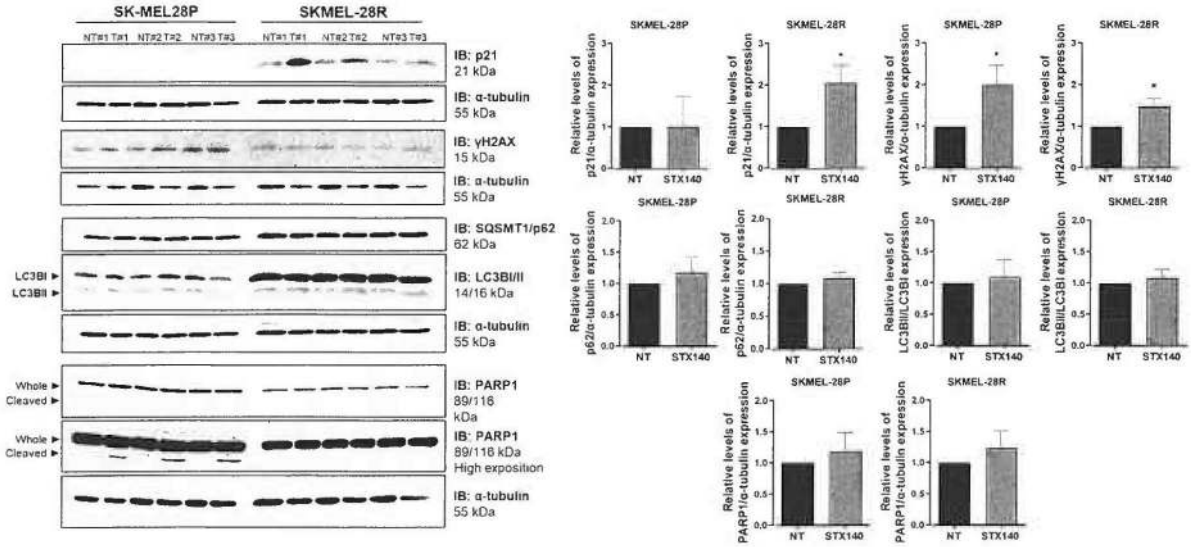

Figure 8. Cell death and senescence-related signaling modulation by STX140 (100 nM) activity in human melanoma cells. Results are expressed as mean of three independent experiments ($n = 3$) ± S.D.; significance is indicated by * $p < 0.05$. NT = no treated cells.

3. Discussion

STX140, classified as a non-estrogenic steroidal bis-sulfamate, has shown antitumor therapeutic potential in vivo and in vitro reported in several studies [24]. STX140 has been shown to disrupt microtubule polymerization when interacting with tubulin leading to cell cycle arrest and apoptosis. In a mouse model of breast cancer, orally administered STX140 outperformed the chemotherapy drug paclitaxel, which also disrupts microtubule dynamics by reducing tumor size and preventing metastasis formation without showing neurotoxicity [29,30]. Also, STX140 showed efficacy when administered orally in both MCF-7^{WT} and MCF-7^{DOX} breast cancer xenograft models, in contrast to paclitaxel and 2-methoxyestradiol [23]. Currently, there are no clinical trials ongoing with STX140, but the compound has already shown many promising results in preclinical studies in oncology [31,32] and also elsewhere, in particular its recent in vivo activity on preventing experimental autoimmune encephalitis [33].

Considering in vitro models, since STX140 is a 2-ME analog, it is worth mentioning that our group previously reported the 2-ME activity against melanoma cells using a bi- and three-dimensional model. Thus, 2-ME inhibited the proliferation, clonogenicity, and invasive potential of $BRAF^{V600E}$ mutated and BRAFi-resistant cells. In the potency evaluation assay, the IC_{50} for the SKMEL-28 cell line was 2.74 μM for 3 days, 6.81 μM for 5 days, and 4.41 μM for 7 days of treatment incubation [17]. As shown here, the 2-ME analog STX140 showed that it could reduce cell proliferation and clonogenicity potential at a nanomolar potency against melanoma cells that harbor $BRAF^{V600E}$ point mutation and its BRAFi-resistant variation (Table 1).

In a study with ovarian, prostate, and breast cancer cells, STX140 inhibited tumor viability with IC_{50} values of 280, 260, and 250 nM, respectively [34]. These values corroborated those presented herein and showed slightly more sensitivity in melanoma cell lines to the effect of STX140, with lower IC_{50} values.

In addition, STX140 demonstrated an inhibition of the migration and invasion capacity of melanoma cells, mainly in SKMEL-28R, as seen in a wound-healing assay, reconstructed skin model, and inhibition of IL-8 expression. Analyzing the results of cell death characterization by Annexin-FITC and PI, we conclude that migration and invasion inhibition is not due to the induction of cell death since STX140 does not significantly induce death features

(apoptosis/necrosis) in melanoma cells. Since other authors have shown that senescent cells commonly block intrinsic and extrinsic apoptosis signaling via antiapoptotic Bcl-2 family proteins [35–37], these results also contribute to the hypothesis that STX140 acts to prevent cell proliferation, migration, and invasiveness phenotype via senescence mechanisms.

Therefore, we evaluated if STX140 induces senescence features in melanoma, as senescence cells highly express the lysosomal enzyme β-galactosidase, classically marked positively using X-gal dye at pH 6 [38,39]. As mentioned before, STX140 led to positive blue staining to SA-β-gal and morphology characteristics such as a flattened cytoskeleton with a prominent nucleus and scattered cytoplasm. In addition to these parameters, quantitative PCR and blotting analysis demonstrated that STX140 upregulated the expression of the senescence gene and proteins related to senescence signaling. GADD45 stimulates proteins that have products that inhibit proliferation in tumor cells and, in response to different stresses, can induce senescence through interaction with p21 [40]. CDKN1A has p21 protein as a product, a potent cyclin-dependent kinase inhibitor that blocks cell cycle progression, and is a highly elevated protein in senescent cells in response to DNA injury. Furthermore, p21 is part of one of the p53/p21 signaling, a crucial main senescence pathway [41,42].

In a cell cycle evaluation, STX140 showed similar results compared to its parent 2-ME. 2-ME and STX140 demonstrated cell cycle arrest in the G2/M phase in human melanoma cells, but STX140 had activity in the nanomolar range. Although cell cycle arrest in G2/M was demonstrated as a microtubule inhibition consequence of 2-ME activity [17], it seems like STX140 is causing G2/M and sub-G1 arrest in response to DNA damage, triggering the senescent machinery activation. Studies have shown that other pharmacological compounds, such as aurora kinases inhibitors (AURK) and PLK1 inhibitors, can induce senescence features by stopping the passage of the G2 phase to the mitosis phase, consequently blocking the G2/M progression of the cell cycle via p21 and γH2AX [43,44].

Beyond the classical cell senescence induction through progressive telomere shortening in response to cell aging and genomic instability prevention, cells can also trigger an irreversible premature senescence signaling that can act as a tumor-suppressive function [45]. Double-stranded DNA breaks are powerful senescent signaling activators, usually inducing activation of H2AX aiming to recruit DNA damage response components [46]. DNA damage response also can act via the cyclin-dependent kinase inhibitor 1A (CDKN1A), triggering the p21 protein and resulting in cell cycle arrest. Even though these molecules related to cell cycle inhibition alone do not necessarily attempt to trigger senescence, these alterations together are an essential part of the hallmarks of the cellular senesce pathway [45,47–50]. In this context, STX140 activity modulated the expression of some of the hallmarks of the cellular senescence pathway, such as upregulating *CDKN1A* and *GADDA45* genes in addition to the elevation of the expression of γH2AX (a phosphorylated form of the histone variant H2AX) and p21 proteins, p53-independently, mostly seen in resistant human melanoma cells.

Moreover, although senescence signaling usually upregulates IL-6 and IL-8 activity in an autocrine manner part of the pro-inflammatory factors termed the senescence-associated secretory phenotype (SASP) [49–51], in melanoma cells, these interleukins are commonly associated with high tumor growth and invasiveness linked to poor prognosis [51,52]. Jobe et al. [53] showed that simultaneous blocking of IL-6 and IL-8 could represent a total inhibition of human melanoma invasiveness, which means that IL-8 downregulation could represent an STX140 anti-invasion pathway activity. Also, patients with a decrease in serum levels of IL-8 could reflect and predict response to anti-PD-1 treatment in melanoma and non-small cell lung cancer [54].

Even though the mechanism of action of STX140 was not tested entirely in non-melanoma cells, we analyzed STX140 cytotoxicity in the 3D reconstructed human skin model. The reconstructed human skin includes a group of samples with no melanoma (Figure 2a) consisting of three primary non-cancer cells (e.g., fibroblasts, keratinocytes, and melanocytes). This group exhibited no STX140 toxicity or morphological changes in the reconstructed human skin tissue.

Moreover, treatment with drugs that induce senescence and inhibit cell proliferation without triggering cell death currently presents an alternative to traditional cytotoxic therapies that can cause serious adverse effects in patients and often develop resistance [36]. The implication of using senotherapeutic drugs (compounds that modulate senescence) to treat cancer are being discussed currently and studied in various stages of development [55–57]. Also, further investigation should be necessary to understand the synergize potential of STX140 with other melanoma drug therapies used in clinics (such as BRAF and MEK inhibitors).

4. Materials and Methods

4.1. STX140 Synthesis, Cell Culture and Reagents

STX140 was synthesized according to the method of Leese et al. [58] (referred to as compound **21**).

Primary human melanocytes and fibroblasts were isolated from foreskin samples donated at the Hospital of the University of São Paulo (CEP/HU-USP 943/09, SISNEP CAAE 0062.0.198.000-9) as approved by the local ethics committee (CEP/FCF-USP 534). They were isolated and cultured according to the protocol previously described by our group [59].

Melanoma SKMEL-28 cell line ($BRAF^{V600E}$ mutation) was kindly donated by Dr. Marisol Soengas (Centro Nacional de Investigaciones Oncológicas, Madrid, Spain). Melanoma cells with BRAF resistance phenotype were prior generated according to Sandri et al. [59] procedure. The parental and vemurafenib-resistant SKMEL-28 melanoma cell lines with $BRAF^{V600E}$ mutation were used as a monolayer cell model. Cultivation was performed in DMEM (Dulbecco's modified Eagle's medium) for the SKMEL-28 cell supplemented with 10% fetal bovine serum (FBS) and antibiotics (25 µg/mL of ampicillin and 100 µg/mL of streptomycin). BRAFi-resistant SKMEL-28 cells were maintained in the presence of vemurafenib (3.0 µM, S1267, Selleck Chemicals, Houston, TX, USA). The cells were maintained in a humid incubator at 37 °C temperature under a 5% CO_2 atmosphere. All cell lines were checked for mycoplasma contaminations by PCR assay.

4.2. Cell Viability Evaluation

Cell viability was accessed by trypan blue (0.4%, 1:1 culture medium) (1.11732, Merck, Temecula, CA, USA) dye exclusion assay. Cells were seeded (2×10^4 cell/well) in a 24-well plate and treated with STX140 at 50 nM, 100 nM, 200 nM, and 400 nM concentrations for 24, 48, and 72 h of incubation. After dye addition, viable cells were counted in Neubauer chambers, considering colored cells as non-viable.

4.3. Clonogenic Assay

Cells were seeded in a 6-well cell culture plate (800 per well) and treated with STX140 at 25, 50, 100, and 200 nM concentrations twice a week during 10–17 days. After this period, cell colonies were stained with crystal violet (0.5% in a 1:1 water/methanol solution). The percentage of the area occupied by the colonies was quantified through a computational analysis.

4.4. Senescence Evaluation with β-Galactosidase Staining

Melanoma cells were seeded (2×10^4 cells/well) in 6-well plates and treated with STX140 (100 nM) concentration for 48 h-incubation. Cells were fixated and stained of senescence-associated β-galactosidase (SA-β-gal) was performed using the Senescence β-galactosidase Staining Kit (9860, Cell Signaling Technology, Danvers, MA, USA) following the manufacturer's instructions. Senescent and non-senescent cells were counted under the microscope in 6 different random fields for each group (treated and not treated).

4.5. Wound-Healing Assay

Melanoma cells were seeded (1×10^4 cells/well) in a 24-well plate and left to reach approximately 90% confluence. Using a 200 µL tip, a vertical scratch was made in the center of each well. Then, cells were washed with phosphate buffered saline (PBS) and treated with STX140 (100 nM) diluted in DMEM culture medium + 1% FBS. The wound-healing process was measured by acquiring twice-per-well scratch images in time 0-, 12-, 24-, and 48-h incubation. Cell-free areas were calculated using Image J 3.7 software.

4.6. IL-6 and IL-8 Quantification

The supernatant of melanoma cell culture prior treated with STX140 (100 nM) was collected and frozen at −80 °C. Interleukins IL-6 (QC193, R&D Systems®, Minneapolis, MN, EUA) and IL-8 (R&D Systems®, AF-208-NA) from samples were quantified by ELISA assay (R&D Systems®) and analyzed according to the instructions of the commercial kit manufacturers. Optical density was immediately measured at 450 nm in a 96-well microplate spectrophotometer detector. Interleukin concentration was determined by interpolation from the standard curve.

4.7. Flow Cytometry—Apoptosis Evaluation

Cell lines were seeded in a 6-well plate (2×10^5 cells/well) and treated with STX140 at 100, 200, and 500 nM concentrations in a 24-h incubation. After treatment, samples were washed twice and resuspended with ice-cold PBS, and stained with FITC Annexin V Apoptosis Detection Kit II (556570, BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's instructions. Fluorescence was measured in a flow cytometer equipment (FACS Cantho, BD Biosciences). For interpretation, cells marked as both FITC Annexin V+/PI+ were considered either in the late stage of apoptosis; FITC Annexin V+/PI− were considered in the early stage of apoptosis; FITC Annexin V−/PI+ were considered already dead or necrosis; both FITC Annexin V−/PI− are alive and not undergoing measurable apoptosis.

4.8. Flow Cytometry—Cell Cycle

Cell lines were seeded in a 6-well plate (2×10^5 cells/well) and treated with STX140 at 100, 200, and 500 nM concentrations in a 24-h incubation. Then, cell samples were washed with PSB solution and resuspended in a permeabilization and stain buffer (PBS containing 4 mg/mL of RNase A, triton x-100 0.1%, and 50 µg/mL of propidium iodide). After 30 min-incubation at room temperature in the dark, DNA quantification was measured by the sample's fluorescence read in a flow cytometer (FACS Cantho, BD Biosciences).

4.9. Gene Expression Evaluation—RT-PCR

Total RNA from samples was extracted using TRIzol reagent (Thermo Fisher Scientific, Rockford, IL, USA). A total of 1 µg of RNA was reverse transcribed into cDNA with the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). Real-time gene amplification was performed on the QuantStudio 3 Real-Time PCR System equipment and the Power SybrGreen reagent (Thermo Fisher Scientific) with specific primers (Table 2). Fifteen nanograms of each cDNA sample were used in the reactions and a negative control, with the addition of water (nuclease-free) in place of cDNA and performed for each pair of primers. HPRT1 and ACTB were used as reference genes. The relative expression was calculated using the formula $2^{-\Delta\Delta CT}$ (PMID:11846609) [60].

Table 2. Primers sequences and gene functions.

Gene	Sequence	Concentration
TP53	FW: GGCGCACAGAGGAAGAGAAT RV: GGAGAGGAGCTGGTGTTGTTG	300 nM
TP73	FW: GCACCACGTTTGAGCACCTCT RV: GCAGATTGAACTGGGCCATGA	300 nM
CDKN1A	FW: TGTCACTGTCTTGTACCCTTGT RV: GCCGGCGTTTGGAGTGGTAG	300 nM
CDKN1B	FW: ACTCTGAGGACACGCATTTGGT RV: TCTGTTCTGTTGGCTCTTTTGTT	300 nM
BBC3	FW: GACCTCAACGCACAGTACGAG RV: AGGAGTCCCATGATGAGATTGT	300 nM
PMAIP1	FW: CGCGCAAGAACGCTCAACC RV: CACACTCGACTTCCAGCTCTGCT	300 nM
BAX	FW: GAGCTGCAGAGGATGATTGC RV: CAGCTGCCACTCGGAAAA	300 nM
CDKN2A	FW: CACCGAATAGTTACGGTCGGA RV: CACGGGTCGGGTGAGAGTG	300 nM
GADD45A	FW: AAGGATGGATAAGGTGGGG RV: CTGGATCAGGGTGAAGTGG	300 nM
SIVA1	FW: TCTTCGAGAAGACCAAGCG RV: TGCCCAAGGCTCCTGATC	300 nM
ACTB	FW: AGGCCAACCGCGAGAAG RV: ACAGCCTGGATAGCAACGTACA	150 nM
HPRT1	FW: GAACGTCTTGCTCGAGATGTGA RV: TCCAGCAGGTCAGCAAAGAAT	150 nM

4.10. Reconstructed Human Skin (RHS)

The reconstructed human skin model was based on the previous description in Brohem et al. [61] with minor changes. In summary, dermis equivalent was constructed in a 6-well plate with fibroblast cells (1.5×10^5 cells/well) plus collagen type I (BD Biosciences). Melanoma cells (5×10^5 cells/well) were incorporated into the epidermis along with keratinocytes and melanocytes (2.5×10^5 and 0.8×10^4 cells/well, respectively). After 24 h-submersion, skin samples were transferred to the air-liquid interface and maintained for 10 days with DMEM/HAM's F-12 supplemented culture medium with 10% FBS. Then, 24 h prior to tissue collection and fixation (4% paraformaldehyde for 4 h and 70% alcohol for 24 h), RHS were treated with STX140 (500 nM). Tissue samples were stained with hematoxylin/eosin (H&E) for histological analysis.

4.11. Statistical Analysis

Data analysis was carried out using GraphPad Prism 8.0 software. Values are expressed as the mean ± standard deviation (S.D.) of three independent experiments (n = 3). Statistical significance (significant when $p < 0.05$) was analyzed using one-way analysis of variance (ANOVA).

5. Conclusions

This study demonstrates that STX140 has potent antiproliferative and anti-invasive effects in the nanomolar range on BRAFi-resistant melanoma cells while having a less pronounced effect on human melanocytes. Additionally, STX140 induced cell cycle arrest in the G2/M phase and sub-G1, reduced migration, and clonogenic potential in monolayer models, and inhibited invasion in a three-dimensional human skin model with melanoma cells. Moreover, STX140 induced senescence features in melanoma and activated the senes-

cence machinery by upregulating the expression of senescence genes, such as CDKN1A e GADD45A in resistant melanoma and proteins related to senescence signaling, such as p21 (only in SKMEL-28R) and γH2AX. Preclinical studies conducted on STX140 demonstrate its potential as a therapeutic agent for the treatment of other cancer types, and clinical trials are necessary to confirm its safety and effectiveness in humans. However, STX140's significant cytostatic and antiproliferative effects underline the need for continued research on this promising compound.

Author Contributions: Conceptualization, Y.A.F., M.O.d.M.J., B.V.L.P., J.A.M.-N. and S.S.M.-E.; methodology, Y.A.F., M.O.d.M.J., L.A.C.C., R.O.d.S., I.H.Y.N. and K.L.; validation, Y.A.F., M.O.d.M.J. and L.A.C.C.; formal analysis, Y.A.F., M.O.d.M.J., L.A.C.C. and K.L.; investigation, Y.A.F., M.O.d.M.J., L.A.C.C., R.O.d.S., I.H.Y.N., K.L. and B.V.L.P.; resources, W.D., B.V.L.P., J.A.M.-N. and S.S.M.-E.; data curation, Y.A.F., M.O.d.M.J., L.A.C.C. and S.S.M.-E.; writing—original draft preparation, Y.A.F. and M.O.d.M.J.; writing—review and editing, Y.A.F., M.O.d.M.J., L.A.C.C., I.H.Y.N., W.D., B.V.L.P., J.A.M.-N. and S.S.M.-E.; visualization, Y.A.F., M.O.d.M.J. and S.S.M.-E.; supervision, L.A.C.C., B.V.L.P. and S.S.M.-E.; project administration, S.S.M.-E.; funding acquisition, B.V.L.P. and S.S.M.-E. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Hospital of the University of São Paulo (CEP/HU-USP 943/09, SISNEP CAAE 0062.0.198.000-9) as approved by the local ethics committee (CEP/FCF-USP 534) on 6 April 2018.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available upon request.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Shain, A.H.; Bastian, B.C. From melanocytes to melanomas. *Nat. Rev. Cancer* **2016**, *16*, 345–358. [CrossRef] [PubMed]
2. Chhikara, B.S.; Parang, K. Global cancer statistics 2022: The trends projection analysis. *Chem. Biol. Lett.* **2023**, *10*, 451.
3. Siegel, R.L.; Miller, K.D.; Wagle, N.S.; Jemal, A. Cancer statistics, 2023. *CA Cancer J. Clin.* **2023**, *73*, 17–48. [CrossRef] [PubMed]
4. Aplin, A.E.; Kaplan, F.M.; Shao, Y. Mechanisms of resistance to RAF inhibitors in melanoma. *J. Investig. Dermatol.* **2011**, *131*, 1817–1820. [CrossRef]
5. Tang, T.; Eldabaje, R.; Yang, L. Current status of biological therapies for the treatment of metastatic melanoma. *Anticancer Res.* **2016**, *36*, 3229–3241.
6. Davies, H.; Bignell, G.R.; Cox, C.; Stephens, P.; Edkins, S.; Clegg, S.; Teague, J.; Woffendin, H.; Garnett, M.J.; Bottomley, W.; et al. Mutations of the BRAF gene in human cancer. *Nature* **2002**, *417*, 949–954. [CrossRef]
7. Pollock, P.M.; Harper, U.L.; Hansen, K.S.; Yudt, L.M.; Stark, M.; Robbins, C.M.; Moses, T.Y.; Hostetter, G.; Wagner, U.; Kakareka, J.; et al. High frequency of BRAF mutations in nevi. *Nat. Genet.* **2003**, *33*, 19–20. [CrossRef]
8. Platz, A.; Egyhazi, S.; Ringborg, U.; Hansson, J. Human cutaneous melanoma; a review of NRAS and BRAF mutation frequencies in relation to histogenetic subclass and body site. *Mol. Oncol.* **2008**, *1*, 395–405. [CrossRef]
9. Guo, Y.; Pan, W.; Liu, S.; Shen, Z.; Xu, Y.; Hu, L. ERK/MAPK signalling pathway and tumorigenesis. *Exp. Ther. Med.* **2020**, *19*, 1997–2007. [CrossRef]
10. Teixido, C.; Castillo, P.; Martinez-Vila, C.; Arance, A.; Alos, L. Molecular markers and targets in melanoma. *Cells* **2021**, *10*, 2320. [CrossRef]
11. Fedorenko, I.V.; Paraiso, K.H.T.; Smalley, K.S.M. Acquired and intrinsic BRAF inhibitor resistance in BRAF V600E mutant melanoma. *Biochem. Pharm.* **2011**, *82*, 201–209. [CrossRef]
12. Cheng, Y.; Zhang, G.; Li, G. Targeting MAPK pathway in melanoma therapy. *Cancer Metastasis Rev.* **2013**, *32*, 567–584. [CrossRef] [PubMed]
13. Chapman, P.B.; Hauschild, A.; Robert, C.; Haanen, J.B.; Ascierto, P.; Larkin, J.; Dummer, R.; Garbe, C.; Testori, A.; Maio, M.; et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N. Engl. J. Med.* **2011**, *364*, 2507–2516. [CrossRef] [PubMed]

14. Kudchadkar, R.R.; Smalley, K.S.; Glass, L.F.; Trimble, J.S.; Sondak, V.K. Targeted therapy in melanoma. *Clin. Dermatol.* **2013**, *31*, 200–208. [CrossRef]
15. Rebecca, V.W.; Herlyn, M. Nongenetic mechanisms of drug resistance in melanoma. *Annu. Rev. Cancer Biol.* **2020**, *4*, 315–330. [CrossRef]
16. Munhoz, R.R.; Postow, M.A. Combinatorial approaches to the treatment of advanced melanoma. *Hematol. Oncol. Clin.* **2021**, *35*, 145–158. [CrossRef]
17. Massaro, R.R.; Faião-Flores, F.; Rebecca, V.W.; Sandri, S.; Alves-Fernandes, D.K.; Pennachi, P.C.; Smalley, K.S.M.; Maria-Engler, S.S. Inhibition of proliferation and invasion in 2D and 3D models by 2-methoxyestradiol in human melanoma cells. *Pharm. Res.* **2017**, *119*, 242–250. [CrossRef]
18. Gorska-Ponikowska, M.; Kuban-Jankowska, A.; Daca, A.; Nussberger, S. 2-methoxyestradiol reverses the pro-carcinogenic effect of L-lactate in osteosarcoma 143B cells. *Cancer Genom. Proteom.* **2017**, *14*, 483–493.
19. Kim, J.; Nam, J.; Kim, A.; Park, M.; Lee, H.; Park, J.; Kim, J.; Lee, Y. 2-methoxyestradiol inhibits radiation-induced skin injuries. *Int. J. Mol. Sci.* **2022**, *23*, 4171. [CrossRef]
20. Hua, W.; Huang, X.; Li, J.; Feng, W.; Sun, Y.; Guo, C. 2-methoxyestradiol inhibits melanoma cell growth by activating adaptive immunity. *Immunopharm. Immunotoxicol.* **2022**, *44*, 541–547. [CrossRef]
21. Ireson, C.R.; Chander, S.K.; Purohit, A.; Perera, S.; Newman, S.P.; Parish, D.; Leese, M.P.; Smith, A.C.; Potter, B.V.L.; Reed, M.J. Pharmacokinetics and efficacy of 2-methoxyoestradiol and 2-methoxyoestradiol-bis-sulphamate *in vivo* in rodents. *Br. J. Cancer* **2004**, *90*, 932–937. [CrossRef]
22. Dahut, W.L.; Lakhani, N.J.; Gulley, J.L.; Arlen, P.M.; Kohn, E.C.; Kotz, H.; McNally, D.; Parr, A.; Parr, A.; Nguyen, D.; et al. Phase I clinical trial of oral 2-methoxyestradiol, an antiangiogenic and apoptotic agent, in patients with solid tumors. *Cancer Biol. Ther.* **2006**, *5*, 22–27. [CrossRef] [PubMed]
23. Newman, S.P.; Foster, P.A.; Stengel, C.; Day, J.M.; Ho, Y.T.; Judde, J.-G.; Lassalle, M.; Prevost, G.; Leese, M.P.; Potter, B.V.L.; et al. STX140 is efficacious *in vitro* and *in vivo* in taxane-resistant breast carcinoma cells. *Clin. Cancer Res.* **2008**, *14*, 597–606. [CrossRef] [PubMed]
24. Potter, B.V.L. Steroid sulfatase inhibition by aryl sulfamates: Clinical progress, mechanism, and future prospects. *J. Mol. Endocrinol.* **2018**, *61*, T233–T252. [CrossRef] [PubMed]
25. Thomas, M.P.; Potter, B.V.L. Estrogen *O*-sulfamates and their analogues: Clinical steroid sulfatase inhibitors with broad potential. *J. Steroid Biochem. Mol. Biol.* **2015**, *153*, 160–169. [CrossRef]
26. Thomas, M.P.; Potter, B.V.L. Discovery and development of the aryl *O*-sulfamate moiety for oncology and women's health. *J. Med. Chem.* **2015**, *58*, 7634–7658. [CrossRef]
27. Day, J.M.; Foster, P.A.; Tutill, H.J.; Newman, S.P.; Ho, Y.T.; Leese, M.P.; Potter, B.V.L.; Reed, M.J.; Purohit, A. BCRP expression does not result in resistance to STX140 in vivo, despite the increased expression of BCRP in A2780 cells in vitro after long-term STX140 exposure. *Br. J. Cancer* **2009**, *100*, 476–486. [CrossRef]
28. Ho, Y.T.; Newman, S.P.; Purohit, A.; Leese, M.P.; Potter, B.V.L.; Reed, M.J. The effects of 2-methoxyoestrogens and their sulphamoylated derivatives in conjunction with TNF-α on endothelial and fibroblast cell growth, morphology, and apoptosis. *J. Steroid Biochem.* **2003**, *86*, 189–196. [CrossRef]
29. Newman, S.P.; Leese, M.P.; Purohit, A.; James, D.R.C.; Rennie, C.E.; Potter, B.V.L.; Reed, M.J. Inhibition of *in vitro* angiogenesis by 2-methoxy- and 2-ethylestrogen sulfamates. *Int. J. Cancer* **2004**, *109*, 533–540. [CrossRef]
30. Jourdan, F.; Leese, M.P.; Dohle, W.; Hamel, E.; Ferrandis, E.; Newman, S.P.; Purohit, A.; Reed, M.J.; Potter, B.V.L. Synthesis, anti-tubulin, and antiproliferative SAR of analogues of 2- methoxyestradiol-3,17-*O*,*O*-bis-sulfamate. *J. Med. Chem.* **2010**, *53*, 2942–2951. [CrossRef]
31. Stengel, C.; Newman, S.P.; Leese, M.P.; Thomas, M.P.; Potter, B.V.L.; Reed, M.J.; Purohit, A.; Foster, P.A. The *in vitro* and *in vivo* activity of the microtubule disruptor STX140 is mediated by Hif-1 alpha and CAIX expression. *Anticancer Res.* **2015**, *35*, 5249–5261. [PubMed]
32. Meyer-Losic, F.; Newman, S.P.; Day, J.M.; Reed, M.J.; Kasprzyk, P.G.; Purohit, A.; Foster, A. STX140, but not paclitaxel, inhibits mammary tumour initiation and progression in C3(1)/SV40 T/t-antigen transgenic mice. *PLoS ONE* **2013**, *8*, e80305. [CrossRef] [PubMed]
33. Hosang, L.; Löhndorf, A.; Dohle, W.; Rosche, A.; Marry, S.; Diercks, B.P.; Müller-Kirschbaum, L.C.; Flügel, L.T.; Potter, B.V.L.; Odoardi, F.; et al. 2-Methoxyestradiol-3, 17-*O*, *O*-bis-sulfamate inhibits store-operated Ca^{2+} entry in T lymphocytes and prevents experimental autoimmune encephalomyelitis. *Biochim. Biophys. Acta Mol. Cell Res.* **2023**, *1870*, 119485. [CrossRef]
34. Foster, P.A.; Ho, Y.T.; Newman, S.P.; Leese, M.P.; Potter, B.V.L.; Reed, M.J.; Purohit, A. STX140 and STX641 cause apoptosis via the intrinsic mitochondrial pathway and down-regulate survivin and XIAP expression in ovarian and prostate cancer cells. *Anticancer Res.* **2009**, *29*, 3751–3758.
35. Wang, E. Senescent human fibroblasts resist programmed cell death, and failure to suppress Bcl-2 is involved. *Cancer Res.* **1995**, *55*, 2284–2292. [PubMed]
36. Sasaki, M.; Kumazaki, T.; Takano, H.; Nishiyama, M.; Mitsui, Y. Senescent cells are resistant to death despite low Bcl-2 level. *Mech. Ageing Dev.* **2001**, *122*, 1695–1706. [CrossRef] [PubMed]
37. Yosef, R.; Pilpel, N.; Tokarsky-Amiel, R.; Biran, A.; Ovadya, Y.; Cohen, S.; Vadai, E.; Dassa, L.; Shahar, E.; Condiotti, R.; et al. Directed elimination of senescent cells by inhibition of Bcl-w and Bcl-xL. *Nat. Commun.* **2016**, *7*, 11190. [CrossRef]

38. Lee, B.Y.; Han, J.A.; Im, J.S.; Morrone, A.; Johung, K.; Goodwin, E.C.; Kleijer, W.J.; DiMaio, D.; Hwang, E.S. Senescence-associated β-galactosidase is lysosomal β-galactosidase. *Aging Cell* **2006**, *5*, 187–195. [CrossRef]
39. Ewald, J.A.; Desotelle, J.A.; Wilding, G.; Jarrard, D.F. Therapy-induced senescence in cancer. *J. Natl. Cancer Inst.* **2010**, *102*, 1536–1546. [CrossRef]
40. Zhang, L.; Yang, Z.; Liu, Y. GADD45 proteins: Roles in cellular senescence and tumor development. *Exp. Biol. Med.* **2014**, *239*, 773–778. [CrossRef]
41. Minamino, T.; Orimo, M.; Shimizu, I.; Kunieda, T.; Yokoyama, M.; Ito, T.; Nojima, A.; Nabetani, A.; Oike, Y.; Matsubara, H.; et al. A crucial role for adipose tissue p53 in the regulation of insulin resistance. *Nat. Med.* **2009**, *15*, 1082–1087. [CrossRef]
42. Stivala, A.L.; Cazzalini, O.; Prosperi, E. The cyclin-dependent kinase inhibitor p21CDKN1A as a target of anti-cancer drugs. *Curr. Cancer Drug Targets* **2012**, *12*, 85–96. [CrossRef] [PubMed]
43. Katayama, H.; Sasai, K.; Kawai, H.; Yuan, Z.; Bondaruk, J.; Suzuki, F.; Fujii, S.; Arlinghaus, R.B.; Czerniak, B.A.; Sen, S. Phosphorylation by aurora kinase a induces MDM2-mediated destabilization and inhibition of p53. *Nat. Genet.* **2004**, *36*, 55–62.
44. Driscoll, D.L.; Chakravarty, A.; Bowman, D.; Shinde, V.; Lasky, K.; Shi, J.; Vos, T.; Stringer, B.; Amidon, B.; D'Amore, N.; et al. Plk1 inhibition causes post-mitotic DNA damage and senescence in a range of human tumor cell lines. *PLoS ONE* **2014**, *9*, e111060. [CrossRef]
45. Muñoz-Espín, D.; Serrano, M. Cellular senescence: From physiology to pathology. *Nat. Rev. Mol.* **2014**, *15*, 482–496. [CrossRef] [PubMed]
46. Ayrapetov, M.K.; Gursoy-Yuzugullu, O.; Xu, C.; Xu, Y.; Price, B.D. DNA double-strand breaks promote methylation of histone h3 on lysine 9 and transient formation of repressive chromatin. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 9169–9174. [CrossRef] [PubMed]
47. Chicas, A.; Wang, X.; Zhang, C.; McCurrach, M.; Zhao, Z.; Mert, O.; Dickins, R.A.; Narita, M.; Zhang, M.; Lowe, S.W. Dissecting the unique role of the retinoblastoma tumor suppressor during cellular senescence. *Cancer Cell* **2010**, *17*, 376–387. [CrossRef]
48. Wang, L.; Lankhorst, L.; Bernards, R. Exploiting senescence for the treatment of cancer. *Nat. Rev. Cancer* **2022**, *22*, 340–355. [CrossRef]
49. Takahashi, A.; Imai, Y.; Yamakoshi, K.; Kuninaka, S.; Ohtani, N.; Yoshimoto, S.; Hori, S.; Tachibana, M.; Anderton, E.; Takeuchi, T.; et al. DNA damage signaling triggers degradation of histone methyltransferases through APC/CCDH1 in senescent cells. *Mol. Cell* **2012**, *45*, 123–131. [CrossRef]
50. Hernandez-Segura, A.; Nehme, J.; Demaria, M. Hallmarks of cellular senescence. *Trends Cell Biol.* **2018**, *28*, 436–453. [CrossRef]
51. Lázár-Molnár, E.; Hegyesi, H.; Tóth, S.; Falus, A. Autocrine and paracrine regulation by cytokines and growth factors in melanoma. *Cytokine* **2000**, *12*, 547–554. [CrossRef]
52. Waugh, D.J.J.; Wilson, C. The interleukin-8 pathway in cancer. *Clin. Cancer Res.* **2008**, *14*, 6735–6741. [CrossRef] [PubMed]
53. Jobe, N.P.; Rosel, D.; Dvorankova, B.; Kodet, O.; Lacina, L.; Mateu, R.; Smetana, K.; Brabek, J. Simultaneous blocking of IL-6 and IL-8 is sufficient to fully inhibit CAF-induced human melanoma cell invasiveness. *Histochem. Cell Biol.* **2016**, *146*, 205–217. [CrossRef]
54. Sanmamed, M.F.; Perez-Gracia, J.L.; Schalper, K.A.; Fusco, J.P.; Gonzalez, A.; Rodriguez-Ruiz, M.E.; Oñate, C.; Perez, G.; Alfaro, C.; Martín-Algarra, S.; et al. Changes in serum interleukin-8 (IL-8) levels reflect and predict response to anti-PD-1 treatment in melanoma and non-small-cell lung cancer patients. *Ann. Oncol.* **2017**, *28*, 1988–1995. [CrossRef] [PubMed]
55. Prasanna, P.G.; Citrin, D.E.; Hildesheim, J.; Ahmed, M.M.; Venkatachalam, S.; Riscuta, G.; Xi, D.; Zheng, G.; Deursen, J.V.; Goronzy, J.; et al. Therapy-induced senescence: Opportunities to improve anticancer therapy. *J. Natl. Cancer Inst.* **2021**, *113*, 1285–1298. [CrossRef]
56. Zhang, L.; Pitcher, L.E.; Yousefzadeh, M.J.; Niedernhofer, L.J.; Robbins, P.D.; Zhu, Y. Cellular senescence: A key therapeutic target in aging and diseases. *J. Clin. Investig.* **2022**, *132*, e158450. [CrossRef] [PubMed]
57. Schmitt, C.A.; Wang, B.; Demaria, M. Senescence and cancer—Role and therapeutic opportunities. *Nat. Rev. Clin. Oncol.* **2022**, *19*, 619–636. [CrossRef] [PubMed]
58. Leese, M.P.; Leblond, B.; Smith, A.; Newman, S.P.; Di Fiore, A.; De Simone, G.; Supuran, C.T.; Purohit, A.; Reed, M.J.; Potter, B.V.L. 2-Substituted estradiol bis-sulfamates, multitargeted antitumor agents: synthesis, *in vitro* SAR, protein crystallography, and *in vivo* activity. *J. Med. Chem.* **2006**, *49*, 7683–7696. [CrossRef] [PubMed]
59. Sandri, S.; Faião-Flores, F.; Tiago, M.; Pennacchi, P.C.; Massaro, R.R.; Alves-Fernandes, D.K.; Berardinelli, G.N.; Evangelista, A.F.; de Lima Vazquez, V.; Reis, R.M.; et al. Vemurafenib resistance increases melanoma invasiveness and modulates the tumor microenvironment by mmp-2 upregulation. *Pharm. Res.* **2016**, *111*, 523–533. [CrossRef]
60. Liu, W.; Meng, J.; Cui, J.; Luan, Y. Characterization and function of microRNA's in plants. *Front. Plant Sci.* **2017**, *8*, 2200. [CrossRef]
61. Brohem, C.A.; Cardeal, L.B.S.; Tiago, M.; Soengas, M.S.; Barros, S.B.M.; Maria-Engler, S.S. Artificial skin in perspective: Concepts and applications. *Pigment Cell Melanoma Res.* **2011**, *24*, 35–50. [CrossRef] [PubMed]

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Silvia Berlanga de Moraes Barros
Retired Professor
School of Pharmaceutical Sciences
University of São Paulo

September 09, 2025. Sao Paulo, Brazil.

USCIS

RE: Letter of Recommendation in Support of Isabella Harumi Yonehara Noma

To whom it may concern,

This letter is to support the application of Dr. Isabella Harumi Yonehara Noma for the EB-1 Extraordinary Ability Category.

I am Silvia Berlanga de Moraes Barros, PhD at the University of Sao Paulo Brazil. At present, I am a retired professor of the Department of Clinical and Toxicological Analysis of the Faculty of Pharmaceutical Sciences of the University of Sao Paulo. I worked at the University from 1974 to 2022, first as Research Assistant and, from 1993 to 2022 as Full Professor of General Pathology. I was also vice-Dean ofthe Faculty of Pharmaceutical Sciences and Coordinator of Undergraduate Studies and the Graduate Program in Toxicology at the same faculty.

I have known Dr. Noma since 2020 when she started her PhD program in the laboratory that I shared with her supervisor, Professor Silvya Stuchi Maria-Engler. From the beginning, she demonstrated high commitment to the laboratory activities concentrated in her PhD thesis. Her work was dedicated to the study of how peroxiredoxin-2 (PRDX-2) modulates the invasive phenotype of NRAS mutated melanomas. Employing a system of reconstructed human skin and in silica analysis she proposed PRDX-2 as a biomarker or therapeutic target for NRAS mutated melanomas. Two papers were produced from her work (Peroxiredoxin-2 represses NRAS-mutated melanoma cells invasion by modulating EMT markers, and Peroxiredoxins 1 and 2 play different roles contributing to tumor heterogeneity and BRAFi resistance in melanoma}. Her first paper discusses how Prx-2 helps to repress the invasion of melanoma cells that carry NRAS mutations. Her contributions with this study are significant because NRAS-mutated melanoma is known for its aggressive behavior and poor prognosis. By modulating EMT markers, Prx-2 may influence the transition of cancer cells from an epithelial to a more migratory mesenchymal state, which is crucial for metastasis. Understanding this mechanism is essential to define new therapeutic strategies aimed at preventing melanoma spread.

The second paper addresses the differential roles of Prx-1 and Prx-2 in contributing to tumor heterogeneity and resistance to BRAF inhibitors (BRAFi). Her work here is significant because BRAF inhibitors are commonly used in treating BRAF-mutant melanoma, but many patients develop resistance over time, by elucidating how these peroxiredoxins contribute to different cellular behaviors and responses to treatment in cancer context. Her work in this research area is noteworthy, as it was presented at the Society of Melanoma Research and awarded with a Participant Fellowship both in 2022 and 2023.

Just after finishing her PhD, she was accepted at the Laboratory of Professor Keiran Smalley at the Moffitt Cancer Center, in Tampa, USA, to study the participation of the Histone

deacetylase 8 in the initiation of UVR-driven melanoma. In this new proposal, she aimed to investigate whether UVR-induced HDAC8 activation allows some melanocytes to evade cell death, enabling cells with pathogenic mutations to survive and potentially develop into melanoma. Her research provides a novel perspective on how UVR-induced epigenetic reprogramming targeting HDAC8 contributes to the persistence of melanocytes and melanoma cells harboring pathogenic mutations, allowing them to grow and evade apoptosis. By addressing the underlying mechanisms of tumor survival and resistance, Dr. Noma's research aids in improving therapeutic strategies, ultimately increasing survival rates and quality of life for patients with melanoma. Additionally, it underscores the importance of considering epigenetic factors in the development of innovative cancer therapies, which will lead to more effective interventions and better patient outcomes in the long term.

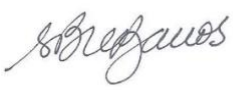
Currently, Dr. Noma is working as a Postdoctoral Fellow in the Tumor Microenvironment and Metastasis Department at the Moffitt Cancer Center, under an O-1A visa, which is granted to individuals with extraordinary ability in their field. This position further solidifies her reputation as a professional of exceptional expertise and international recognition in the area of melanoma and cancer research.

Further, Dr. Noma also had other publications that contribute to the field of epigenetics, which is growing in scientific research. She studied methylation related to nutrition, Alzheimer's disease, obesity, aging, diabetes, and COVID-19. She has contributed significantly to several areas in health science, particularly in skin cancer. She presented posters twice at the International Congress of the Society for Melanoma Research, as well as at the symposium of the graduate program in pharmacy in Brazil, and the Brazilian Congress of Toxicology, focusing on melanoma and peroxiredoxin. These are all prestigious conferences and events, which illustrate her standing in this field. Additionally, these works demonstrate her diverse contributions and the impact of her presentations on cancer research.

Dr. Noma has an excellent profile of publications that are not only derived from her MSc and PhD thesis but include also papers in collaboration with other laboratories indicating her ability to interact with other teams of research.

I will be pleased to answer any further questions about Ms. Noma if needed. My personal e-mail is silviaberlanga@gmail.com and my Whatsapp number 5511991029301.

Kind Regards,



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Education

University of Siena, 1990, Postdoctoral training on Free Radical Research
University of São Paulo, 1980, PhD in Toxicology
University of São Paulo, 1976, MSc Analytical Toxicology
University of São Paulo, 1972, PharmD

Professional Experience

2024 - Member of the Advisory Group to to recommend priorities for potential cancer hazards agents to be evaluated by the Monographs Programme of the International Agency for Research on Cancer.
2020 - Member of 90th JOINT FAO/WHO Expert Committee on Food Additives (JECFA).
2016 - 2024 Member **of** Expert Advisory Panel on Food Safety WHO.
2008-2014 – Organizer and faculty member of the Latin America Risk Assessment Workshop (I,II,III,IV,V,VI), Brazil.
2004 - International Regulatory Consultant of Spherix Consulting Group, USA, Maryland, United States of America
2003- 2022 - Senior Professor, School of Pharmaceutical Sciences, University of São Paulo, SP, Brazil
1993-2003 - Full Professor, Department of Clinical Chemistry and Toxicology, University of São Paulo, SP, Brazil
1986-1993 - Associate Professor, Department of Clinical Chemistry and Toxicology, University of São Paulo, SP, Brazil
1974-1986 - Assistant Professor, Department of Clinical Chemistry and Toxicology, University of São Paulo, SP, Brazil

Teaching Experience

School of Pharmaceutical Sciences University of São Paulo Graduate Program
2018 - 2022: Updates and Review of Skin Models
2008 - 2022: Topics in Physiopathology and Toxicology I and II
2006 - 2012: Pharmaceutical Strategies to Prevent Skin Damage by Solar Radiation: Natural Antioxidants
2002 - 2008: Seminars in Toxicology
1986 - 2005: Mechanisms of Hepatotoxicity
1986 - 1991: Seminar in Clinical Laboratory Analysis

School of Pharmaceutical Sciences University of São Paulo - Undergraduate Program in Pharmacy
1980 - 2006: General Pathology

School of Pharmacy and Biochemistry Oswaldo Cruz, São Paulo, Brazil
1983 - 1987: General Pathology

Course at the Latin America Risk Assessment Workshop sponsored by the Brazilian Society of Toxicology and the International Union of Toxicology 2008, 2009, 2010, 2011, 2012, 2014: Coordinator and lecture on Immunotoxicity in the risk assessment process

Risk Assessment for environmental Health Hazards Continuing Education Virtual Course sponsored by the Federal University of Paraná and the Brazilian Society of Toxicology 2021: Coordination and lectures on General Toxicity evaluation, Immunotoxicology and Study Case

Professional Society Membership

- Brazilian Society of Toxicology, since 1977
- Society of Toxicology USA, since 2001

Specialty Sections: Dermal Toxicology; *In Vitro* and Alternative Methods; Risk Assessment
Special Interest Groups: Hispanic Organization of Toxicologists; Women in Toxicology

- Associação Brasileira de Ciências Farmacêuticas, since 2005.

Participation in professional and scientific associations

International Society for the Study of Xenobiotics
1993 - 1995 Councilor
ILSI-Brazil
2004 - 2007 Member of the executive committee of the ILSI-Brazil Scientific and Administrative Council
Toxicology Education Foundation
2010 - 2022 Member of the Board of Trustees of the Toxicology Education Foundation
National Research Council Brazil
2004-2017 Fellow of the National Research Council in Brazil
International Union of Toxicologists (IUTOX)
2016 - 2022 Board of IUTOX Directors
2004 - 2010 Board of IUTOX Directors
2016 - 2022 Chair of the IUTOX Developing Countries Committee
2023 - 2024 Advisor at the IUTOX Education, Membership and Developing Countries committees
Brazilian Society of Toxicology (BSTox)
1990-1991 Secretary-General
2004-2005 Secretary-General
1992-1993 President
University of São Paulo
2005-2011 Representing at the GPEN (Globalization of Pharmaceutical Education Network)
Society of Toxicology
2001 Membership Committee and the Global Strategic Task Force
2014-2016 Councilor of the Hispanic Organization of Toxicologists (HOT)
2016 Vice president elected of the HOT
2017 Vice president of the HOT
2018 President of the HOT
2023 External Advisor at the Scientific Committee of HOT
2023 Nominated as Income-Chair of the SOT/Scientific Liaison Coalition

World Health Organization
2016 - 2024 Expert Advisory Panel on Food Safety
2024 Member of the Advisory Group to to recommend priorities for potential cancer hazards agents to be evaluated by the Monographs Programme of the International Agency for Research on Cancer

Honors

- Secretary-General of the Brazilian Society of Toxicology, 1989-1990/2003-2005
- President of the Brazilian Society of Toxicology, 1991-1992
- Vice-Dean, Department of Clinical Chemistry and Toxicology, School of Pharmaceutical Sciences University of São Paulo, 1993-1996
- Councilor of the International Society for the Study of Xenobiotics (1993-1995)
- Dean, Department of Clinical Chemistry and Toxicology, School of Pharmaceutical Sciences University of São Paulo, 1996-2000
- Vice-Dean, School of Pharmaceutical Sciences University of São Paulo, 2000-2003
- Associate Vice-Rector Undergraduate Studies, University of São Paulo, 1998 -2001
- Director International Union of Toxicology, 2004-2011, 2016-2019
- Member of the Scientific council of ILSI Brazil, 2004-2009
- Member of the Strategic Task Force of SOT, 2009-2011
- Member of the board of the Hispanic Organization of Toxicology 2014-2016
- Vice-president elected Hispanic Organization of Toxicology 2016-2017
- Vice-President Hispanic Organization of Toxicology 2017-2018
- Member of the board of the Toxicology Education Foundation, 2012-2022.
- Member of the board of the ITEHPEC (Institute of Technology and Studies on Personal Care, Hygiene, Perfumery and Cosmetics Products
- Member of the Membership Committee SOT 2015-2018
- Pharmaceutical Excellence, Conselho Regional do Estado de São Paulo, 2005
- Sphere Award, Dow Chemical USA, 2001
- Medal Profa Ester de Camargo Fonseca Moraes – Brazilian Society of Toxicology, 2013
- Distinguish Toxicologist Award, Hispanic Organization of Toxicologists, SOT SIG, 2021.
- Heinz Maurer Award for Dermatological Research, Sebapharma GmbH&Co.KG, Germany, 2022
- Education Award, Society of Toxicology USA, 2022.

Research Interests

- Oxidative stress mediated mechanism of hepatotoxicity, photoprotective and anticarcinogencic activity of natural products, UV radiation/pesticides interactions and air pollution in skin lesions development, alternative methods to animal experimentation for drugs and cosmetics safety evaluation and risk assessment.
- KEYWORDS: UV radiation; pesticides; natural products; skin; alternative methods to animal experimentation; cutaneous melanoma; oxidative stress; photoquimioprevention; air pollution.

Publications in peer-reviewed journals

1. HIEDA, D.S; CARVALHO. L.A.C.; VAZ DE MELLO, B.; OLIVEIRA, E.A.; ASSIS, S.R.; WU, J.; DU-THUMM, L.; VIANA DA SILVA, C.L.; ROUBICEK, D.A.; MARIA-ENGLER, S.S.; **BARROS, S.B.M.** Air particulate matter induces skin barrier dysfunction and water transport alteration on a reconstructed human epidermis model. JOURNAL OF INVESTIGATIVE DERMATOLOGY, v. 140, p. 2343-2352.e3, 2020.

2. BROHEM,C.A. ; DA SILVA CARDEAL,L.B.. ; TIAGO,M. ; SOENGAS,M.S.; **BARROS, S. B. M.;** MARIA-ENGLER,S.S. Artificial skin in perspective: concepts and applications. PIGMENT CELL & MELANOMA RESEARCH, v.24, p.35-50, 2011.
3. TORRES,L. L. ; QUAGLIO, N. B. ; SOUZA, G.T .; GARCIA, R.T. ; DATI, L.M.M. ; MOREIRA, W.L. ; LOUREIRO, A. P.M. ; SOUZA-TALARICO, J.N. ; SMID, J. ; PORTO, C.S. ; BOTTINO, C. M.C. ;NITRINI,R.; **BARROS,S.B.M.**; CAMARINI,R. ; MARCOURAKIS,T. Peripheral oxidative stress biomarkers in mild cognitive impairment and Alzheimer's disease. JOURNAL OF ALZHEIMER'S DISEASE, v. 26, p. 59-68, 2011.
4. VIDELA, L.A; **BARROS, S.B.M.**; JUNQUEIRA, V.B.C. Lindane-induced liver oxidative stress - review. FREE RADICAL IN BIOLOGY AND MEDICINE, v. 9, p. 169-179, 1990.
5. JUNQUEIRA, V.B.C.; SIMIZU,K.; VIDELA,L.A. **BARROS,S.B.M.** Dose dependent study of the effects of acute lindane administration on rat liver superoxide anion production, antioxidant enzymes activities and lipid peroxidation.. TOXICOLOGY, v. 41, p. 193-204, 1986.

Patricia A. Possik PhD.
Program of Immunology and Tumor Biology
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September 09th, 2025. Rio de Janeiro, Brazil.

United States Department of Homeland Security
U.S. Citizenship and Immigration Services

This letter is to support Dr. Isabella Harumi Yonehara Noma's in her EB-1 VISA application. I had the pleasure of meeting Dr. Noma during her PhD studies at the University of Sao Paulo and when I visited the Moffitt Cancer Center, in Tampa. As an investigator working on the same field and with current scientific collaborations with her supervisor Dr. Keiran Smalley, I am familiar with her work through her publications and scientific presentations and can evaluate the significance of her research.

I am a group leader at the Brazilian National Cancer Institute (INCA), in Rio de Janeiro, Brazil, since 2016. I am also a professor at the Postgraduate Program in Oncology at INCA and Royal Society - Newton Advanced fellow. At INCA, I am head of the Functional Biology of Tumors laboratory whose main focus is to study the cellular and molecular biology of tumors, especially melanomas, in order to identify new therapeutic targets and biomarkers. This effort is being supported by the Medical Research Council (UK), The Royal Society/ Newton Fund (UK), The Melanoma Research Alliance (USA), ICGEB (Italy), CNPq and FAPERJ (Brazil).

Dr. Noma successfully completed her PhD in 2023 with a scholarship by the National Council for Research Scientific Development, Brazil. She received in her Master's degree, and Bachelor's degree scholarship by CAPES (Brazilian Federal Foundation for Support and Evaluation of Graduate Education - Ministry of Education). She also received post doctorate fellowship from Cnpq-Brazil in 2024 to her visiting research in USA - Florida, which is also a very competitive award/grant. She demonstrated great potential, scientific merit and commitment during her scientific training, which resulted in several peer-reviewed publications. Her successful completion of the PhD was followed by an invitation to join Dr. Keiran Smalley's group as a postdoctoral fellow. Her previous laboratory experience and the scientific knowledge acquired during her postgraduate training are certainly contribute to her success and to the advancement of these studies, whose main objective is to benefit society.

Dr. Noma has made contributions with regard to melanoma cancer with three studies she published: Peroxiredoxin-2 represses NRAS-mutated melanoma cells invasion by modulating EMT markers, 2- Methoxyestradiol-3,17-O,O-bis-sulfamate (STX140) Inhibits Proliferation and Invasion via Senescence Pathway Induction in Human BRAFi-Resistant Melanoma Cells, and Peroxiredoxins 1 and 2 play different roles contributing to tumor heterogeneity and BRAFi resistance in melanoma. Her first study highlights how Peroxiredoxin-2 can suppress the invasion of melanoma cells with NRAS mutations. By modulating epithelial-mesenchymal transition {EMT) markers, it

underscores the importance of oxidative stress response proteins in cancer progression and suggests therapeutic targets for aggressive melanoma. Dr. Noma's second research indicates that STX140 can inhibit both proliferation and invasion of BRAFi-resistant melanoma cells by inducing cellular senescence. This research has opened up avenues for exploring senescence as a therapeutic strategy against resistant melanoma, which is a significant hurdle when it comes to treatment. Her third paper emphasizes the distinct roles of Peroxiredoxins 1 and 2 in contributing to tumor heterogeneity and resistance to BRAF inhibitors (BRAFi) in melanoma. All publications indicated new treatments to overcome resistance mechanisms and improve treatment outcomes, such as proposing new strategies that explore antioxidants or redox balance with less toxicity and more efficacy. Exploring redox balance can address the hallmarks of cancer, which are significant challenges today in cancer treatment, including resistance and metastasis. Both of her studies demonstrated efficacy through antioxidants, representing a new strategy to pursue. Moreover, antioxidants have lower toxicity compared to conventional treatments such as chemotherapy and radiotherapy. Her research works together demonstrate that Dr. Noma has been contributing to the melanoma cancer area, looking for treatments and new strategies for cancer patients.

Among the several contributions in epigenetics role: Eight Weeks of Physical Training Decreases 2 Years of DNA Methylation Age of Sedentary Women, 14-weeks combined exercise epigenetically modulated118 genes of menopausal women with prediabetes, Sample Preparation to Bioinformatics Analysis of DNA Methylation: Association Strategy for Obesity and Related Trait Studies, and Gene signatures of autopsy lungs from obese patients with COVID-19. Her collaborations in important studies based in diabetes, COVID-19, and obesity. That impact in several issues important to national health care. She presented posters with her results in 19th and 20th International Congress of the Society for Melanoma Research, in Edinburgh, Scotland, 2022 and Philadelphia in 2023. Also, she participated of the XXII Brazilian Congress of Toxicology, Balneario Camburiu, Brazil, 2022, presenting her results.

Dr. Noma published 19 manuscripts, and 80 citations. She had important international collaborations with a professor from Newcastle University, and researchers from Moffitt Cancer Center, that result in her publication in Biomedicine and Pharmacotherapy, and Antioxidants (journals with impact higher than 6). Publications in collaboration in journals with 3-4 impact factor. For these reasons, I strongly support Dr. Isabella Yonehara Noma's application for the EB-1 VISA.

Please do not hesitate to contact me should you need any further information.

Sincerely,
Patricia Abrão Possik, PhD
Brazilian National Cancer Institute
Principal Investigator (Associate Professor level)
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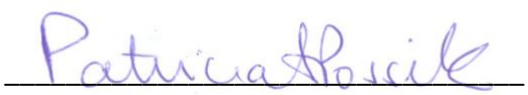
Phone: +55 21 990046873



PATRICIA ABRÃO POSSIK

Professional Address: Brazilian National Cancer Institute (INCA)
Rua André Cavalcanti, 37. Rio de Janeiro, Brazil, CEP: 20231-050
Office Phone #: +55-21-32076534
Corresponding e-mail: ppossik@inca.gov.br

CURRENT APPOINTMENT:

2016-ongoing: Associate Investigator, Brazilian National Cancer Institute, Division of Experimental and Translational Research, Program of Immunology and Tumor Biology, Rio de Janeiro, Brazil.

EDUCATION AND TRAINING:

2009-2015: Post-doctoral fellow, The Netherlands Cancer Institute, Amsterdam, Netherlands.
2006-2008: Visiting Scientist, The Wistar Institute, Philadelphia, USA.
2004-2008: PhD in Sciences/Oncology, Fundação Antônio Prudente, Ludwig Institute for Cancer Research, São Paulo, Brazil.
2001-2003: MSc in Genetics and Evolution, Federal University of São Carlos, São Carlos, Brazil.
1997-2001: BSc in Biological Sciences, Federal University of São Carlos, São Carlos, Brazil.

FELLOWSHIPS AND AWARDS:

2025 (awarded): Wellcome International Fellowship, UK
2020: Royal Society – Newton Advanced Fellowship, UK
2015-2017: Young Talent Fellowship, National Council for Scientific and Technological Development (CNPq), Brazil.
2006-2007: "Sandwich" PhD Fellowship for training abroad (PDEE), Coordination of Improvement of Higher Education Personnel (CAPES), Brazil.
2004-2008: PhD Fellowship, São Paulo Research Foundation (FAPESP), Brazil.
2001-2003: MSc Degree Fellowship, CNPq, Brazil.

CURRENT FUNDING:

- National Council for Scientific and Technological Development (CNPq, Brazil) #442091/2023-0 "Therapeutic strategies directed to the genetic profile of acral melanoma" Role: PI. Duration: 2023-2026.

- Chan-Zuckerberg Initiative - Ancestry Networks for the Human Cell Atlas # 2021-240108 (5022). "A Human Cell Map of Latin American Diversity". Role: Co-PI. Duration: 2022-2024.

- Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ, Brazil) E-26/211.321/2021. "Study of metastatic potential: molecular characterization of the tumor and the microenvironment". Role: Co-PI. Duration: 2021-2025.

- Melanoma Research Alliance – Pilot Award (825924). "Defining mediators of metastatic spread in acral melanoma". Role: Co-PI. Duration: 2021-2023.

- Royal Society – Newton Advanced Fellowship (NAF02163). "Exploring synthetic lethal interactions with therapeutic potential in Acral Lentiginous Melanoma". Role: PI. Duration: 2020-2024.

- Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ, Brazil) E-26/010.001645/2019. "Chk1 as a therapeutic target in melanomas". Role: PI. Duration: 2020-2023.

- Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ, Brazil), E26/010.002187/2019. "Molecular studies in Genomic Instability: causes and consequences". Role: Co-PI. Duration: 2019-2023.

SELECTED PUBLICATIONS:

1. Carvalho LAD, Aguiar FC, Smalley KSM, **Possik P.A**. Acral melanoma: new insights into the immune and genomic landscape. Neoplasia. 2023 Dec;46:100947. Epub 2023 Oct 31.

2. Primo, R. B. B.; Nobre, Aretha B; Santos, B. N.; Nunes, L. F.; Santos, R. F.; **Possik, P. A.***; Bernardes, Sara S*. Impact of clinical and histopathological characteristics on the disease-free survival of stage I-II acral melanoma patients. *International Journal of Dermatology*, v.x, p.x - 2023. *equal contribution

3. **Possik, Patricia A**. Obstacles and opportunities in studying melanoma in Latin America. *Nature Cancer*, v. 1, p. 1, 2023.

4. Birkeälv, Sofia; Harland, Mark; Matsuyama, Larissa Satiko Alcântara Sekimoto; Rashid, Mamun; Mehta, Ishan; Laye, Jonathan P.; Haase, Kerstin; Mell, Tracey; Iyer, Vivek; Robles'espinoza, Carla Daniela; Mcdermott, Ultan; Van Loo, Peter; Kuijjer, Marieke L.; **Possik, P. A.**; Maria Engler, Silvya Stuchi; Bishop, D. Timothy; Newton'bishop, Julia; Adams, David J. Mutually exclusive genetic interactions and gene essentiality shape the genomic landscape of primary melanoma. *Journal of Pathology*, v.x, p.1 -, 2022.

5. Bernardes, Sara S; Ferreira, Ingrid; Elder, David E; Nobre, Aretha B; Martínez'said, Héctor; Adams, David J; Robles'espinoza, Carla Daniela; **Possik, P. A.**; More than just acral melanoma: the controversies of defining the disease. *The Journal of Pathology: Clinical Research*. v.1, p.cjp 2.233 - 01, 2021.

6. Basurto'lozada, Patricia; Molina'aguilar, Christian; Castañeda'garcia, Carolina; Vázquez'cruz, Martha Estefanía; Garcia'salinas, O. Isaac; Álvarez'cano, Alethia; Martínez'said, Héctor; Roldán'marín, Rodrigo; Adams, David J.; **Possik, P. A.***; Robles'espinoza, Carla Daniela*. Acral lentiginous melanoma: Basic facts, biological characteristics and research perspectives of an understudied disease. *Pigment Cell & Melanoma Research* (Online), v.34, p.59 - 71, 2020. *equal contribution

7. Jorge, Natasha A. N.; Cruz, Jéssica G. V.; Pretti, Marco Antônio M.; Bonamino, Martín H.; **Possik, P. A.** *; Boroni, Mariana*. Poor clinical outcome in metastatic melanoma is associated with a microRNA-modulated immunosuppressive tumor microenvironment. *Journal of Translational Medicine.*, v.18, p.1 - 17, 2020. *equal contribution

8. Mueller, J.; Krijgsman, O.; Tsoi, J.; Robert, L.; Hugo, W.; **Possik, P. A.**; Cornelissen- Steijger,P.; Geukes, M; Kemper, K.; Goding, C.; Mcdermott, U.; Blank, C.; Haanen, J.; Graeber, T.; Lo, R. S.; Ribas, A.; Peeper. D.S. Low MITF/AXL ratio predicts early resistance to multiple targeted drugs in melanoma. *Nature Communications*., v.5, p.5712 , 2014.

9. **Possik, P. A.**; Mueller, J.; Gerlach, C.; Kenski, J. C. N.; Huang, X.; Shahrabi, A.; Krijgsman, O.; Song, J.; Smit, M.A; Gerritsen, B.; Liefting, C.; Kemper, K.; Michaut, M.; Beijersbergen, R. L.; Wessels, L.; Schumacher, T.; Peeper, D. S. Parallel in vivo and in vitro melanoma RNAi screens reveal synthetic lethality between hypoxia and the DNA damage response inhibition. *Cell Reports*., v.9, p.1 - 12, 2014.

10. Vredeveld, L. C. W. *; **Possik, P. A**. *; Smit, M. A.; Meissl, K.; Michaloglou, C.; Horlings, H. M.; Ajouaou, A.; Kortman, P. C.; Dankort, D.; Mcmahon, M.; Mooi, W. J.; Peeper, D. S. Abrogation of BRAFV600E-induced senescence by PI3K pathway activation contributes to melanomagenesis. *Genes & Development*, v.26, p.1055 - 1069, 2012. *equal contribution

* For a complete list of published work see
https://www.ncbi.nlm.nih.gov/pubmed/?term=Possik+PA+or+possik+P

CERTIFICATE OF COMPLETION

The Vice President of Research and Innovation at the University of São Paulo, in the exercise of his duties, hereby certifies that ISABELLA HARUMI YONEHARA NOMA, BRAZILIAN, holder of an identity document (RG) number 108737174, born on SEPTEMBER 3, 1996, in the State of Paraná, has completed the POSTDOCTORAL PROGRAM carried out in the Department of Clinical and Toxicological Analysis at the School of Pharmaceutical Sciences.

PROJECT: THE ROLE OF HDAC8 IN UVR-DRIVEN MELANOMA INITIATION

SUPERVISOR: PROFESSOR DR. SILVYA STUCHI MARIA ENGLER

PERIOD: 08/01/2023 to 07/31/2024

CNPq - National Council for Scientific and Technological Development

CREDIT HOURS: 1440 HOURS

Paulo Alberto Nussenzveig

VICE PRESIDENT OF RESEARCH AND INNOVATION

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Activities Performed

Cell culture and animal management (320 hours)

Cloning cells with HDAC8 overexpression (160 hours)

Functional assays (320 hours)

Generation of UVR-driven skin cancers (160 hours)

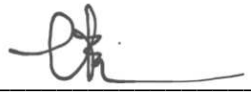
Protein expression (HDAC8, p53, Acetyl-p53) in melanocytes (160 hours)

Protein expression (HDAC8, p53, Acetyl-p53, other markers) in melanoma cells (160 hours)

shRNA-mediated HDAC8 knockdown in melanoma cells (160 hours)

Document issued at 1:36:09PM on 07/16/2025 (Brasília date and time).

I, Carolina Favero da Silva, telephone number 415 425-2508, mailing address P.O. Box 90487, San Diego, CA 92169, certify that the professional translation of this document from Portuguese to English has been performed by myself, a qualified translator fluent in both languages, and that the following is an accurate and complete translation of the document.


_____ Date: August 29, 2025.

ATESTADO DE CONCLUSÃO

O Pró-Reitor de Pesquisa e Inovação da Universidade de São Paulo, no uso de suas atribuições, atesta que ISABELLA HARUMI YONEHARA NOMA, de nacionalidade BRASILEIRA, portador(a) do documento tipo RG número 108737174, nascido(a) em 3 de SETEMBRO de 1996 e natural do Estado de Paraná, concluiu o PROGRAMA DE PÓS-DOUTORADO, realizado no Departamento de Análises Clínicas Toxicológicas da Faculdade de Ciências Farmacêuticas.

PROJETO: THE ROLE OF HDAC8 IN UVR-DRIVEN MELANOMA INITIATION

SUPERVISOR: PROFA. DRA. SILVYA STUCHI MARIA ENGLER

PERÍODO: 01/08/2023 a 31/07/2024

CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico

CARGA HORÁRIA: 1440 HORAS

Paulo Alberto Nussenzveig

PRÓ-REITOR DE PESQUISA E INOVAÇÃO

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Atividades Desenvolvidas

Cell culture and animal management (320 horas)

Clonning cells with HDAC8 overexpression (160 horas)

Functional assays (320 horas)

Generate UVR-driven skin cancers (160 horas)

Proteins expression (HDAC8, p53, Acetyl-p53) in melanocytes (160 horas)

Proteins expressions (HDAC8, p53, Acetyl-p53, other markers) for melanoma cells (160 horas)

shRNA to HDAC8 knockdown in melanoma cells (160 horas)

UNIVERSIDADE DE SÃO PAULO
Departamento de Análises Clínicas e Toxicológicas

FACULDADE DE CIÊNCIAS FARMACÊUTICAS

Laboratório Biologia da Pele
Centro de inovação tecnológica em Pele
iNovaPele

September 24, 2025
São Paulo, Brazil

United States Department of Homeland Security
U.S. Citizenship and Immigration Services

To Whom It May Concern,

I am writing to express my **strongest recommendation** for **Dr. Isabella Harumi Yonehara Noma** in support of her **EB-1 visa application**. I had the privilege of supervising Dr. Noma during her doctoral studies at the University of São Paulo (USP), where she completed her PhD in Pharmaceutical Sciences with outstanding performance. She is currently a **postdoctoral fellow at the Moffitt Cancer Center** in Florida, USA, under the supervision of **Dr. Keiran Smalley**. This position follows her earlier one-year fellowship at Moffitt as a CNPq scholar, a highly competitive grant awarded by the Brazilian National Council for Scientific and Technological Development, where she also worked under my co-supervision.

My laboratory specializes in **melanoma biology** and the use of **human reconstructed 3D skin models** to investigate mechanisms of **metastasis and tumor resistance**. These models are essential for both fundamental and translational research in dermatology and oncology. During her PhD, Dr. Noma made notable contributions by applying **2D and 3D models** to study **melanoma cell invasion**, a key step in the metastatic process. Her work advanced our understanding of **how molecular pathways drive cancer cell migration**, contributing to the identification of potential biomarkers and therapeutic targets.

Dr. Noma presented her research at major national and international scientific conferences. Notably, at the **Society for Melanoma Research (SMR)** Congress, she presented *"PRDX2 as a possible phenotype switcher in NRAS-mutated melanomas"*, which generated significant academic discussion. She also presented *"Gliotoxin and its potential cytotoxicity against NRAS-mutated melanoma"* at the **XXII Brazilian Congress of Toxicology (CBTox 2022)**, one of Brazil's leading toxicology meetings, highlighting the interdisciplinary nature and impact of her research.

Her doctoral work resulted in the publication of a first-author paper entitled *"Peroxiredoxin-2 represses NRAS-mutated melanoma cells invasion by modulating EMT markers"* (*Biomedicine & Pharmacotherapy*, 2024; PMID 38955087; DOI 10.1016/j.biopha.2024.116953*). This study elucidated how **Peroxiredoxin-2 (PRDX2)** regulates epithelial-mesenchymal transition (EMT) markers, revealing its role as a suppressor of invasion in NRAS-mutated melanoma cells. This discovery provides a foundation for **novel therapeutic approaches** aimed at limiting tumor spread and may inform strategies for other malignancies with similar oncogenic pathways.

In addition to her strong publication record—**19 articles indexed in Web of Science** with nearly **100 citations**—Dr. Noma has demonstrated excellence in securing competitive funding throughout her career. She was a recipient of prestigious **CAPES** and **CNPq** fellowships across all academic levels, from undergraduate through postdoctoral training, evidencing consistent recognition of her scientific merit. Her achievements have been acknowledged with **honorable mentions** in national symposia and repeated invitations to present at international conferences, including the **SMR 2022 (Scotland)** and **SMR 2023 (Philadelphia)** meetings.

During her postdoctoral tenure at Moffitt, Dr. Noma has expanded her expertise into **epigenetics and UV-induced melanoma**. Her research uncovered that **HDAC8 overexpression** following UV irradiation enhances the survival of damaged melanocytes and melanoma cells, demonstrating a novel mechanism of **UV-induced epigenetic reprogramming** contributing to tumor persistence and resistance to apoptosis. This work opens new perspectives for **melanoma prevention and therapy** by targeting the epigenetic consequences of UV exposure.

Dr. Noma's scientific rigor, creativity, and independence make her an **exceptional young investigator** poised to make impactful contributions to biomedical research in the United States. I have no doubt that she will continue to advance the fields of oncology and molecular biology through innovative, high-quality science.

Please feel free to contact me for any additional information.

Sincerely,

Prof. Silvya Stuchi Maria-Engler, PhD
Full Professor, School of Pharmaceutical Sciences
University of São Paulo (USP)
Email: silvya@usp.br

Curriculum Vitae for: Silvya Stuchi Maria-Engler, Ph.D.

Current Position:

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School of Pharmaceutical Sciences
Bldg 17, room 114
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São Paulo, SP, 05508-000 Brazil
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Google Schoolar:
H index: 31
Citation number: 3056
Web of Science
H index: 26
Citation number: 1870

Education:

1988-1992	B.Sc. (Hons.)	Biology, Paulista State University, SP, Brazil
1992-1994	M.Sc	Cell biology, University of Campinas, Sp, Brazil
1994-1998	Ph.D.	Biochemistry and Molecular Biology, University of Campinas, SP, Brazil

Postgraduate Training and Fellowship Appointments

1998-1999	Postdoctoral Fellowship in the laboratory of Prof. Nancy Boudreau, Surgery Department, University of California, San Francisco, USA.
2000-2002	Postdoctoral Fellowship in the laboratory of Prof. Mari Cleide Sogayar, Chemistry Institute, University of São Paulo, SP, Brazil.
2007-2008	Research Fellowship in the laboratory of Dr Marisol Soengas, Dermatology Department, University of Michigan, USA

Academic Appointments and Employment:

2003-2010	Assistant Professor, University of são Paulo, SP, Brazil (with tenure)
2010-2019	Associate Member (with tenure), University of São Paulo, SP, Brazil
2019	Full Professor

Teaching Experience
University Courses:
Pharmacy Undergraduate Course

2003- present	**Course Coordinator and Lecturer:** Clinical Cytopathology 80 hours/semester
2008-present	**Course Coordinator and Lectures:** Neoplasia in Pathophysiology Course 40 hours/semester

Patophysiology Master and Ph.D. Programs

2003-present	**Course Coordinator and Lecturer:** Cancer invasion process 40 hours every two years

Teaching and Training Experience (over 70 students in different levels)

Graduate training: Supervisor for Masters

1. 2002- 2005 **TATIANA CAROLINE SILVEIRA CORRÊA**. Fellowship CNPq
2. 2003-2006 **LAURA BEATRIZ SILVA CARDEAL**. Fellowship CNPq
3. 2006-2009 **RENATO RAMOS MASSARO**. Fellowship FAPESP.
4. 2009-2011 **MANOELA TIAGO DOS SANTOS.** Fellowship CNPq
5. 2009-2011 **ERIKA REGINA MATHEUS.** Fellowship CNPq
6. 2009-2011 **RAQUEL HAGA. Fellowship** FAPESP
7. 2010-2012 **FERNANDA B. FILLIPIN** Fellowship Fapesp.
8. 2012-2015 **CAROLINA MOTTER CATARINO** Fellowship Fapesp
9. 2013-2016 **NAYANE DOS SANTOS** Fellowship CNPq
10. 2016-2019 **LUIS WATANABE** Fellowship CNPq
11. 2016-2019 **STEPHANIE CRISTINE CARVALHO DOS SANTOS** Fellowship CNPq
12. 2019-2022 **YLANA ADAMI FRANCO- NO FELLOWSHIP- JANSEN EMPLOYEE.**

Graduate training: Supervisor for PhD

1. 2005-2010 TATIANA CAROLINE SILVEIRA CORRÊA. Fellowship FAPESP
2. 2005-2010 LAURA BEATRIZ DA SILVA CARDEAL. Fellowship FAPESP -sandwich 1 year Zena Werb-UCSF
3. 2006-2001 CARLA ABDO BROHEM Fellowship FAPESP-sandwich 1 year Marisol Soengas, CNIO, Spain
4. 2010-2014 RENATO RAMOS MASSARO Fellowship FAPESP-sandwich 1 year Keiran Smalley, Moffitt Cancer Center, Tampa, USA
5. 2011-2015 PAULA COMUNE PENNACCHI Fellowship FAPESP-sandwich 1year Lóreal, France
6. 2011-2015 MANOELA TIAGO DOS SANTOS Fellowship FAPESP-sandwich 1 year Keiran Smalley, Moffitt Cancer Center, Tampa, USA
7. 2011-2015 TATIANA PEDROSA Fellowship FAPESP- Dr Yves Poumay, Namur University, Belgium
8. 2011-2015 DÉBORA K. ALVES Fellowship FAPESP- -sandwich 6 months at Dr. Ashani Weeraratna, Wistar Institute, USA
9. 2017-2022 LARISSA S. SEKIMOTO MATUSYAMA -Fellowship CAPES- sandwich 18months, Dr David Adams, Welcome Sanger Institute, Cambridge, UK.

Undergraduate Students

1. 2000-2002 **DENISE LOLI.** Fellowship Fapesp.
2. 2005-2006 **KAIO VITIZEL.** Fellowship CNPq-PIBIC.
3. 2003-2006 **CARLA ABDO BROHEM.** Fellowship Fapesp.
4. 2004-2006 **RENATO RAMOS MASSARO.** Fellowship Fapesp.
5. 2009-2010 **MARINA LÍGIA M. GUTIERREZ.** Fellowship Cnpq- PIBIC.
6. 2010-2010 **RAFAELA CHRISTINA DE AMORIM SILVA**
7. 2010-2011 **MARIANA T. BARTIÉ.** Fellowship CNPq
8. 2011-2012 **TAÍSSA FARIA.** Fellowship Fapesp
9. 2011-2013 **MARIA CLARA CREPALDI** Fellowship Fapesp
10. 2012-2014 **OCTÁVIO GOMES** Fellowship Fapesp
11. 2013-2014 **NAYANE DE SOUZA** Fellowship CNPq
12. 2013-2016 **GUSTAVO TAKASHI** Fellowship Fapesp
13. 2014-2016 **GABRIELA LONGO LOCRETO DE TOLEDO** Fellowship CNPq
14. 2014-2015 **SILVIA CARDOSO TRATINIK** Fellowship CNPq
15. 2016-2018 **VANESSA VALENTINI** Fellowship PROCAD CAPES
16. 2016-2018 **BARBARA VAZ** Fellowship PROCAD CAPES

17. 2017-2020 **ADRIANA UENOHARA** Fellowship Fapesp
18. 2019- **RODRIGO GONÇALVEZ QUEIJO**, Fellowship Fapesp
19. 2022- **SOPHIA TAVERES DE SOUZA**, Fellowship Fapesp
20. 2022 **MARIANA GIANELLA**, Fellowship Fapesp

Post-Doctoral Supervision

1. 2006-2007 **TÂNIA SAWADA** - Fellowship CNPq
2. 2010-2011 **CARLA ABDO BROHEM** - Fellowship FAPESP
3. 2010-2011 **PAULA ANASTÁCIA FERREIRA**, Fellowship ProDoc- Capes
4. 2012-2014 **SILVANA SANDRI,** Fellowship Prodoc-CAPES.
5. 2012-2015 **MICHELLE GARCIA DISCACCIATI DE CARVALHO** Fellowship FAPESP
6. 2013-2017 **FERNANDA FAIÃO FLORES** Fellowship FAPESP
7. 2014-present **ÉRICA OLIVEIRA** Fellowship PRODOC CAPEs/ FAPESP
8. 2014-2017 **THALITA BOLDRIN ZANONI** Fellowship FAPESP
9. 2014-2017 **MÁRCIA CONSOLARO**
10. 2018-2021 **LARISSA ANASTÁCIO DE CARVALHO-** Fellowship Fapesp
11. 2018-2021 **ANA LUÍSA MILLÁS MASSAGUER** Fellowship Natura
12. 2021- **DENISSE CAMARENA, Fellowship USP and CNPq**
13. 2021 **ANDRESSA PROENÇA ANTUNES, Fapesp Fellwship**

Honors and Awards

1998	Stanford University visit by CAPES Studentship.
2000	Gordon Conference Registration Award.
2010	Nomination to Women in Science, Claudia Magazine, São Paulo
2010	São Paulo Scientist of the Year, Veja São Paulo Magazine, Editora Abril.
2011	Best Poster Prize-Melanoma in skin reconstructed as alternative method to test a new proteasome inhibitor, International Association for Pharmaceutical Technology and Skin Forum, Germany
2015-10-28	Best oral Presentation, University of São Paulo, School of Pharmaceutical Sciences
2014-2017	Scientific Productivy of Pharmacy Committee fellowship, Level 2, CNPq.
2018-	Scientific Productivity of Pharmacy Committee fellowship, Level 1D, CNPq.
2020	Science Innovation, Natura and Co.(Avon, The Body Shop and Aesop) Innovati Prize,Skin bioprinted for the cosmetic development platform.
2022	Heinz Maurer Award for Dermatological Basic Research, Sebapharm, Germany.

Research Support

Coordination of Current Projects:

Thematic Project FAPESP 17/04926-6:
Melanoma and Chemoresistance: in vitro and in silico models to exploit new therapeutical targets.

Past Coordination over 60 Research Project and Fellowship supported by FAPESP and CNPq.

Service

University of São Paulo

Committees:

2011-2014	Coordinator of School of Third Age, University of São Paulo
2011-2017	Scientific Board of ABIHPEC – Brazilian Association of the Cosmetic, Toiletry and Perfumery Industry, São Paulo, Brazil
2016-2019	Head of Department of Clinical and Toxicological Analyses
2010-present	Member of Animal Experimentation Council, Federal Government, Brazil
2012-present	Member of the Graduate Education Committee, University of São Paulo
2012-present	Member of Director Council of National Network of Alternative Methods, Federal Government, Brazil.
2011-present	Coordinator of ANIMAL Imaging Core Facility, School of Pharmaceutical Sciences, University of São Paulo

Other Service

Conference organizer:

Member of Scientific Board of National and International Conferences

2008-present	Meeting Organizer for the Melanoma Workshop every 2 years local scientific meeting with 100 participants

Grant Reviewer:

2003 - present	FAPESP *Ad Hoc*
2003 - present	CNPq *Ad Hoc*
2010 - present	Yorkshire Cancer Research
2018 - present	The Croatian Science Foundation (HRZZ)
2018 - present	KU Leuven, Belgium
2018 - present	Inserm, France
2019 - present	Frei University of Berlin
2020 - present	Austrian Science Fund (FWF)

Peer Review panels

2006 - present	Brazilian Journal of Medical and Biological Research
2005 - present	Journal of Endocrinology
2006 - present	European Journal of Cancer
2006 - present	RBCF. Revista Brasileira de Ciências Farmacêuticas
2007 – present	Cytometry. Part A
2009 – present	Photochemistry and Photobiology
2006 – present	Journal of Cellular Biochemistry (Print)
2011 – present	Naturalia (São José do Rio Preto)
2012 – present	BMC Cancer (Online)
2012 – present	Plos One
2012 – present	Archives of Dermatology Research
2012 – present	Cellular Physiology and Biochemistry
2012 – present	Journal of Tissue Engineering and Regenerative Medicine
2012 – present	Journal of Biomedicine and Biotechnology (Print)
2012 - present	Virology Journal
2012 - present	Pigment cell and melanoma research
2010 – present	Laboratory Investigation (Print)
2010 – present	International Journal of Gynecology & Obstetrics. Supplement
2010 - present	Tissue Engineering

Editorial Boards and Editor in chief:

2013-present	Editor-in-chief *Brazilian Journal of Pharmaceutical* Sciences
2015-present	Editorial Board Member for Pharmacological Research
2019-present	Editrial Board Member for Pigment Cell and Melanoma Research

Professional Association Memberships

2000–present	**Member**, Brazilian Society of Cell Biology
2014-present	**Member**, Brazilian Society of Pharmaceutical Science
2016-present	**Member**, Society of Melanoma Research

Peer-Reviewed Publications (over 90 publications)

5 most relevant papers

1. Redox-Related Proteins in Melanoma Progression. Carvalho LAC, Queijo RG, Baccaro ALB, Siena ÁDD, Silva WA Jr, Rodrigues T, **Maria-Engler SS.** Antioxidants (Basel). 2022 Feb 22;11(3):438. doi: 10.3390/antiox11030438.PMID: 35326089

2. Mutually exclusive genetic interactions and gene essentiality shape the genomic landscape of primary melanoma.Birkeälv S, Harland M, Matsuyama LSAS, Rashid M, Mehta I, Laye JP, Haase K, Mell T, Iyer V, Robles-Espinoza CD, McDermott U, van Loo P, Kuijjer ML, Possik PA, **Maria Engler SS**, Bishop DT, Newton-Bishop J, Adams DJ.J Pathol. 2022 Oct 11. doi: 10.1002/path.6019.

3. HDAC inhibition enhances the in vivo efficacy of MEK inhibitor therapy in uveal melanoma. Faião-Flores F, Emmons MF, Durante MA, Kinose F, Saha B, Fang B, Koomen JM, Chellappan SP, Maria-Engler SS, Rix U, Licht JD, Harbour JW, Smalley KSM. Clin Cancer Res. 2019 Jun 21. pii: clincanres.3382.2018. doi: 10.1158/1078-0432.CCR-18-3382.

4. ER stress promotes antitumor effects in BRAFi/MEKi resistant human melanoma induced by natural compound 4-nerolidylcathecol (4-NC). Alves-Fernandes DK, Oliveira ÉA, Faião-Flores F, Alicea-Rebecca G, Weeraratna AT, Smalley KSM, Barros SBM, Maria-Engler SS. Pharmacol Res. 2019 Mar;141:63-72. doi: 10.1016/j.phrs.2018.12.006. Epub 2018 Dec 11. JCR 4.81

5. Targeting the Hedgehog transcription factors GLI1 and GLI2 restores sensitivity to vemurafenib-resistant human melanoma cells. Faiao-Flores F, Alves-Fernandes D, Pennacchi P, Sandri S, Vicente AL, Scapulatempo-Neto C, Vazquez V, Reis R, Chauhan J, Goding C, Smalley K, Maria-Engler SS. Oncogene, aceito para publicação em 16 de agosto de 2016. JCR 7.932

Book Editor

Consolaro, M.E.L. ; **Maria-Engler, Silvya Stuchi** . Citologia Clínica cérvico-vaginal texto e atlas. 1. ed. São Paulo: Gen Roca, 2012. 270p .
Schafer- Korting, M & **Maria-Engler, S. S**. Handbook of Experimental Pharmacology (Organotypic Models in Drug Development), Springer- Nature, 2020

Book Chapters

1. Munhoz, D. ; CARDEAL, L. B. S. ; **Maria-Engler, Silvya Stuchi** .Carcinoma Escamoso e Atipias. In: Consolaro; Maria-Engler. (Org.). Citologia Clínica cérvico-vaginal texto e atlas. São Paulo: Gen Rocas, 2012, v. 1, p. 143-190.

2. CARDEAL, L. B. S. ; ANDRIOLO, A. ; **Maria-Engler, Silvya Stuchi** . Marcadores Tumorais. In: Consolaro; Maria-Engler. (Org.). Citologia Clínica cérvico-vaginal texto e atlas. 1ed.São Paulo: Gen Roca, 2012, v. 1, p. 247-256.

3. Haga R. ; **Maria-Engler, Silvya Stuchi** .Molecular targets: inhibition of tumor invasion. In: Aleksandar Lazinica. (Org.). Glioma/ Book 3. : Intech International Offices, 2011, v. , p. -.

4. LOJUDICE, F. H. ; **Maria-Engler, S. S.** ; KROGH, K. ; OLIVEIRA, E. M. C. ; AITA, C. A. M. ; NORONHA, I. L. ; ELIACHEWITZ, F. ; SOGAYAR, M. C. . Terapia celular para o diabetes. In: E. Jeckel-Neto. (Org.). Avanços em Biologia Celular, 2002, v. , p.

5. MELLO, M. L. S. ; VIDAL, B. C. ; **Maria-Engler, S. S.** . Morte celular. In: Hernandes F Carvalho; Laurecir Gomes;. (Org.). Biologia celular., 2000

International Work

1. Chair Person/ oral presentation: The 10th World Congress on Alternatives and Animal Use in the Life Sciences will be held at the Washington State Convention Center, Seattle(http://wc10seattle.org/2017/General-Information/Congress-Committees.aspx).
2. Chair Person/ oral presentation: SIMPAC. 2016 (http://simpac.wixsite.com/simpac1) Dr. Bruce Horwitz Brigam and Women's Hospital/ Harvard Medical School Regulation of inflammatory gene transcription by IL-10; 2) Pankaj Karande, Rensselaer Polytechnic Institute, NY, EUA "3D Biofabrication of Vascularized Human Skin for Efficacy Studies Module" 3.Progress in alternative in vitro methods for the safety assessment of chemicals and their impact in human health: International and Brazilian View. http://prope.unesp.br/eventos/fapesp/index.php. Reitoria da UNESP, 2015.
3. Chair Person/ oral presentation:Challenges and perspectives in research on alternatives to animal testing (http://www.fapesp.br/9310) Thomas Hartung, Center for Alternatives to Animal Testing of the Johns Hopkins Univ Hospital (CAAT); Tox21: A U.S. Federal Collaboration to Improve the Human Hazard Characterization of Chemicals Raymond Tice - National Institute of Environmental Health Science (USA); Validation of Alternative in Vitro Assays for Toxicity Evaluation Chantra Eskes - European Society of Toxicol in vitro (ESTIV).
4. Chair Person Colin Goding in the Conference XVIII Meeting of the Brazilian Society for Cell Biology, São Paulo-SP, 13 a 16 de julho 2016.
5. Chair Person/ oral presentation: Congresso of the Society of Melanoma Research 2011, Tampa, Florida, a qual pertenço como membro associado, http://onlinelibrary.wiley.com/doi/10.1111/j.1755-148X.2011.00943.x/full a convite do Dr Keiran Smalley.
6. Maria-Engler, S.S. Chair Person Marisol Soengas in the Conference Meeting of the Brazilian Society for Cell Biology, São Paulo-SP, 2007.

Oral presentation as invited speaker:
1. Targeting the SHH transcription factors Gli1 and Gli2 restores sensitivity to vemurafenib-resistant human melanoma cells IX International Melanoma Workshop, June 7-9, 2017, Sestri Levante, Italy invited by Dr Colin Goding.
2. Skin reconstruction and melanoma invasion; Surrey University, UK, invited by Dr Tao Chen, Fev, 2017.

3. Human Reconstructed Skin in vitro: National Laboratory of London, UK, Fev, 2017.
4. Pan-American Conference for Alternative Methods, 12 a 14 de abril de 2016. Johns Hopkins, CAAT. Baltimore, Maryland, USA, invited by Dr Thomas Hartung;
5. Pieles artificiales como modelo para la evalucion de nuevos fármacos y cosméticos Maria-Engler, S.S. Salazar, L., Farias, J., Abdalla, D.S.P., Hirata, M.H., Suarez-Kurtz, G. Simpósio Biotecnologia e Innovacion Farmacêutica. Santiago-Chile, 23
6. Melanoma Resistance; Bavarian Research network on Laton America, Erlagen, Germany, 2019
7. Skin reconstruction and melanoma invasion; Freie University of Berlin, Germany, 2018
8. Melanoma and Chemoresistence, Freie University of Berlin, Germany, 2022

9. Melanoma and Chemoresistence, Society of Melanoma Research, Edinburg, 2022

Media Coverage & Interviews

May 2014-05-15 Skin reconstructed and banning of animals, Radio França Internacional
http://www.portugues.rfi.fr/geral/20140507-pele-artificial-criada-por-laboratorio-da-usp-evitara-testes-com-animais-no-brasil?ns_campaign=reseaux_sociaux&ns_source=FB&ns_mchannel=social&ns_linkname=emission&aef_campaign_ref=partage_aef&aef_campaign_date=2014-05-07

October 2013 Alternative methods to animal testing . Globo News 24.

July 2012 Treatment on skin - substance extracted from plant shows effectiveness against melanoma and can be combined with targeted therapies . Science Today , Rio de Janeiro , p . 60-61 01

April 2012 Finger in the Wound . Cosmetic actuality , Toronto , p . 56-60 01

March 2012. Compound prevents advancement of melanoma. Agência FAPESP , São Paulo - SP 01

January 2012. Artificial skin as organotypic culture model . Newsletter WBG 02

June 2011 First stories . S / A 01.

December 2010. Paulistanos who shone in 2010 . Veja São Paulo , São Paulo , p . 56-56 15

December, 2010 Researchers study center creating alternative tests for cosmetics. FCF News , Publication College Astronomy Science , p . 1-1 01

October 2010. http://veja.abril.com.br/noticia/ciencia/um-futuro-mais-humano-ate-para-os-animais . SEE 20

July 2010. Claudia Award Finalists 2010. Cláudia Magazine , Brazil , p . 152-152 01

April 2010 Artificial skin will facilitate beauty product testing . Brazil 25.

February 2010 Autophagy for survival. FAPESP (Printed) , São Paulo , p . 42-47 01.

December 2009 Recreated skin. FAPESP , p . 64-67 01.

November 2008 FCF develops artificial skin . FCF News , USP , p . 1 01 August 2009.. Innovation that can save lives . Journal of USP 22

December 2008 Question of " artificial" skin. Journal of Vegetarians .

December 2008 Scientists recreate in the laboratory the skin . Journal of Culture .

RESEARCH ARTICLE

Editorial Process: Submission:01/14/2021 Acceptance:04/16/2021

Association of Righ-Risk Human Papillomavirus and *Ureaplasma parvum* Co-Infections with Increased Risk of Low-Grade Squamous Intraepithelial Cervical Lesions

Isabella Harumi Yonehara Noma, Cristiane Suemi Shinobu-Mesquita, Tamy Taianne Suehiro, Fabricio Morelli, Maria Vitória Felipe de Souza, Edilson Damke, Vânia Ramos Sela da Silva, Marcia Edilaine Lopes Consolaro*

Abstract

Objective: The present report investigated the rates of coinfections between high-rik human papillomavirus (hrHPV) and the most important human mycoplasmas including *Mycoplasma hominis, M. genitalium, Ureaplasma urealyticum* and *U. parvum* in cervical samples of asymptomatic brazilian population. **Methods:** Were included a total of 283 women aged 25–64 years screened by Papanicolaou smears for determining cervical abnormalities, single-target polymerase chain reaction (PCR) and real-time PCR (rt-PCR) for hrHPV and mycoplasmas, respectively. **Results:** A total of 273 (94.5%) women were negative for intraepithelial lesions or malignancy cytology (NILM) and 10 (3.5%) presented abnormal cytology, all low-grade intraepithelial lesions (LSIL). The prevalence of hrHPV was 12.7% and 53.7% for mycoplasmas. *U. parvum* was the most frequently bacteria detected, followed by *Mycoplasma hominis* and *U. urealyticum. M. genitalium* was not detected. Women positive for *U. parvum* presented a 5-fold increased risk of LSIL (OR = 5.33; 95% CI = 1.09-26.04, P = 0.02) and co-infections between *U. parvum* and hrHPV increased the risk for LSIL (OR = 3.88; 95% CI = 1.75-8.58, P = 0.0003). However, these associations were not dependent on the concentration of the bacteria. **Conclusion:** Our results reinforced the hypothesis that some mycoplasmas may play a role as cofactors in HPV-mediated cervical carcinogenesis, at least in some populations.

Keywords: Cervical cancer- high-risk HPV- Co-factors- mycoplasmas- risk

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Introduction

Cervical cancer is the fourth leading cause of cancer in women worldwide, despite the existence of highly effective prevention and screening methods (Bray et al., 2018). Persistent high-risk human Papillomavirus (hrHPV) infection is the central factor in the development of squamous cell cervical carcinoma and hrHPV is a prerequisite for progression to high-grade squamous intraepithelial lesions (HSIL) (Kjaer et al., 2010). hrHPV infections are most eventually cleared, but few cases progress to HSIL (10% of HPV infections) and squamous cervical cancer (< 1% of HPV infections) (Ho et al., 1998; Tota et al., 2011; Monsego et al., 2015). The reasons for this variable natural history are poorly understood, but it is generally assumed that other causes or cofactors are important for the development of neoplasia in HPV-infected women (Bodily et al., 2011; Tota et al., 2011). The identification of these cofactors will improve the understanding of cervical carcinogenesis and will help to identify new opportunities for cervical cancer prevention and treatment (Adebamowo et al., 2017).

Several possible risk factors were associated with persistent hrHPV and contributed to cervical carcinogenesis (Moreno et al., 2002; Castle et al., 2003, Appleby et al., 2006; Carrillo-Gárcia et al., 2014). Among them, coinfection with sexually transmitted infections (STI) (de Abreu et al., 2016) as genital mycoplasmas, are gradually becoming more important (Ye et al., 2018). Genital mycoplasmas are groups of small free-living pathogenic bacterium belonging to class Mollicutes that lives on the ciliated epithelial cells of the urinary and genital tracts in humans (Tully et al., 1981), generally transmitted through direct interaction between hosts—venereally through genito-genital or oro-genital contact and vertically from mother to child (either in utero or at birth) (Taylor-Robinson, 2017). Genital mycoplasmas often refer to six species that colonize the genital tract, involving *Ureaplasma urealyticum, U. parvum, Mycoplasma hominis, M. genitalium, M. primatum*, and

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M. spermatophilum. M. primatum and M. spermatophilum are considered non-pathogenic for humans (Ljubin-Sternak and Mestrovic, 2014). Mycoplasmas have been associated with some asymptomatic and symptomatic genital tract conditions, including cervicitis, bacterial vaginosis and endometritis. Cervicitis, an acute or chronic inflammation of the uterine cervix, may promote the infection by human immunodeficiency virus and other STIs, including HPV, by the disruption of epithelial barrier integrity, and subsequent recruitment of inflammatory cells (Patel and Nyirjesky, 2010; Herfs et al., 2011; Silva et al., 2014). However, the results of previous studies on the association between prevalent mycoplasmas and hrHPV infections have been contradictory (biernat-Sudolska et al., 2011; Magaña-Contreraas et al., 2015; de Abreu et al., 2016; Kim et al., 2016; Klein et al., 2020). Therefore, it is still under discussion whether genital mycoplasmas may be a cofactor associated with hrHPV in cervical carcinogenesis.

The real prevalence of co-infections between hrHPV and mycoplasmas in cervical samples is not clear. The overall prevalence of genital mycoplasmas is an under-recognized condition in several populations despite its clinical importance, which is likely because of difficulties in the diagnoses that depend on traditional methods, such as culture (Daley et al., 2014). The recent development and introduction of cultivation-independent molecular-based techniques, such as polymerase chain reaction (PCR), revolutionized the diagnosis of genital tract infections (DÍnzeo et al., 2017) including mycoplasmas. Although studies have investigated cervical infections in Brazilian women, to date, few studies evaluated the coinfections between hrHPV and mycoplasmas in the Brazilian female population. Therefore, the present report investigated the rates of coinfections between hrHPV and the most important human mycoplasmas including *M. hominis, M. genitalium, U. urealyticum* and *U. parvum* in cervical samples of asymptomatic brazilian population. We found that *U. parvum* was the primary pathogen associated with hrHPV for increasing the risk of low-grade squamous intraepithelial lesions in this population, suggesting a possible synergistic action in cervical lesion development.

Materials and Methods

Study population

This cross-sectional study enrolled asymptomatic women for STI and without a history of abnormal cytology residing in Maringá city/Brazil attending at the Public Health System to cervical cancer screening consult between March to October 2016. Women aged 25-64 years old were recruited because this is the recommended screening cohort by the Brazilian Cancer Institute. Women with any of the following factors were excluded: pregnancy, post-partum, previous hysterectomy, vaginal bleeding, previous history of cancer, sexual inactivity, recent treatment for any pathological condition of the urogenital tract, ablative or excisional therapy for the cervix within the previous 12 months, receiving treatment with antimicrobials (oral or topical) within the previous 4 weeks, and those using intrauterine devices or contraceptives delivered directly to the vaginal mucosa. Prior to undergoing gynecological examination, all women signed a written informed consent. Each woman voluntarily agreed to provide a sample of uterine cervix for Pap screening, hrHPV test and molecular mycoplasmas detection. This study was conducted with the supervision and approval of the Committee for Ethics in Research Involving Humans at the State University of Maringá (UEM)/Paraná/Brazil (CAAE: 37091114.2.0000.0104).

Study procedures

Demographic and baseline characteristics were obtained from analysis of the data from the standard registration form of each woman. Cytological samples were obtained from asymptomatic women for cervical cancer screening consult. Ecto/endocervical samples were collected with an Ayre's spatula and cytobrush and were used for conventional Pap smear preparation. For hrHPV and mycoplasmas DNA analyses, samples were immediately suspended in liquid medium (HC2 DNA Collection Device; Digene, Gaithersburg, MD, USA) and stored at −10°C until analysis.

Cytological diagnosis

Cytological examination was conducted without the knowledge of hrHPV status at an accredited clinical laboratory (Clinical Cytology Laboratory of UEM) and reported according to the Bethesda 2001 nomenclature (Solomon and Nayar, 2015). The threshold of abnormal cytology was used to define all abnormal cytological findings including low-grade squamous intraepithelial lesions (LSIL), HSIL and cervical cancer. Negative for intraepithelial lesion or malignancy (NILM) was used to define normal cytological findings.

HPV detection and typing by Cobas HPV test

Cervical specimens (2–5 mL of the liquid medium with sample) were tested for hrHPV DNA using the cobas HPV test (Roche Molecular Systems, USA) run on the fully automated cobas 4800 testing platform. The Cobas HPV test is a DNA test for hrHPV, and the results are reported in three separate channels: HPV16 individually, HPV18 individually, and a pool of 12 other hrHPV genotypes [11 definite high-risk, cancer-associated, HPV types (HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) plus one possibly hrHPV genotype (HPV66)]. A fourth channel measures β-globin for specimen adequacy. Inadequate specimens were retested and if still inadequate, results were treated as positive for patient safety purposes.

Single-target polymerase chain reaction (sPCR) for mycoplasmas detection

sPCR was conducted for qualitative detection of *Ureaplasma* spp., *M. hominis* and *M. genitalium*. DNA was extracted using the AxyPrep Body Fluid Miniprep Kit (Axygen, California, USA) according to the manufacturer's instructions. The quality and quantity of the purified DNA were determined by spectrophotometry on a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Massachusetts, USA).

For the design of primers (supplementary Table 1),

specificity was checked against all sequences in GenBank and primers were aligned by using the Pairwise Sequence Alignment. Subsequently, the primers were evaluated by the OligoAnalyzer Tool (Integrated DNA Technologies) and selected according to melting temperatures, amplification temperature and amplicon size. All primers were evaluated performing a Basic Local Alignment Search Tool (BLAST) analysis against the sequences in the National Center for Biotechnology Information (NCBI) database. To assess the specificity of the primers, all primers were tested in sPCR reactions with different samples and positive control.

The optimized protocol for each assay was a mixture of 25 µL containing 2.5 mM of each deoxynucleotide triphosphates (dNTP), 1U of Taq DNA polymerase (Invitrogen, Carlsbad, CA), 0.6 mM of $MgCl_2$, 25 mM of each primer and 50 ng of the extracted DNA for a final volume of 15 mL. The PCR conditions were comprised of thirty-five amplification cycles; denaturation for 10 min at 94°C, annealing for 1 min at 55°C, extension for 1 min at 72°C, and final extension for 10 min at 72°C (Thermal cycler, Biosystem,CA, USA). PCR products were electrophoresed on 1.0% agarose gel, stained with 1 mg/mL UniSafe Dye (Uniscience, USA), and photodocumented under UV light. Amplification of the human β-globin gene was performed as an internal control using primers GH20 and PC04. Positive controls for all studied bacteria were derived from positive clinical detected samples using reference methods (culture). Two types of controls were also included in each reaction, a "no-DNA" (negative control) and "positive DNA" for each bacteria (positive control). Samples positive for Ureaplasma spp. by sPCR were differentiated in U. urealyticum or *U. parvum* by real-time PCR as follows.

Real-time PCR (rt-PCR) assay for Ureaplasma spp. identification and mycoplasmas quantification

rt-PCR was conducted for the identification of Ureaplasma spp. in *U. urealyticum* or *U. parvum* and for all mycoplasmas quantification using the standardized kits: Bio Gene Research Max *Mycoplasma* spp., Bio Gene Research Max *Ureaplasma parvum*, and Bio Gene Research Max *Ureaplasma urealyticum* (Bioclin, Brazil), following the manufacturer's instructions. This assay has a detection range of 2 to $2X10^5$ copies/µL of each bacteria. For the analysis, we inferred a low concentration of bacteria between 2 and $2x10^2$ copies/µL and as high those between $2X10^3$ and $2X10^5$ copies/µL.

Statistical analysis

Different variables were evaluated for comparisons between hrHPV positive and negative findings for analytical calculations, and between NILM and LSIL women. A two-sided Fisher's exact test for 2×2 contingency table was performed to evaluate the statistical significance between different groups. Crude odds ratios (OR, relative risk) with 95% confidence intervals (CI) were calculated to estimate the association of HPV and mycoplasmas positive findings with socio-demographic data. The age was expressed as a mean and standard deviation (SD). Statistical significance was defined as P values <0.05. All statistical analyses were performed using GraphPad Prism 6.0 (San Diego, California, USA).

Results

Baseline features

Table 1 outlines the baseline features of the study cohort. A total of 360 women were attended during the study period for cervical cancer screening. Of these, 283 women fit the inclusion criteria of the study and were included with a mean (SD) age of 45.84 ± 11.04 years old (range 25-64). Most women exhibited the following characteristics: between 31-50 years old (51.2%); were married (68.2%); had education level ≥8 years (61.1%); were white (55.1%); and had 1-2 children (50.5%).

A total of 273 (96.5%) women presented normal cytology (NILM) and 10 (3.5%) presented LSIL (Table

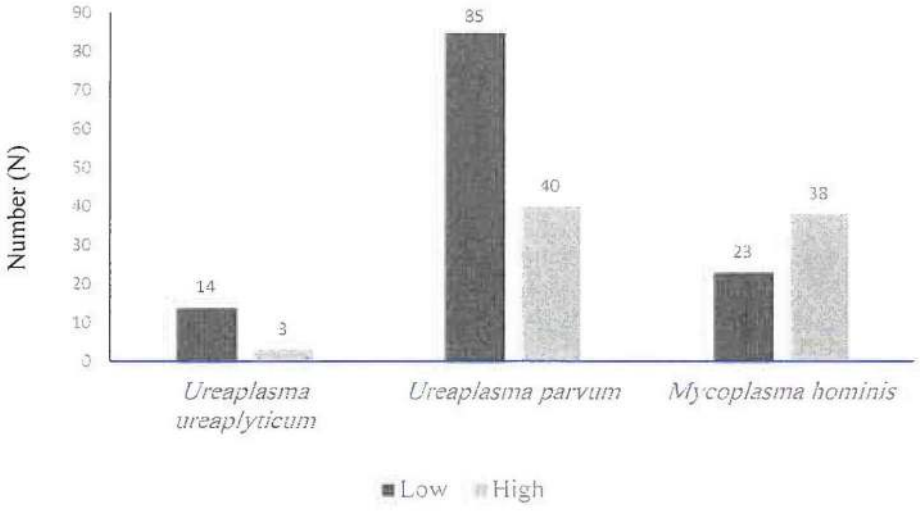

Figure 1. Quantification of Mycoplasmas in Copies/µL by rt-PCR as Low (between 2 and $2x10^2$ copies/µL) and high (between $2X10^3$ and $2X10^5$ copies/µL) Concentration.

Table 1. Overall Baseline Features of the Study Cohort

Characteristics	Overall (n=283) n (%)	hrHPV+ (n=36) n (%)	hrHPV- (N=247) n (%)	OR (95%CI)	P value	*Ureaplasma* spp. + (n=142) n(%)	*Ureaplasma* spp. - (n=141) n (%)	OR (95%CI)	P value	*Mycoplasma hominis*+ (n=61) n (%)	*Mycoplasma hominis*- (n=222) n (%)	OR (95%CI)	P value
Age groups													
≤30 years	33 (11.7)	5 (13.9)	28 (11.34)	2.47 (0.71-8.52)	0.13	16 (11.3)	17 (12.1)	1.09 (0.49-2.41)	0.81	8 (13.1)	25 (11.3)	1.19 (0.47-3.01)	0.70
31-40 years	60 (21.2)	12 (33.3)	48 (19.43)	3.46 (1.25-9.58)	0.01	37 (26.0)	23 (16.3)	1.87 (0.97-3.62)	0.06	16 (26.2)	44 (19.8)	1.35 (0.64-2.85)	0.42
41-50 years	85 (30.0)	12 (33.3)	73 (29.55)	2.28 (0.84-6.13)	0.09	40 (28.2)	45 (31.9)	1.03 (0.58-1.84)	0.90	15 (24.6)	70 (31.5)	0.79 (0.38-1.66)	0.54
>50 years	104 (36.8)	7 (19.4)	97 (39.27)	1	-	48 (33.8)	56 (39.7)	1	-	22 (36.1)	82 (36.9)	1	-
No related	1 (0.3)	0 (0.0)	1 (0.40)	-	-	1 (0.7)	0 (0.0)	-	-	0 (0.0)	1 (0.5)	-	-
Marital status													
Married	193 (68.2)	20 (55.6)	173 (70.0)	1	-	93 (65.5)	100 (70.9)	1	-	36 (59.0)	157 (70.7)	1	
Unmarried	87 (30.7)	15 (41.7)	72 (29.2)	1.80 (0.87-3.73)	0.11	47 (33.1)	40 (28.4)	1.26 (0.76-2.10)	0.37	25 (41.0)	62 (27.9)	1.75 (0.97-3.18)	0.06
No related	3 (1.1)	1 (2.7)	2 (0.8)	-	-	2 (1.4)	1 (0.7)	-	-	0 (0.0)	3 (1.4)	-	
Education level													
<8 years	101 (35.7)	11 (30.6)	90 (36.4)	1.19 (0.55-2.57)	0.65	55 (38.7)	46 (32.6)	1.29 (0.79-2.12)	0.30	21 (34.4)	80 (36.0)	1.03 (0.56-1.89)	0.90
≥8 years	173 (61.1)	22 (61.1)	151 (61.1)	1	-	83 (58.5)	90 (63.8)	1	-	37 (60.7)	136 (61.3)	1	-
No related	9 (3.2)	3 (8.3)	6 (2.5)	-	-	4 (2.8)	5 (3.6)	-	-	3 (4.9)	6 (2.7)	-	-
Ethnic group													
White	156 (55.1)	18 (50.0)	138 (55.9)	1	-	78 (54.9)	78 (55.3)	1		32 (52.5)	124 (55.9)	1	-
No white	118 (41.7)	15 (41.7)	103 (41.7)	1.11 (0.53-2.32)	0.77	59 (41.6)	59 (41.9)	1		28 (45.9)	90 (40.5)	1.20 (0.67-2.14)	0.52
No related	9 (3.2)	3 (8.3)	6 (2.4)	-	-	5 (3.5)	4 (2.8)	-	-	1 (1,6)	8 (3.6)	-	-
Number of children													
0	25 (8.8)	4 (11.1)	21 (8.5)	1.32 (0.40-4.31)	0.64	12 (8.5)	13 (9.2)	1.11 (0.47-2.60)	0.81	8 (13.1)	17 (7.7)	2.59 (0.98-6.82)	0.04
1 or 2	143 (50.5)	18 (50.0)	125 (50.6)	1	-	65 (45.8)	78 (55.3)	1	-	22 (36.1)	121 (54.5)	1	-
≥3	93 (32.9)	10 (27.8)	83 (33.6)	0.83 (0.37-1.90)	0.67	52 (36.6)	41 (29.1)	1.52 (0.89-2.58)	0.11	24 (39.3)	69 (31.1)	1.91 (0.99-3.68)	0.04
No related	22 (7.8)	4 (11.1)	18 (7.3)	-	-	13 (9.1)	9 (6.4)	-	-	7 (11.5)	15 (6.7)	-	-

hrHPV, high-risk human papilomavirus

Table 2. Association between hrHPV and Mycoplasmas with Cytological Findings

	Overall (n = 283) n (%)	Normal (n = 273) n (%)	LSIL (n = 10) n (%)	OR (95%CI)	P -value
hrHPV					
Positive	3 (12.7)	26 (9.5)	10 (100.0)	-	< 0.0001
Negative	247 (87.3)	247 (90.5)	0 (0.0)	1	
Ureaplasma parvum					
Positive	125 (44.2)	117 (42.9)	8 (80.0)	5.33 (1.09-26.04)	0.02
Negative	158 (55.8)	156 (57.1)	2 (20.0)	1	
Ureaplasma urealyticum					
Positive	17 (6.0)	17 (6.3)	0 (0.0)	-	> 0.99
Negative	266 (94.0)	256 (93.7)	10 (100.0)	1	
Mycoplasma hominis					
Positive	61 (21.5)	59 (21.6)	2 (20.0)	1.10 (0.26-5.27)	> 0.99
Negative	222 (78.5)	214 (78.4)	8 (80.0)	1	

hrHPV, high-risk human papillomavirus; LSIL, low-grade squamous intraepithelial lesions

Table 3. Overall Rates of hrHPV and Mycoplasmas co-Infections in Women Included in the Study

Mycoplasmas	Overall (n=283) n (%)	hrHPV + (n=36) n (%)	hrHPV – (n=247) n (%)	OR (95%CI)	P- value
Ureaplasma parvum					
Positive	125 (44.2)	26 (72.2)	99 (40.1)	3.88 (1.75-8.58)	0.0003
Negative	158 (55.8)	10 (27.8)	148 (59.9)	1	
Ureaplasma urealyticum					
Positive	17 (6.0)	2 (5.6)	15 (6.1)	0.91 (0.19-4.16)	0.90
Negative	266 (94.0)	34 (94.4)	232 (93.9)	1	
Mycoplasma hominis					
Positive	61 (21.6)	8 (22.2)	53 (21.5)	1.04 (0.45-2.43)	0.92
Negative	222 (78.4)	28 (77.8)	194 (78.5)	1	

hrHPV, high-risk human papillomavirus

2). Women with NILM and LSIL had similar ages (45.90 ± 10.99 and 44.10 ± 12.84 years old, respectively; P = 0.30).

hrHPV positivity

Thirty-six women (12.7%) were positive for hrHPV (Tables 2 and 3). HPV16 was detected in 4 (11.1%) and HPV18 in 2 (5.6%) women. In the remaining 30 (83.3%) women, other types of hrHPV were detected. Twenty nine (10.6%) women with NILM and all (100%) women with LSIL were hrHPV positive. Thus, hrHPV was associated with LSIL (P < 0.0001) (Table 2).

hrHPV positive women were younger than hrHPV negative women (41.42 ±10.52 vs. 46.49 ±10.99, respectively, p-value: 0.004) and most (51.2%) had between 31-40 years (P = 0.01) (Table 1).

Table 4. Rates of hrHPV and *U. parvum* co-Infections in Relation to Bacteria Concentrations

U. parvum concentration	hrHPV+ (n=26) n (%)	hrHPV- (n=99) n (%)	OR (95%CI)	P value
Low	23 (88.5)	92 (92.9)	1	-
High	3 (11.5)	7 (7.1)	1.71 (0.45-7.07)	0.43

hrHPV, high-risk human papillomavirus; Low, between 2 and $2x10^2$ copies/µL of *U. parvum*; High, between $2X10^3$ and $2X10^5$ copies/µL of U. parvum.

Cervical mycoplasmas positivity and quantification

The PCR results for cervical mycoplasmas presented high rates of these bacteria (n = 152/283, 53.7%), with 142/283 (50.2%) positive women for *Ureaplasma* spp. and 61 (21.6%) for *Mycoplasma* spp. (Tables 2 and 3). In 51/152 of the women positive for mycoplasmas (33.6%). *Ureaplasma* spp. and *Mycoplasma* spp. were detected simultaneously (OR = 7.34, 95%CI = 3.36-16.00, P < 0.0001). For *Mycoplasma* spp. (n = 61), all samples (100%) were positive for *M. hominis* and none positive for M. genitalium.

Women positive for *Ureaplasma* spp. had similar mean age to negative women (44.84 ± 10.65 vs. 46.85 ± 11.37 respectively, P = 0.07). Women positive for *M. hominis* also had similar mean age to negative women (45.05 ± 11.09 vs. 46.06 ± 11.04 respectively, P = 0.26). Women positive for *M. hominis* were associated with no children and with ≥3 children (P = 0.04 for both).

By rt-PCR, the samples of *Ureaplasma* spp. (n = 142) were identified as *U. parvum* (n = 125, 88%)

and *U. urealyticum* (n = 17, 12%). None of the women was positive to both bacteria. Thus, *U. parvum* (n = 125/152, 82.2%) was the most frequently bacteria detected, followed by M. hominis (n = 61/152, 40.1%) and U. urealyticum (n = 17/152, 11.2%).

Regarding cytological findings, women positive for *U. parvum* have a 5-fold increased risk of LSIL (OR = 5.33; 95% CI = 1.09-26.04, P = 0.02) (Table 2).

Figure 1 represents the quantification of mycoplasmas in copies/μL by rt-PCR as low and high concentration. It was possible to observe that despite *U. parvum* being the most detected mycoplasma it was quantified mainly in low concentrations (n = 85/125, 68%). On the other hand, the second most prevalent bacteria (*M. hominis*) was found mainly in higher concentrations (n = 38/61, 62.3%). *U. urealyticum* was quantified mainly in low concentrations (n = 14/17, 82.4%).

hrHPV and mycoplasmas co-infections

Women positive for *U. parvum* had a 4-fold increased risk of hrHPV positivity (OR = 3.88; 95% CI = 1.75-8.58, P = 0.0003) (Table 3). Although we have evidenced the association between *U. parvum* with hrHPV, these associations were not dependent on the concentration of the bacteria, as shown in Table 4.

Discussion

This study investigated the rates of co-infections between hrHPV and important human mycoplasmas in cervical samples of asymptomatic Brazilian population. We found that *U. parvum* was the primary mycoplasma associated with hrHPV for increasing the risk of LSIL in this population. Therefore, *U. parvum* might play a synergistic role in the initial stage of cervical lesions caused by hrHPV infection.

Previous reports presented high rates of ureaplasmas and *M. hominis* detection in asymptomatic sexually mature females (Taylor-Robinson et al., 2017). Epidemiological data showed that *Ureaplasma* spp. can be detected in the cervix or vagina of 40-80% of sexually mature asymptomatic females, whereas *M. hominis* and M. genitalium only in 20-50% and 0-5%, respectively (Morse et al., 2010). Our results are consistent with these evidences since *Ureaplasma* spp. was detected in 142 (50.2%) women, *M. hominis* in 61 (21.6%), and *M. genitalium* was not detected. Additionally, we found that *U. parvum* was the most frequent bacterial agent detected (n = 125/283, 44.2%). Our results are similar to obtained in a recent study, which *U. parvum* was the most prevalent mycoplasma in the cervix (Carneiro et al., 2020). However, for these authors, *U. parvum*, *M. hominis* and *U. urealyticum* were detected at much lower rates (14.9%, 8.5% and 4.2%, respectively) than in our study, and *M. genitalium* was also not detected. Foschi et al., (2018) also found that *U. parvum* was the most prevalent mycoplasma (67.3%), followed by *M. hominis* (14.3%), U. urealyticum (9.2%) and *M. genitalium* (3.1%) in the group of asymptomatic healthy women.

HPV is necessary, but not sufficient, to cause cervical cancer, which supports the influence of additional factors in carcinogenesis associated with hrHPV (Ho et al., 1998; Kjaer et al., 2010; Tota et al., 2011; Ko et al., 2015; Monsonego et al., 2015). Mycoplasmas have been suggested as a possible risk factor for the development of cervical lesions (Ekiel et al., 2009) and cervical cancer (Biernat-Sudolska et al., 2011), and with HPV infections in samples with abnormal cytology (Biernat-Sudolska et al., 2011; Kawaguchi et al., 2012). Our results are in agreement with these findings once women positive for *U. parvum* showed a 5-fold increased risk of LSIL. Additionally, we observed significant association between *U. parvum* and hrHPV infections, indicating that women with both infections might be more likely to develop cervical lesions. Our results are consistent with the hypothesis that mycoplasmas can exert a synergistic action with hrHPV and possibly act as cofactors in the development of cervical lesions (Ho et al., 1998; Bodily et al., 2011; Tota et al., 2011). Therefore, detecting and eradicating *U. parvum* infection might contribute to reduce HPV-related carcinogenesis (Drago et al., 2016).

However, our results also suggest that there exist differences between different mycoplasmas regarding the potential to act as a cofactor for hrHPV since only *U. parvum* was associated with LSIL and hrHPV. These results are in agreement with Drago et al., (2016) which found a strong correlation between *U. parvum*-HPV co-infection and CIN1. According to these authors, this data indicates that *U. parvum* may be involved in HPV persistence and in promoting the development of cytological abnormalities. Additionally, in a recent meta-analysis, *U. parvum* was also associated with a significantly increased risk of abnormal cervical cytopathology (Ye et al., 2018). Finally, Camporiondo et al., (2016) showed that the probability of HPV-positive women being infected by *U. parvum* was 2.5 fold higher than in HPV-negative women.

The association between *U. urealyticum* and *M. hominis* with abnormal cervical cytopathology and with hrHPV was not observed in our results, in contrary to other authors (Magaña-Contreras et al., 2015; de Abreu et al., 2016; Kim et al., 2016; Kim et al., 2018; Klein et al., 2020). This can be explained, at least in part, by the differences between the populations evaluated in the different studies as well as in the techniques used to detect and identify mycoplasmas. Thus, it is very important that future studies should be carried out in different populations with similar clinical conditions to elucidate the participation of different mycoplasmas as cofactors of hrHPV in cervical carcinogenesis.

Although we have evidenced the association between *U. parvum* with LSIL and with hrHPV, these associations were not dependent on the concentration of the bacteria. This data may indicate that possibly the presence of the bacteria regardless of their concentration in the cervical environment would be sufficient to act synergistically with hrHPV in the development of cervical lesions. In a recent study, Klein et al., found different results regarding the mycoplasmas concentration and cervical lesions. More specifically, these authors showed that *M. hominis* prevalence was similar despite the severity of cervical lesions; however the abundance of *M. hominis* increased significantly in women with cervical lesions. Therefore,

the role of *U. parvum* concentration as a risk factor for the development of cervical lesions has not been completely elucidated and requires further investigation.

The present study had no data on the history of hrHPV and vaginal infections as well as mycoplasmas agents in the women prior to enrollment in the study or during follow-up, which limits our interpretations on the influence of hrHPV persistence. As we evaluated mycoplasmas and hrHPV infections at the same time, we were not able to rule out reverse causation. It is important to note that our study did not aim to assess the carcinogenicity mechanisms of the synergistic action between hrHPV and mycoplasmas agents, but assessed the possible occurrence of this synergistic action.

In conclusion, this study provided an opportunity to determine the rates of co-infection between hrHPV and important mycoplasmas in different cytological cervical samples. We found that *U. parvum* was the primary pathogen associated with hrHPV for the increased risk of cervical abnormalities, particularly for LSIL in an asymptomatic population. Our results reinforce the hypothesis that some mycoplasmas may play a role as cofactors in hrHPV-mediated cervical carcinogenesis. Further cross-sectional studies are needed in different populations mainly in those with high rates of HSIL and cervical cancer.

Author Contribution Statement

IHY Noma, CS Shinobu-Mesquita, TT Suehiro, MEL Consolaro, and F Morelli designed and wrote the study protocol; IHYNoma, MVF de Souza, and E Damke were in charge of HPV analysis; IHY Noma, VRS da Silva, and CS Shinobu-Mesquita managed the data and performed the statistical analysis; and IHY Noma, CS Shinobu-Mesquita, TT Suehiro, F Morelli, MVF de Souza, E Damke, VRS da Silva, and MEL Consolaro wrote the manuscript. All of the authors have read and approved the final manuscript.

Acknowledgments

This study was the result of a research project approved by the review board of the the Committee for Ethics in Research Involving Humans at the State University of Maringá. The authors would like to thank all participants.

Funding Statement

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Approvation by scientific Body/part of an student thesis: This study was part of an approved student thesis (Isabella Harumi Yonehara Noma).

Ethics approval and consent to participate

Prior to undergoing gynecological examination, all women signed a written informed consent. Each woman voluntarily agreed to provide a sample of uterine cervix for Pap screening, hrHPV test and molecular mycoplasmas detection. This study was conducted with the supervision and approval of the Committee for Ethics in Research Involving Humans at the State University of Maringá (UEM)/Paraná/Brazil (CAAE: 37091114.2.0000.0104).

Statement conflict of Interest

The authors declare no conflicts of interest or industry supports of the project in this study.

References

Adebamowo SN, Ma B, Zella D, et al (2017). Mycoplasma hominis and Mycoplasma genitalium in the vaginal microbiota and persistent high-risk human papillomavirus infection. *Front Public Health*, **5**, 140.

Appleby P, Beral V, et al (2006). Carcinoma of the cervix and tobacco smoking: Collaborative reanalysis of individual data on 13,541 women with carcinoma of the cervix and 23,017 women without carcinoma of the cervix from 23 epidemiological studies. *Int J Cancer*, **118**, 1481-95.

Biernat-Sudolska M, Szostek S, Rojek-Zakrzewska D, et al (2011). Concomitant infections with human papillomavirus and various mycoplasma and ureaplasma species in women with abnormal cervical cytology. *Adv Med Sci*, **56**, 299-303.

Bodily J, Laimins LA (2011). Persistence of human papillomavirus infection: keys to malignant progression. *Trends Microbiol*, **19**, 33-9.

Bray F, Ferlay J, Soerjomataram I, et al (2018). Global cancer statistics 2018. GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*, **68**, 394–424.

Camporiondo MP, Farchi F, Ciccozzi M, et al (2016). Detection of HPV and co-infecting pathogens in healthy Italian women by multiplex real-time PCR. *Infez Med*, **24**, 12-7.

Carneiro FP, Darós AC, Darós ACM, et al (2020). Cervical cytology of samples with Ureaplasma urealyticum, Ureaplasma parvum, Chlamydia trachomatis, Trichomonas vaginalis, Mycoplasma hominis, and Neisseria gonorrhoeae detected by multiplex PCR. *Biomed Res Int*, **2020**, 7045217.

Carrillo-Garcia A, Ponce-de-León-Rosales S, Cantú-de-León D, et al (2014). Impact of human papillomavirus coinfections on the risk of high-grade squamous intraepithelial lesion and cervical cancer. *Gynecol Oncol*, **134**, 534-9.

Castle PE, Giuliano AR (2003). Chapter 4: Genital tract infections, cervical inflammation, and antioxidant nutrients-assessing their roles as human papillomavirus cofactors. *J Natl Cancer Inst Monogr*, **31**, 29-34.

D'Inzeo T, De Angelis G, Fiori B, et al (2017). Comparison of Mycoplasma IES, Mycofast Revolution and Mycoplasma IST2 to detect genital mycoplasmas in clinical samples. *J Infect Dev Ctries*, **11**, 98-101.

Daley GM, Russell DB, Tabrizi SN, et al (2014). Mycoplasma genitalium: a review. *Int J STD AIDS*, **25**, 475-87.

de Abreu ALP, Malaguti N, Souza RP, et al (2016). Association of human papillomavirus, Neisseria gonorrhoeae and Chlamydia trachomatis co-infections on the risk of high-grade squamous intraepithelial cervical lesion. *Am J Cancer Res*, **6**, 1371–83.

Discacciati MG, Gimenes F, Pennacchi PC, et al (2015). MMP-9/RECK imbalance: a mechanism associated with high-grade cervical lesions and genital infection by human papillomavirus and chlamydia trachomatis. *Cancer Epidemiol Biomarkers Prev*, **24**, 1539-47.

Drago F, Herzum A, Ciccarese G, et al (2016). Ureaplasma parvum as a possible enhancer agent of HPV-induced cervical intraepithelial neoplasia: preliminary results. *J Med*

Virol, **88**, 2023-24.
Ekiel AM, Friedek DA, Romanik MK, et al (2009). Occurrence of Ureaplasma parvum and Ureaplasma urealyticum in women with cervical dysplasia in Katowice, Poland. *J Korean Med Sci*, **24**, 1177-81.
Foschi C, Salvo M, D'Antuono A, et al (2018). Distribution of genital Mollicutes in the vaginal ecosystem of women with different clinical conditions. *New Microbiol*, **41**, 225-9.
Herfs M, Hubert P, Moutschen M, et al (2011). Mucosal junctions: open doors to HPV and HIV infections?. *Trends Microbiol*, **19**, 114-20.
Ho GYF, Bierman R, Beardsley L, et al (1998). Natural history of cervicovaginal papillomavirus infection in young women. *N Engl J Med*, **338**, 423-8.
Kawaguchi S, Shigehara K, Sasagawa T, et al (2012). Liquid-based urine cytology as a tool for detection of human papillomavirus, Mycoplasma spp., and Ureaplasma spp. in men. *J Clin Microbiol*, **50**, 401-6.
Kim HS, Kim TJ, Lee IH, et al (2016). Associations between sexually transmitted infections, high-risk human papillomavirus infection, and abnormal cervical Pap smear results in OB/GYN outpatients. *J Gynecol Oncol*, **27**, e49.
Kim S II, Yoon JH, Park DC, et al (2018). Co-infection of Ureaplasma urealyticum and human papilloma virus in asymptomatic sexually active individuals. *Int J Med Sci*, **15**, 915-20.
Kjær SK, Frederiksen K, Munk C, et al (2010). Long-term absolute risk of cervical intraepithelial neoplasia grade 3 or worse following human papillomavirus infection: role of persistence. *J Natl Cancer Inst*, **102**, 1478-88.
Klein C, Samwel K, Kahesa C, et al (2020). Mycoplasma co-infection is associated with cervical cancer risk. *Cancers (Basel)*, **12**, 1093.2020.
Koh W-J, Greer BE, Abu-Rustum NR, et al (2015) Cervical Cancer, Version 2.2015. *J Natl Compr Cancer Netw*, **13**, 395-404.
Ljubin-Sternak S, Meštrović T (2014). Chlamydia trachomatis and genital mycoplasmas: pathogens with an impact on human reproductive health. *J Pathog*, **2014**, 183167.
Magaña-Contreras M, Contreras-Paredes A, Chavez-Blanco A, et al (2015). Prevalence of sexually transmitted pathogens associated with HPV infection in cervical samples in a Mexican population. *J Med Virol*, **87**, 2098-2105.
Monsonego J, Cox JT, Behrens C, et al (2015). Prevalence of high-risk human papilloma virus genotypes and associated risk of cervical precancerous lesions in a large U.S. screening population: data from the ATHENA trial. *Gynecol Oncol*, **137**, 47-54.
Moreno V, Bosch FX, Muñoz N, et al (2002). Effect of oral contraceptives on risk of cervical cancer in women with human papillomavirus infection: the IARC multicentric case-control study. *Lancet*, **359**, 1085-92.
Morse SA, Holmes K, King K, Ballard R, Ronald C (2010). Atlas of sexually transmitted diseases and AIDS: Expert Consult. 4th Edition, Saunders 2010 [Published: 17 Sep 2010].
Patel MA, Nyirjesy P (2010). Role of Mycoplasma and Ureaplasma species in female lower genital tract infections. *Curr Infect Dis Rep*, **12**, 417-22.
Silva J, Cerqueira F, Medeiros R (2014). Chlamydia trachomatis infection: implications for HPV status and cervical cancer. *Arch Gynecol Obstet*, **289**, 715-23.
Solomon D, Nayar R (2015). The Bethesda system for cervicovaginal cytology. definitions, criteria, and explanatory notes. *Anticancer Res*, **35**, 5708.
Taylor-Robinson D (2017). Mollicutes in vaginal microbiology: Mycoplasma hominis, Ureaplasma urealyticum, Ureaplasma parvum and Mycoplasma genitalium. *Res Microbiol*, **168**, 875-81.
Tota JE, Chevarie-Davis M, Richardson LA, et al (2011). Epidemiology and burden of HPV infection and related diseases: implications for prevention strategies. *Prev Med*, **53**, S12-21.
Tully JG, Taylor-Robinson D, Cole RM, Rose DL (1981). A newly discovered mycoplasma in the human urogenital tract. *Lancet*, **1**, 1288-91.
Ye H, Song T, Zeng X, et al (2018). Association between genital mycoplasmas infection and human papillomavirus infection, abnormal cervical cytopathology, and cervical cancer: a systematic review and meta-analysis. *Arch Gynecol Obstet*, **297**, 1377-87.

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Maringa, Brazil, September 08, 2025

Beneficiary: Dr. Isabella Harumi Yonehara Noma

Form I-140: Petition for EB-1A Classification

To whom it may concern,

I am writing in enthusiastic support of an application by Dr. Isabella Harumi Yonehara Noma for an EB-1 visa, and to attest to her outstanding reputation as a scientist and researcher of excellence in the field of Melanoma, Molecular biology.

I am currently Associate Professor C in the Department of Clinical Analysis and Biomedicine at State University of Maringa (UEM). Current coordinator of the Graduate Program in Biosciences and Pathophysiology/UEM (Pharmacy/CAPES) and also from 2012-2016. Director of Graduate Studies at UEM from 2018-2022. Principal researcher in CNPq research groups, editor and reviewer of scientific journals. Coordinator of national and international projects funded by CNPq, CAPES, Fundacao Araucaria, DECIT/MS, National Institute of Health/NIH-USA, UK RESEARCH AND INNOVATION-UKRI, Medecins Sans Frontieres Operational Center Barcelona/MSF-OPB and Oxford University. I had supervised more than 100 students in their CBT, IC, master's, doctorate and post-doctorate studies and has published at least 131 scientific papers. I have experience in Pharmacy, with an emphasis on HPV and cervical cancer; sexually transmitted infections; and arboviruses.

I supervised Dr. Noma in her Master's degree, and she worked with HPV and infections. She produced her dissertation, and an article entitled: "Association of High-Risk Human Papillomavirus and Ureaplasma parvum Co-Infections with Increased Risk of Low-Grade Squamous Intraepithelial Cervical Lesions." Her work was an original contribution to the field of Melanoma and Molecular Biology, showing how high-risk HPV and other infections are able to increase the risk of low-grade squamous cervical lesions, with an important contribution in cervical cancer. Interestingly, this study is the first to be published about this relationship between HPV and other infections in cervical cancer. Moreover, this study had citations that impacted recent publications in the area in Frontiers, and MDPI journals, important to share knowledge.

Based on my long scientific career, and my vast experience in evaluating fellowships, grant applications, faculty candidates, graduate students' dissertations, and the research publications, I feel very confident in my ability to

evaluate Dr. Noma's scientific skills and judge her contributions to the field of Molecular biology and Cancer.

I became acquainted with Dr. Noma when she was a graduate student and Master candidate at the State University of Maringa. She performed the experimental work for her dissertation under my supervision, in the Health Science Program, at the Health Science Center. During that time, she utilized very creative approaches to conducting research and actively participated in the organization of multiple institutional activities, including the VI International Meeting of Biosciences and Physiopathology. After she graduated, she was recruited for her PhD to come to University of Sao Paulo to join in the School of Pharmaceutical Sciences, led by cancer famous researcher Dr. Silvya Stuchi, as well-known in her area in Brazil and other countries.

Unlike most researchers, her scientific and technical skills span the whole spectrum of research from basic fundamental biology to applied research. Owing to her training in multidisciplinary environments, Dr. Noma has shown the ability to integrate concepts of synthetic biology, molecular biology, systems biology, and oncology in the design and development of novel insights to therapeutic approaches for the treatment of human cancers.

Later in her career, she was awarded the prestigious grants from her project about PRDX2 and melanoma had high translational impact in the field of oncology in the wellknown congress in Melanoma area called Congress from the Society for Melanoma Research. Her writing and analytic skills are excellent.

In summary, Dr. Nomas's contributions to the cancer gene therapy communities are of significant relevance. She has extraordinary abilities and skills in developing new methodologies as well as applying previously described approaches. These characteristics, along with her remarkable communication skills, passion for science, and her appreciation of diversity, make her a valuable asset for the US scientific community. Without question, Dr. Noma has proven herself as an outstanding scientific researcher and I have no doubt that she will continue to make significant contributions to the cancer field and in so doing, benefit US public health.

I, therefore, strongly and unreservedly endorse Dr. Isabella's EB-1 visa petition to remain contributing substantially to her field in the United States. Please do not hesitate to contact me to confirm Dr. Isabella's credentials and qualifications.

Best Regards,

_____
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Last CV update on 04/17/2026

Pharmaceutical-biochemistry from the State University of Maringá (UEM) (1992), master (1996) and doctor (2005) in Cell Biology also from UEM. He completed a post-doctoral internship (2016) at the Department of Clinical and Toxicological Analysis, Faculty of Pharmaceutical Sciences, University of São Paulo/USP. She is currently Associate Professor C at the Department of Clinical Analysis and Biomedicine at UEM. Current coordinator of the Postgraduate Program in Biosciences and Pathophysiology/UEM (Pharmacy/CAPES) and also from 2012-2016. Director of Postgraduate Studies at UEM from 2018-2022. Principal researcher for CNPq research groups, editor and reviewer of scientific journals. Coordinator of national and international projects with funding from CNPq, CAPES, Fundação Araucária, DECIT/MS, National Institute of Health/NIH-USA, UK RESEARCH AND INNOVATION-UKRI, Médecins Sans Frontieres Operational Center Barcelona/MSF-OPB and Oxford University. Guided more than 140 TCC, IC, master's students doctorate and post-doctorate and published more than 14 scientific articles. He has experience in the field of Pharmacy, with an emphasis on HPV, cervical cancer and sexually transmitted infections. **(Text informed by the author)**

Identification

Name

Marcia Edilaine Lopes Consolaro

Name in bibliographic citations

CONSOLARO, M. AND. L.;Lopes-Consolaro, M. AND.;Consolaro, Marcia Edilaine Lopes;Consolaro, Márcia Edilaine Lopes;Consolaro, Márcia EL.;Lopes-Consolaro, Marcia E.;Lopes-Consolaro, Márcia Edilaine;Consolaro, Márcia E. L.;Lopes-Consolaro, Márcia E.;Consolaro, ME;CONSOLARO, MARCIA E;Consolaro, MEL;Consolaro, MEL.;Lopes-Consolaro, ME;Lopes-Consolaro, ME;MEL. Consolaro;M. AND. L. Consolaro;Consolaro, Marcia Lopes;CONSOLARO, MARCIA E. LOPES;CONSOLARO, MARCIA EL.;CONSOLARO, MARCIA E. L.;CONSOLARO, MARCIA EL;LOPES CONSOLARO, MARCIA EDILAINE;CONSOLARO, M.;Consolarob,Marcia EL;MARCIA CONSOLARO;CONSOLARO, MARCIA

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Country of Nationality

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Av. Colombo, 1179, Clinical Cytology Laboratory, block B09
Zone 07
87020900 - Maringá, PR - Brazil
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Homepage URL: http://www.uem.br

Academic training/title

2003 - 2005

Phd in Biological Sciences (Cellular Biology).
State University of Maringá, UEM, Brazil.
Title: Phenotypic aspects, susceptibility to antifungals and virulence potential of Candida albicans vaginal isolates and their relationship with clinical conditions, Year of obtaining: 2005.
Advisor: Rosane Marina Peralta.
Keywords: Candidiasis; virulence factors; Proteinases; non-C. albicans; C. albicans.
Large area: Health Sciences / Area: Pharmacy / Subarea: Clinical Analysis / Specialty: Mycology.
Large Area: Biological Sciences / Area: Biochemistry / Subarea: Biochemistry of Microorganisms.
Large Area: Health Sciences / Area: Pharmacy.
Sectors of activity: Care for People's Health; Other.

1994 - 1996

Master's degree in Biological Sciences (Cellular Biology).
State University of Maringá, UEM, Brazil.
Title: Cytogenetic characterization of different southern Brazilian populations of Centella Asia (L.) Urban (Umbelliferae), Year of Obtainment: 1996.
Advisor: Maria Suely Pagliarini.
Scholarship holder from(a): Coordination for the Improvement of Higher Education Personnel, CAPES, Brazil.
Keywords: Centella asiatica; Cytogenetics.
Large area: Biological Sciences / Area: Genetics.
Activity sectors: Plant Production.

2002 - 2003

Specialization in Specialization in Clinical Cytology.
Brazilian Society of Clinical Cytology, SBCC, Brazil.
Title: Clinical Cytology.

1988 - 1993

Graduation in Pharmacy.
State University of Maringá, UEM, Brazil.

Postdoctoral and Free Teaching

2013

Free teaching.
State University of Maringá, UEM, Brazil.
Title: Descriptive memorial of activities at UEM, Year of obtaining: 2013.
Keywords: Competition; Teaching; Pharmacy.
Large area: Health Sciences / Area: Pharmacy.
Large Area: Health Sciences / Area: Pharmacy / Subarea: Clinical Analysis.
Large Area: Health Sciences / Area: Pharmacy / Subarea: Clinical Analysis / Specialty: Clinical Cytology.
Sectors of activity: Scientific research and development; Other professional, scientific and technical activities.

2015 - 2016

Postdoctoral.
University of São Paulo, USP, Brazil.
Large area: Health Sciences / Area: Pharmacy.
Large Area: Health Sciences / Area: Pharmacy / Subarea: Clinical Analysis.
Large Area: Health Sciences / Area: Pharmacy / Subarea: Pathophysiology and laboratory diagnosis.

Professional performance

University of Florida, UF, United States.

Institutional link

2024 - Current

Bond: , Functional Frame:

Ministry of Health, MS, Brazil.

Institutional link

2017 - Current

Bond: , Functional Frame:

University of São Paulo, USP, Brazil.

Institutional link

2014 - Current

Link: Partnership in Research Project, Functional Framework: None

State University of Maringá, UEM, Brazil.

Institutional link

2016 - Current

Link: Public servant, Functional Framework: Associate Professor B, Workload: 40, Regime: Exclusive dedication.

Institutional link

2013 - Current

Link: Public servant, Functional Framework: Associated teacher A, Workload: 40, Regime: Exclusive dedication.

Institutional link

2011 - Current

Link: Public servant, Functional Framework: Adjunct teacher level D, Workload: 40, Regime: Exclusive dedication.

Institutional link

2007 - 2009

Link: Public servant, Functional Framework: Adjunct teacher C, Workload: 40, Regime: Exclusive dedication.

Institutional link

2005 - 2007

Link: Public servant, Functional Framework: Adjunct teacher B, Workload: 40, Regime: Exclusive dedication.

Institutional link

1997 - 2005

Link: Public servant, Functional Framework: Assistant teacher level C, Workload: 40, Regime: Exclusive dedication.

Institutional link

1996 - 1997

Link: CLT, Functional Framework: Assistant teacher A, Workload: 40

Other information

I continue working at that institution

04/2008 - Current

Teaching, Specialization in Clinical Analysis, Level: Specialization

Subjects taught
Crevico-vaginal cytopathology and semen analysis

12/2007 - Current

Research and development, Health Sciences Center.

Search lines
HPV and carcinogenesis

08/2007 - Current

Teaching, Health Sciences, Level: Postgraduate

Subjects taught
Vulvovaginal candidiasis
Inflammatory processes of the cervix and vagina

03/2007 - Current

Councils, Committees and Consultancy, Health Sciences Center, Department of Clinical Analysis.

Position or function
Effective member of the Board of the Master's degree in Clinical Analysis-PAN/UEM.

06/2006 - Current

Teaching, Clinical Analysis, Level: Postgraduate

Subjects taught
Cervical-vaginal cytology in the diagnosis and prevention of sexually transmitted diseases
APPLICATION OF CYTOLOGICAL DIAGNOSIS IN THE PREVENTION OF CERVICAL-UTERINE CANCER

5/2006 - Current

Research and development, Center for Biological Sciences, Department of Clinical Analysis.

Search lines
Sexually transmitted diseases

5/2005 - Current

University extension , Center for Biological Sciences, Department of Clinical Analysis.

Extension activity carried out
Participant of the extension project: Prevention of gynecological cancer.

11/2004 - Current

Teaching,

Subjects taught
Permanent teaching project: Internship in laboratory practices, process 2850/1990, UEM

08/1999 - Current

Councils, Committees and Consultancy, Health Sciences Center, Department of Clinical Analysis.

Position or function
Participant of the Clinical Analysis Department's Timetable Committee.

07/1998 - Current

University extension , Health Sciences Center, Department of Clinical Analysis.

Extension activity carried out
Participation in the permanent extension project: Prevention of gynecological cancer, process 1508/1990,DEX/UEM.

1/1998 - Current

Research and development, Center for Biological Sciences, Department of Clinical Analysis.

Search lines
Clinical Cytology

8/1997 - Current

Councils, Committees and Consultancy, Center for Biological Sciences, Department of Clinical Analysis.

Position or function
Participation in the Timetable Committee.

5/1997 - Current

Teaching, Pharmacy, Level: Undergraduate

Subjects taught
Clinical Cytology
Supervised Internship for Pharmaceutical-Biochemical

5/1997 - Current

Specialized technical services , Center for Biological Sciences, Department of Clinical Analysis.

Service performed
Supervision and issuance of reports on Cytopathological, Urinary and Human Semen exams.

07/1996 - Current

Teaching,

Subjects taught
Teaching project: Teaching and Research Laboratory in Clinical Analysis-LEPAC/UEM

06/2007 - 12/2007

Councils, Committees and Consultancy, Health Sciences Center, Department of Clinical Analysis.

Position or function
Member of the PAN-postgraduate Selection Committee in Clinical Analysis-master's degree.

04/2006 - 08/2006

Teaching, Specialization in Clinical Analysis, Level: Specialization

Subjects taught
Cervico-vaginal cytopathology and semen analysis

03/2005 - 08/2005

Teaching, Specialization in Clinical Analysis, Level: Specialization

Subjects taught
Skin and mucous membrane infections
Cytopathology of the female genital tract
Functional analysis and metabolic diseases
Analysis of Cavity Liquids

8/2001 - 10/2004

University extension , Center for Biological Sciences, Department of Clinical Analysis.

Extension activity carried out
Extension project coordinator: gynecological cancer prevention.

8/1999 - 8/2001

University extension , Center for Biological Sciences, Department of Clinical Analysis.

Extension activity carried out
Participation in an extension project: Prevention of Gynecological Cancer. Workload of 4 hours per week.

5/1996 - 4/1997

Teaching, Pharmacy, Level: Undergraduate

Subjects taught
Clinical Cytology
Supervised internship for Biochemical pharmac6eutic

Universidade Paranaense, UNIPAR, Brazil.

Institutional link

1993 - 1997

Link: Hired teacher, Functional Framework: Master's student teacher, Workload: 12

Activities

8/1993 - 3/1997

Teaching, Pharmacy, Level: Undergraduate

Subjects taught
Deontology and Pharmaceutical Legislation
Supervised Internship for Pharmacists
Clinical Cytology
Supervised Internship for Biochemical Pharmacist

3/1994 - 5/1995

Specialized technical services , Qualification in Clinical Analysis, Clinical Analysis Laboratory.

Service performed
Implementation and standardization of the UNIPAR Clinical Analysis Laboratory.

Search lines

1.

Clinical Cytology

Objective: Scientific studies covering the area of Clinical Cytology, especially Cervico-vaginal Cytology.
Large area: Health Sciences / Area: Pharmacy / Subarea: Clinical Analysis.
Large Area: Health Sciences / Area: Public Health / Subarea: Public Health.
Large Area: Health Sciences / Area: Medicine / Subarea: Maternal and Child Health / Specialty: Gynecology.
Sectors of activity: Human Health; Health Care for Human Populations; Care for People's Health.
Keywords: colpocytology; Pap smear; cervical cancer; cervical-vaginal infections; HPV.

TWO.

Sexually transmitted diseases

Objective: Contribution of cytology to the diagnosis and prevention of STDs.
Large area: Health Sciences / Area: Pharmacy / Subarea: Clinical Analysis / Specialty: Mycology.
Large Area: Health Sciences / Area: Pharmacy / Subarea: Clinical Analysis / Specialty: Clinical Cytology.
Large Area: Health Sciences / Area: Medicine / Subarea: Maternal and Child Health / Specialty: Gynecology.
Sectors of activity: Human Health; Health Care for Human Populations; Care for People's Health.
Keywords: STD; Pap smear cytology; Colpocytological diagnosis; cervical-vaginal infections.

3.

HPV and carcinogenesis

Objective: Scientific studies on HPV and carcinogenesis.
Large area: Health Sciences / Area: Pharmacy / Subarea: Clinical Analysis / Specialty: Clinical Cytology.
Large Area: Health Sciences / Area: Pharmacy.
Large Area: Health Sciences / Area: Pharmacy / Subarea: Clinical Analysis.
Sectors of activity: Human Health; Care for People's Health; Research and scientific development.
Keywords: cervical-uterine cancer; contraceptive methods; HPV; biomarkers.

Research projects

2024 - Current

Hypericin nanoparticles combined with chemotherapy for cervical cancer photochemotherapy

Description: Cervical cancer is the fourth most common cancer in the global female population

and the third in Brazil, also presenting high mortality rates. Usual treatments have high failure rates in advanced, metastatic or recurrent cancer. The use of combined therapies has been extremely important to achieve multiple targets, maximize therapeutic results and overcome resistance to chemotherapy drugs. Photodynamic therapy (PDT) is a promising modality, due to its selectivity and reduction of systemic effects, and in association with chemotherapy it can significantly increase the effectiveness of its antitumor action. Several studies have already demonstrated the activity of hypericin after photoactivation in different tumor cells. However, due to its hydrophobic nature, the hypericin molecule tends to undergo self-aggregation in an aqueous solution, which compromises its availability and reduces its photodynamic effect. Additionally, the main chemotherapy drugs used to treat cervical cancer, such as acisplatin and doxorubicin, have limitations in relation to blood decirculation time and trigger systemic side effects. To overcome the physical-chemical limitations of both photosensitizing agents and chemotherapy drugs, and co-deliver both, medication delivery systems can be used. Among these platforms, pluronics stand out for their ability to solubilize and stabilize drugs of different degrees of hydrophobicity and have low systemic toxicity. Our research team previously produced solid evidence of the selective antitumor effectiveness of hypericin encapsulated in pluronic P-123 in cervical cancer cells, but to date There are no reports of the application of this nanosystem associated with chemotherapy drugs. Therefore, this temporary study aims to analyze the effect of photochemotherapy with hypericin associated with cisplatin or doxorubicin, nanoencapsulated in pluronic P-123 front cell alignments derived from human cervical cancer, compared to non-tumorigenic human epithelial lineage. To this end, cellular internalization of the nanosystem, its cytotoxicity and phototoxicity, the pathway of death induced by experimental treatment, as well as its action on the demigration and metastasis capacity of tumor cells, will be evaluated. With this work, we hope to contribute to the elucidation of effective therapeutic alternatives to treat cervical cancer through PDT combined with chemotherapy..
Status: In progress; Nature: Research.
Students involved: Doctorate: (1) .

Members: Marcia Edilaine Lopes Consolaro - Coordinator / Lucimara Rodrigues Carobeli - Member.
Financier(s): National Council for Scientific and Technological Development - Bolsa.

2024 - Current

IN VITRO THREE-DIMENSIONAL MODELS BASED ON ORGANOIDS WITH A FOCUS ON CERVICAL CANCER

Description: This Sandwich Doctoral PROJECT Abroad aims to enable international collaboration with Professor Dr. Rodrigo Cristofoletti/University of Florida-USA and his research group, as a way of training and creating conditions so that new research using organoids in the development of medicines and disease studies, especially cervical cancer, can be developed in Brazil, contributing to the country's science and technology..
Status: In progress; Nature: Research.

Students involved: Undergraduate: (0) / Specialization: (0) / Academic master's degree: (0) / Professional master's degree: (0) / Doctorate: (1) .

Members: Marcia Edilaine Lopes Consolaro - Coordinator / Lucimara Rodrigues Carobeli - Member / Rodrigo Cristofoletti - Member.
Financier(s): Coordination for the Improvement of Higher Education Personnel - Scholarship.

2023 - Current

Self-collection for HPV testing as a strategy to promote equity and reduce morbidity and mortality from cervical cancer in black women from different Brazilian macroregions

Description: Although cervical cancer screening based on the Pap smear (Pap) is provided by the Unified Health System (SUS) in an opportunistic manner, many women, especially those with social vulnerability, do not participate in this screening due to lack of access to the SUS, social factors such as race/color and intra/interpersonal barriers, resulting in a high incidence and mortality from this cancer in these women. Among them, black women stand out, who have the highest incidence and mortality from cervical cancer in the country, which is progressive from 2002 to 2021. It has already been proven that tests to detect human Papillomavirus (HPV), the main causal agent of cervical cancer, are more effective in screening than Pap tests, which are recommended by the World Health Organization (WHO). Hpv tests have the additional advantage of being able to detect the virus in self-collected samples enabling access and adherence for socially vulnerable women such as black women, and thus representing an important support tool for changing the extremely negative panorama of cervical cancer in our country. However, national guidelines recommend tracking by the SUS with the Pap, not allowing the introduction of self-collection in the SUS. Despite our experience in developing research funded by DECIT/MS on this topic, the studies were aimed at untreated women as a whole regardless of race/color and thus, to date, do not reflect the reality of the possibility of implementing self-collection /HPV test in screening in black women. These facts motivated us to submit this proposal, which proposes to evaluate the acceptability and adherence of urban and quilombola black women to self-collection in the three different Brazilian macro-regions reaching out to these women via health education and training of community health workers (CHAs) and community leaders..
Status: In progress; Nature: Research.
Students involved: Academic master's degree: (6) Doctorate: (1) .

Members: Marcia Edilaine Lopes Consolaro - Coordinator / Sandra Marisa Pelloso - Member / Vânia Ramos Sela da Silva - Member / Valquíria do Carmo Alves Martins - Member / Janaina Cristiana de Oliveira Crispim Freitas - Member / Fábio Juliano Negrão - Member / Idalina Cristina Ferrari - Member / Karolina Reis dos Santos Lukachaki - Member / Jacinto da Costa Silva Neto - Member / Isabel Cristina Scarinci-Searles - Member.
Financier(s): DECIT-MS - Aid financial.

2021 - 2022

Strategies for improvements to the stricto sensu postgraduate system at the State University of Maringá-UEM

Description: Strategies for improvements to the system stricto sensu postgraduate studies State University of Maringá-UEM.
Status: Completed; Nature: Research.
Students involved: Academic master's degree: (10) Doctorate: (10) .

Members: Marcia Edilaine Lopes Consolaro - Coordinator / Luis Fernando Cótica - Member.
Financier(s): Araucária Foundation to Support Scientific and Technological Development - Aid financial.

2021 - 2021

Diagnosis of Oxygen Requirement in non-severe SARS-CoV-2 infections-PRIORITISIS

Description: Identify clinical and biochemical diagnostic markers in adults with laboratory-confirmed SARS-CoV-2 infection who do not require oxygen supplementation, with the aim of: Aiding safe discharge from a healthcare facility (i.e., high NPV); In the short-term impact on COVID-19 interventions in limited resource configurations (i.e., simple clinicodemographic variables and biochemical markers for which POCTs close to the Patient/POCTs are commercially available or in the final stage of development).
Status: Completed; Nature: Research.

Members: Marcia Edilaine Lopes Consolaro - Member / André M. Siqueira - Member / Arjun Chandna - Coordinator / Nicholas Day - Member / Arjen Dondorp - Member / Sakib Burza - Member / Yoel Lubell - Member / Paul Turner - Member / Sophie Yacoub - Member / Endeshaw Aderi - Member / Carolina Jimenez - Member / Mikhael Yosia - Member.
Financier(s): MÃ©decins Sans FrontiÃ¬reres Operational Center Barcelona Athens - Other / University of Oxford - Other.

2021 - Current

Effectiveness of COVID-19 Vaccination in Brazil: Case-Control Study

Description: This research project is a study on the effectiveness of the vaccination program whereas the effectiveness of licensed vaccines, demonstrated in controlled studies, needs it be verified in the routine of immunization services across the country, with their regional diversity including different vaccination coverage and circulation of SARS-CoV-2 variants, in addition to factors individual, such as age group and presence of comorbidities. The performance of immunization programs in susceptible population generates groups exposed and not exposed to the COVID-19 vaccine. Hypothetical cohorts underlying vaccinated and unvaccinated cannot be clearly enumerated, and the strategy is reconstruct the experience of exposure (vaccination) and disease (COVID-19) in this population..
Status: In progress; Nature: Research.
Students involved: Doctorate: (10) .

Members: Marcia Edilaine Lopes Consolaro -

Coordinator / Luciano Pamplona G. Cavalcanti - Member / Moacyr Jesus Barreto de Melo Rêgo - Member / Julio Croda - Member / Fábio Juliano Negrão - Member / André Machado de Siqueira - Member / Franciana Maria Rosa da Silva - Member / Tatiana Noronha - Member.
Financier(s): DECIT-MS - Other.

2020 - 2022

Genomic approach to investigate genetic variations of Sars-CoV-2 (coronavirus) and the human host

Description: General ObjectiveStudy the phylogeography of SARS-CoV-2 lineages present in a sample of the Brazilian population, in addition to investigating the impact of genetic changes in SARS-CoV-2 and canonical and non-canonical receptors on the host infection rate; finally, identify genomic signatures of mutations, gene expression and miRNAs to establish correlations with the clinical manifestations of a cohort of 150 individuals diagnosed with COVID-19.Study the phylogeography of SARS-CoV-2 strains and identify genomic signatures in a cohort of 150 patients to establish correlations with the clinical severity of COVID-19.2.1. Specific objectivesObjective 1. Investigate whether genetic changes in SARS-CoV-2 and its receptors are correlated with the clinical evolution of patients with COVID-19.Objective 2. Classify, using a genomic approach, the SARS-CoV-2 subtypes in a sample of patients from the states of Paraná and São Paulo.Objective 3. To evaluate, by structural modeling and molecular docking, the functional impact of variants in the interaction regions of host ACE2 and SARS-CoV-2 Spike proteins. Objective 4. Characterize the global expression profile of miRNAs and mRNAs of lymphocytes from patients in the cohort. Objective 5. Identify expression signatures of miRNAs, mRNAs and mutations related to symptom variability in groups of patients with mild, moderate and severe clinical manifestations. Objective 6. Determine the discriminatory power of molecular signatures in relation to the three clinical groups of the cohort, as defined in objective 4..
Status: Completed; Nature: Research.
Students involved: Doctorate: (7) .

Members: Marcia Edilaine Lopes Consolaro - Member / David Livingstone - Coordinator / Edson Arpini Miguel - Member / Paulo Roberto Donadio - Member / Jeane EL Visentainer - Member.
Financier(s): SETI - Aid financial / Guarapuava City Hall - Aid financial.
Number of productions C, T & A: 1

2020 - 2021

Multicenter study of the natural history of the new SARS-CoV-2 coronavirus in Brazil (REBRACOVID)

Description: Multicenter study of the natural history of the new SARS-CoV-2 coronavirus in Brazil.
Status: Completed; Nature: Research.
Students involved: Undergraduate: (10) / Academic master's degree: (10) / Doctorate: (10) .

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Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

14-Week exercise training modifies the DNA methylation levels at gene sites in non-Alzheimer's disease women aged 50 to 70 years

Guilherme da Silva Rodrigues [a,1], Natália Yumi Noronha [b,*,1], Isabella Harumi Yonehara Noma [c], João Gabriel Ribeiro de Lima [a], Andressa Crystine da Silva Sobrinho [a], Marcela Augusta de Souza Pinhel [a], Mariana Luciano de Almeida [d], Lígia Moriguchi Watanabe [e], Carla Barbosa Nonino [e], Carlos Roberto Bueno Júnior [a,f]

[a] Department of Internal Medicine, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil
[b] Department of Gynecology and Obstetrics, University Medical Center Groningen, Groningen, the Netherlands
[c] Department of Clinical and Toxicological Analyses, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil
[d] University of São Paulo at Ribeirão Preto College of Nursing, Brazil
[e] Department of Health Sciences, Ribeirão Preto Medical School, Ribeirão Preto, São Paulo, Brazil
[f] School of Physical Education of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

ARTICLE INFO

Section Editor: Emanuele Marzetti

Keywords:
Epigenetic
Combined training
Women
Physical activity

ABSTRACT

Exercise training emerges as a key strategy in lifestyle modification, capable of reducing the risk of developing Alzheimer's disease (AD) due to risk factors such as age, family history, genetics and low level of education associated with AD. We aim to analyze the effect of a 14-week combined exercise training (CT) on the methylation of genes associated with AD in non-alzheimer's disease women. CT sessions lasted 60 min, occurring three times a week for 14 weeks. Forty non-Alzheimer's disease women aged 50 to 70 years (60.7 ± 4.1 years) with a mean height of 1.6 ± 0.1 m, mean weight of 73.12 ± 9.0 kg and a mean body mass index of 29.69 ± 3.5 kg/m2, underwent two physical assessments: pre and post the 14 weeks. DNA methylation assays utilized the EPIC Infinium Methylation BeadChip from Illumina. We observed that 14 weeks of CT led to reductions in systolic ($p = 0.001$) and diastolic ($p = 0.017$) blood pressure and improved motor skills post-intervention. Among 25 genes linked to AD, CT induced differentially methylated sites in 12 genes, predominantly showing hypomethylated sites (negative β values). Interestingly, despite hypomethylated sites, some genes exhibited hypermethylated sites (positive β values), such as *ABCA7*, *BDNF*, and *WWOX*. A 14-week CT regimen was adequate to induce differential methylation in 12 CE-related genes in healthy older women, alongside improvements in motor skills and blood pressure. In conclusion, this study suggest that combined training can be a strategy to improve physical fitness in older individuals, especially able to induce methylation alterations in genes sites related to development of AD. It is important to highlight that training should act as protective factor in older adults.

1. Introdution

Alzheimer's disease (AD) stands as the most prevalent form of dementia among the older population, representing 60 to 80 % of diagnosed cases in the world (Duthey, 2013). Global prevalence estimates exceed 45 million individuals. With an aging global population, a threefold rise in disease prevalence by 2050 is anticipated (Jakovljevic et al., 2021; El-Hayek et al., 2019). The economic impact of AD is substantial, evidenced by an estimated cost of $818 billion due to clinical and caregiving expenses (El-Hayek et al., 2019).

Therefore, it is essential to allocate efforts to research and find effective interventions for disease prevention. Exercise training emerges as a key strategy in lifestyle modification, capable of reducing the risk of developing AD arising from risk factors such as age, familial history, genetics, and low educational attainment associated with AD (Crous-Bou et al., 2017). Neurological and vascular adaptations during exercise

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[1] These authors have contributed equal to this work.

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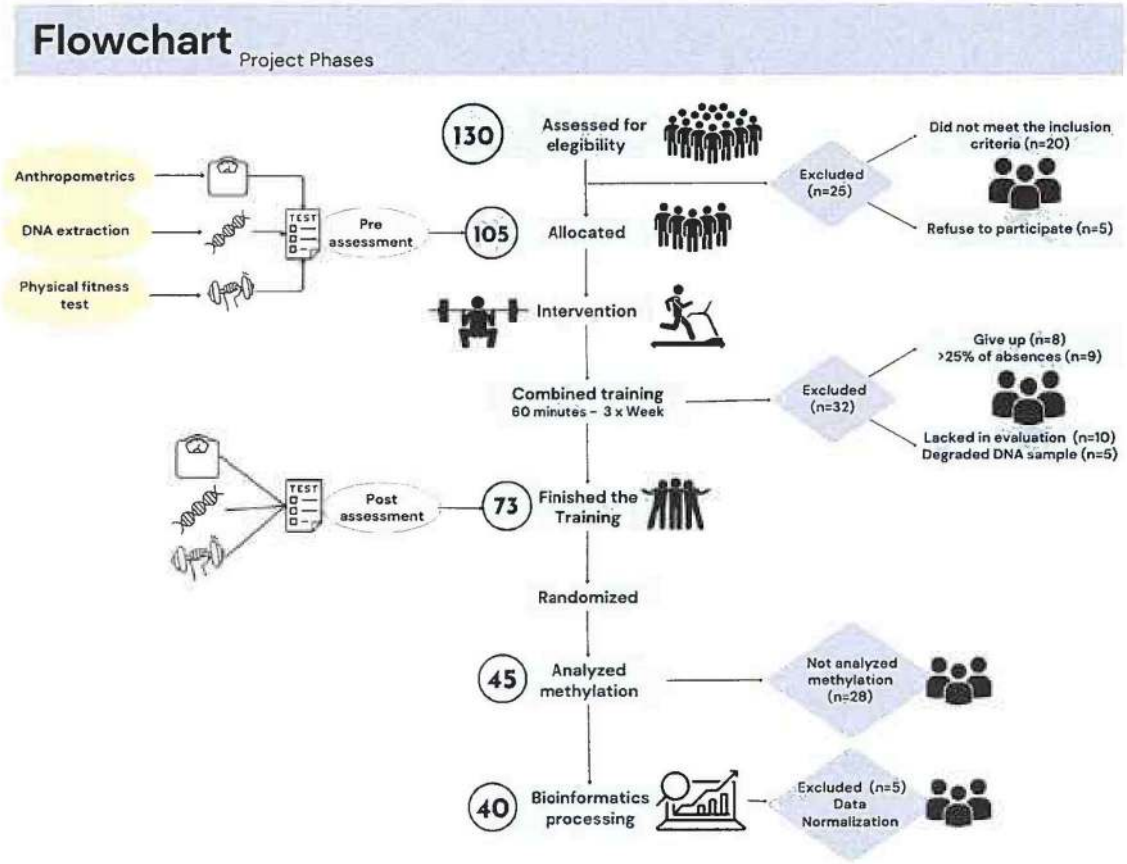

Fig. 1. Flowchart and experimental design.

training foster neurogenesis, and synaptic plasticity, diminish inflammatory processes, and mitigate cellular damage due to oxidative stress, important processes in prevention AD prevention (Foster et al., 2011).

A recent meta-analysis highlights that engaging in moderate-intensity aerobic and resistance exercises for at least 45 min, multiple times weekly, enhances cognition in adults aged 50 and above (Northey et al., 2018). Additionally, exercise training contributes to AD prevention even in cases of high genetic risk, an unmodifiable factor (Hamer and Chida, 2009). The meta-analysis that analyzed 16 studies (n = 160,000 participants) found a 45 % reduction in the risk of developing AD due to the regular practice of physical activity (Hamer and Chida, 2009). After 3.5 years of monitoring 716 older subjects, one study reported that individuals with low daily physical activity levels were 53 % more likely to suffer AD than those who reported more active lives (Buchman et al., 2012). It is still estimated, that 3 % of dementia cases could be prevented by increasing levels of free-living physical activity (Liang et al., 2020; Livingston et al., 2020).

It is well know that exercise training and others external factors such as dietary habits, smoking, or alcohol consumption interact with the genome and affect the phenotype via modifications in epigenetic mechanisms (DNA methylation, histone modifications, chromatin remodeling and non-coding RNA), which can suppress or increase gene expression (Wu et al., 2023).

Potentially, DNA methylation is the epigenetic mechanism that has the greatest impact on the regulation of gene expression, being related to the aging process, health parameters, and the increase or reduction in the risk of developing cardiovascular and neurological diseases, cancers, among other complications (Wu et al., 2023; Barrón-Cabrera et al., 2019). Current dementia research increasingly delves into the role of epigenetics in AD development, a crucial mechanism in memory formation and regulation (Higgins-Chen et al., 2021).

Recently, it was presented in the literature that eight weeks of combined exercise training showed changes in methylation patterns compared to baseline values, as well as rejuvenating the epigenetic clock by two years in older women (da Silva et al., 2023a). Conversely, a year-long aerobic training study in 320 healthy older women revealed no influence of exercise training on DNA methylation in specific breast cancer-related genes (Boyne et al., 2018). To date, evidence remains lacking regarding whether any form of exercise training can modify methylation levels in genes linked to AD among healthy seniors. A recent meta-analysis delineating AD risk loci implicated in Aβ, tau, immunity, and lipid processing identified 25 genes associated with AD development, emphasizing the need to elucidate how exercise training impacts the epigenetics of these genes to bolster AD prevention in conjunction with training (Verheijen and Sleegers, 2018).

It is important to investigate alternative ways of preventing AD. The literature shows that exercise can be used as a tool, but it is still not clear how exercise acts on DNA methylation in healthy older people when we observe genetic sites related to AD, being able to understand whether the exercise would actually act as a protective factor for the development of AD in healthy women aged 50 to 70 years.

Our investigation aims to examine the effects that combined exercise training carried out for 14 weeks has on DNA methylation in genes associated with AD in healthy non-Alzheimer's disease women aged 50 to 70 years, understanding how exercise would act in an epigenetic way to prevent AD.

2. Methods

The research project underwent review by the Research Ethics Committee involving Human Subjects at EEFERP-USP (CAAE: 79582817.0.0000.5659), adhering to Resolution 466/12 by the Ministry of Health, as regulated by the National Health Council. This study was registered in the Brazilian Clinical Trials Registry (REBEC) under registration number RBR-3g38dx.

2.1. Eligibility criteria

Women aged between 50 and 70 years old were included, with medical authorization to practice physical exercise and identified as physically inactive using the Modified Baecke Questionnaire (QBMI) for the Elderly. The QBMI measures the three domains of physical activity (domestic, sporting, and free time) usually carried out by the elderly, scores lower than 9.11 consider those assessed to be physically inactive (Florindo et al., 2003). Another criterion chosen for eligibility not to include people with cognitive impairment was achieving >26 points on the Montreal Cognitive Assessment (Wang et al., 2022), the minimum score for cognitively normal older adults in this cognitive test. This instrument was developed as a brief instrument for screening mild cognitive impairment. The Montreal Cognitive Assessment stands out for evaluating different cognitive domains such as: attention and concentration, executive function, memory, language, visual-constructive capacity, abstract summary, calculation, and orientation (Wang et al., 2022).

Participants were excluded based on criteria such as physical limitations that make it difficult to perform exercises or motor tests used in the study, current smokers or those who smoked in the last six months, degraded DNA at the time of bioinformatics processing, individuals who consumed caffeine within 24 h prior to blood collection and those diagnosed with Alzheimer's disease (Fig. 1).

2.2. Participants

The sample size required for the experiment was calculated using the GPower software. We used tests for independent samples to analyze the results of post-intervention changes. With an alpha error probability of 0.05 and power adjusted to 0.8 %, we estimated that a minimum of 35 participants would be necessary to detect a difference between the means.

We recruited more participants than necessary due to dropouts during the intervention process and exclusion of participants after normalization in the processing of methylation data, we calculated a sample with 28 % more, totaling 40 participants in the final group (Fig. 1).

2.3. Combined training protocol

The combined exercise training protocol consisted of sessions lasting 60 min, conducted three times a week over a period of 14 weeks. The training sessions were a combination of cardiovascular exercises (treadmill or bicycle) lasting 30 min and muscular strength exercises, also lasting 30 min (Rodrigues et al., 2023; da Silva et al., 2023b).

We adopted eight exercises for strength training, comprising five exercises for the upper limbs (inclined bench press, leg curl, leg extension, leg press 45, triceps bar, rower, arm curl, and lat pull-down) and three for the lower limbs (leg curl, leg extension, and leg press at 45°) (da Silva et al., 2023b). During the initial two weeks, participants performed two sets of 15 to 17 maximum repetitions for strength exercises and spent 30 min on a treadmill or stationary bicycle using 50 % of their heart rate reserve. From weeks 3 to 14, two sets of 10 to 12 maximum repetitions were completed, along with 30 min on a treadmill or stationary bicycle at 70 % of the heart rate reserve (Rodrigues et al., 2023). Training intensities were also assessed using the Borg Rating of Perceived Exertion (Borg and Noble, 1974). The Polar Team2 from Finland was utilized for heart rate measurements (Rodrigues et al., 2021).

2.4. Assessment

Participants underwent two rounds of physical assessments: before and after the 14 weeks of combined training. We assessed anthropometric variables (height (cm), weight (kg), body mass index (kg/m^2), waist circumference (cm) and hip circumference (cm)) (Rodrigues et al., 2023), and systolic and (SBP) diastolic blood (DBP) pressure pressures were measured using a previously calibrated automatic digital blood pressure gauge (OMRON®, Jundiaí, Brazil, model HEM-7113, SBH, 2010).

The motor variables evaluated included arm curl, chair stand, agility, 6-Min walk test (Rikli and Jones, 1999), maximum strength of the upper limbs measured by inclined bench press, and maximum strength of the lower limbs assessed by the leg press 45° (Izquierdo et al., 2002).

2.5. DNA extraction and methylation analysis

Peripheral blood samples were collected in tubes with EDTA at two different time points: before and after 14 weeks of training. Subsequently, genomic DNA was isolated from peripheral blood using the salting-out method (Shokrzadeh and Mohammadpour, 2018). The quality and integrity of the DNA were assessed by spectrophotometry (Bulla et al., 2016).

DNA methylation assays were conducted using the array technique, employing the EPIC Infinium Methylation BeadChip from Illumina. The fluorescence of the Infinium BeadChip was detected using the Illumina iScan system (Noronha et al., 2022). Intensities were converted into Beta values of methylation and exported as raw data. Subsequently, data processing was carried out using Rstudio software (R version 3.6.3) (Noronha et al., 2022), analyzing methylation patterns following the recommendations of the chAMP package from Bioconductor (Tian et al., 2017; Correia et al., 2023).

In the DMP function, we modified the command to pheno = myLoad $pd.$Sample_Group and conducted compare group to analyze the pre- and post-intervention moments. We adopted a significance threshold of $p < 0.05$ and subsequently filtered hypermethylation and hypomethylation values in the gene list, considering the beta values (Correia et al., 2023), as a method of adjusting the p value, the default = BH (Benjamini-Hochberg) recommended by ChAMP was adopted (Li and Barber, 2019).

2.6. Enrichment analysis (String)

We enriched our gene list using the STRING software. It's worth noting that the desired specificity cutoff for functional associations in STRING approximately corresponds to the annotation granularity of KEGG pathway maps (Kanehisa et al., 2017). We performed cytosine-phosphate-guanine enrichment through the epigenome-wide association study (EWAS), as it is becoming increasingly significant in identifying associations between epigenetic variations and the different biological characteristics that can be presented (Li et al., 2019).

2.7. Statistical analysis

The motor and anthropometric data collected were entered into an Excel file by two researchers and subsequently cross-checked to eliminate potential errors. We utilized GraphPad Prism 8 for statistical analyses, employing the Shapiro-Wilk test for normality assessment.

To compare values before and after the intervention, we used the Student's t-test for symmetrically distributed data. For asymmetrically distributed data, we employed the Mann-Whitney test. Across all statistical analyses, we set the significance level at $p < 0.05$.

Table 1
Means and standard deviations of the variables adopted for sample characterization ($n = 40$).

Variables	Pre	Post	*P*-value
YEARS OF EDUCATION (years)	10.6 ± 3.3		
AGE (years)	60.7 ± 4.1		
HEIGHT (m)	1.6 ± 0.1		
BW (Kg)	73.12 ± 9.0	73.46 ± 8.5	0.887
BMI (Kg/m2)	29.69 ± 3.5	29.35 ± 3.1	0.714
WC (cm)	94.56 ± 8.0	96.35 ± 9.0	0.467
HC (cm)	106.8 ± 7.5	106.1 ± 6.8	0.718
SBP (mmHg)	134.1 ± 14.7	121.0 ± 10.6	0.001*
DBP (mmHg)	80.15 ± 8.4	75.17 ± 5.5	0.017*

Note - BW: body weight; BMI: body mass index; WC: waist circumference; HC: hip circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; Normality test: Shapiro-Wilk; Student's t-test $p < 0.05$.

3. Results

3.1. Anthropometric data and blood pressure

Below are presented the results as mean and standard deviation of the 40 participants, regarding anthropometric comparisons and pre- and post-training blood pressure. We observed that 14 weeks of combined training lead to reductions in both SBP ($p = 0.001$) and DBP ($p = 0.017$) women with an average age of 60.7 ± 4.1 years, but no significant differences were found in anthropometric variables (Table 1).

3.2. Physical fitness tests

Physical improvements were observed after 14 weeks across all motor tests employed in this study. Significant enhancements and a considerable increase in strength were evident in the arm curl (Fig. 2A), chair stand (Fig. 2B), upper limb maximum repetition (Fig. 2E), and lower limb maximum repetition (Fig. 2F) tests when comparing the initial assessment to the final evaluation. The physical outcomes demonstrate that the combined training regimen yielded improvements in both strength tests and the assessment of aerobic fitness (6-min walk, Fig. 2D), as well as agility (Fig. 2C).

3.3. Genetic enrichment

We conducted enrichment analysis of the list of 25 genes used in this study to comprehend their potential relationships (Fig. 3). Following comparisons between the two assessment points, we observed differentially methylated sites in 12 genes (Fig. 4).

In the comparison of beta values, we observed that the vast majority of genes exhibited hypomethylated sites (negative β values). Despite these hypomethylated sites, some genes still displayed hypermethylated sites (positive β values), such as *ABCA7*, *BDNF*, and *WWOX* (Table 2).

We performed Cytosine-phosphate-Guanine enrichment of the 12 genes that were differentially methylated after 14 weeks of training to identify which gene ontology they were linked to (Fig. 5).

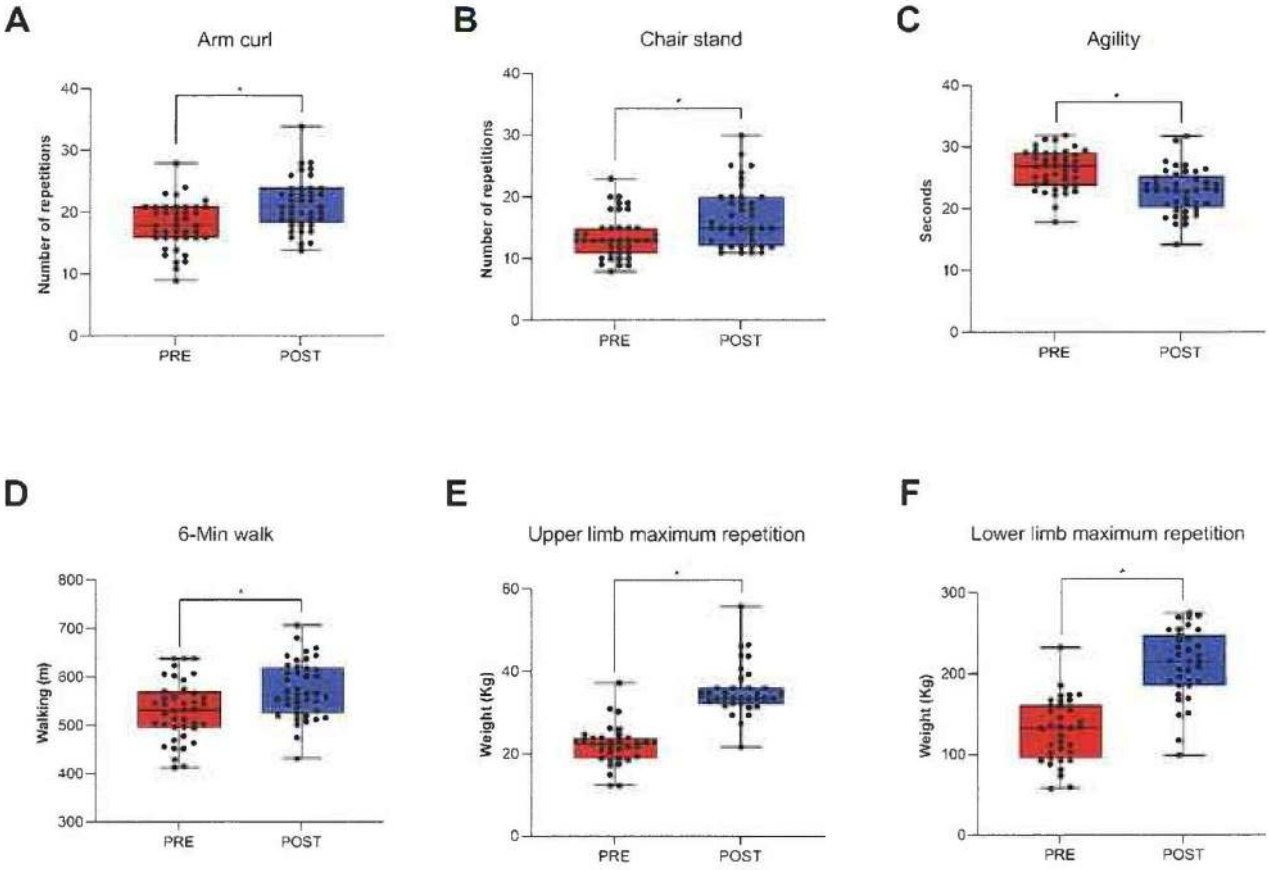

Fig. 2. Pre- and Post-Exercise Intervention Comparisons for Motor Variables ($n = 40$).
Note - Normality test: Shapiro-Wilk; Mann-Whitney test $p < 0.05$.

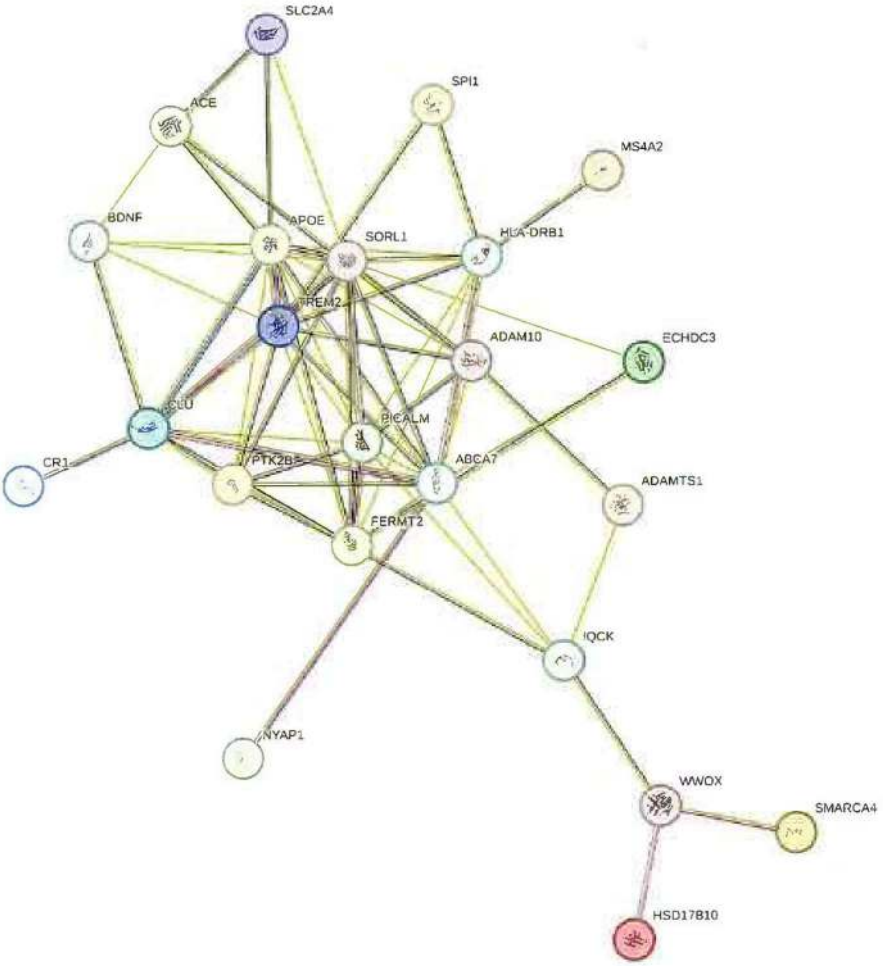

Fig. 3. Genetic enrichment of the list of genes adopted in the study.

4. Discussion

The present study aimed to investigate the effect that 14 weeks of combined training has on three distinct evaluation moments at sites of genes related to AD in women aged 50 to 70 years. Our data presented that 14 weeks of combined training were insufficient to obtain significant differences in anthropometric variables. The combined training is effective in improving health, however some recent studies have shown it may not be as effective in improving anthropometric data in older people (Rodrigues et al., 2023; da Silva et al., 2023b). These findings align with a 2020 study conducted by Brandão et al. (Brandao et al., 2020) that assessed the effects of an 8-week combined training program in overweight or obese older women. Although combined training was effective in improving cardiorespiratory fitness and muscular strength of participants, there was no significant change in body mass index (BMI) and waist circumference (Brandao et al., 2020).

The literature shows that similar training periods also did not result in changes in anthropometric data, such as the 2023 (Rodrigues et al., 2023) study comparing fourteen weeks of multicomponent training versus combined training in physically inactive older women, which did not show significant changes in anthropometric data like BMI and body fat percentage (Rodrigues et al., 2023). Therefore, other intervention strategies (such as nutritional ones) may be necessary in association to enhance the response magnitude of these data in older people (Amati et al., 2010).

According to our observations, combined training is an effective strategy to reduce systolic and diastolic blood pressure in older people, achieving significant results in 14 weeks of training in this study. When compared with the literature, we found a 2019 study by Leando et al. (Leandro et al., 2019) that assessed the effects of an eight-week combined training program on the blood pressure of hypertensive older women. The study showed that combined training significantly reduced systolic and diastolic blood pressure compared to a control group that did not undergo physical training. Furthermore, a 2019 study conducted by Ruangthai and colleagues (Ruangthai and Phoemsapthawee, 2019) evaluated the effects of a 12-week combined training program on the blood pressure of pre-hypertensive older women and men. The results indicated that the hypotensive and antioxidant effects of combined training were significantly high. The combined training is effective in reducing systolic and diastolic blood pressure, as well as providing other health benefits, similar to our findings. Our study had a longer training period than the studies presented above, which may have potentiated the results due to the broader range of stimuli.

There is an association between epigenetic modifications and the loss of phenotypic plasticity. Epigenetic patterns contribute to the etiology of Alzheimer's disease (AD) and other neurodegenerative disorders (Fuso, 2013). Various external factors, including stressors (physical and behavioral), nutritional elements, pollutants, pesticides, chemicals, metals, physical exercise, mental activity, and lifestyle, can exert effects that might contribute to the onset and progression of numerous diseases.

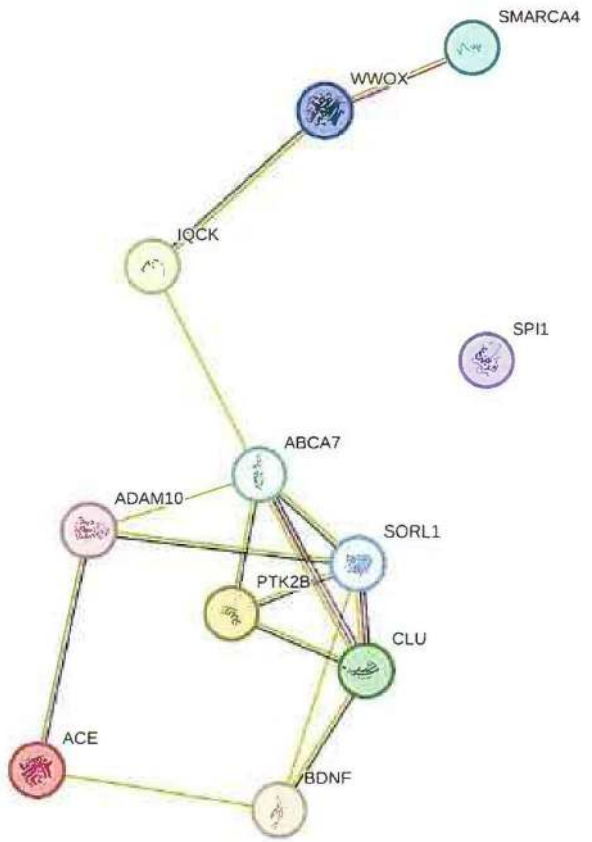

Fig. 4. Enrichment of genes with differentially methylated sites in training comparisons.

Physical exercise has been linked to a reduced risk and protective effects against mild cognitive impairment and AD (Nicolia et al., 2015). Studies have shown that moderate physical activity in animal models induces positive physiological and cognitive effects by altering transcriptional profiles through epigenetic modifications (Kaliman et al., 2011). A meta-analysis of 13 studies has confirmed the impact of physical activities on positive cognitive effects and their protective role against AD (Jia et al., 2019).

A literature review has indicated that physical activity, ranging from mild to moderate intensity, can decrease the risk of dementia and AD. This is in line with our findings since the studied population was healthy. Furthermore, potential benefits were observed for patients already diagnosed with AD (Cass, 2017), and physical activities may provide benefits for the treatment or prevention of AD.

The capacity of exercise to induce alterations in genes and proteins through epigenomic manifestations that regulate synaptic and cognitive plasticity is fundamental in controlling neurological disturbances (Fernandes et al., 2017). Shireby et al. (2022) conducted a DNA methylation profiling in cortical regions associated with AD neuropathology. They identified differentially methylated positions associated with AD, utilizing measures of neuropathology to identify epigenetic signatures of AD, underscoring the importance of characterizing the disease (Shireby et al., 2022). Despite several studies demonstrating the relationship between exercise and AD, our results showcased epigenetic modulation in crucial genes to prevent AD in healthy patients.

Epigenetic biomarkers could potentially serve as a helpful tool in patient screening in the future, as changes in methylation in specific genes have already been identified as biomarkers in AD using peripheral blood leukocytes (Hou et al., 2013). Additionally, an association has been observed between diet, exercise, and improved health in AD patients related to epigenetic modifications (Athanasopoulos et al., 2016).

After 14 weeks of exercise, modulation of gene methylation correlated with AD (*ABCA7, ACE, ADAM10, BDNF, CLU, INPP5D, IQCK, PTK2B, SMARCA4, SORL1, SPI1, WWOX*) was evident. Studies have demonstrated that methylation in ABCA7 is linked to AD, as decreased *ABCA7* levels are associated with the condition. Hence, increasing functional *ABCA7* expression levels could be a valuable therapeutic approach (De Roeck et al., 2019). Our results indicated hypomethylation in *ABCA7* after exercise, suggesting a potential preventive role against AD. The *WWOX* gene's loss of function has been associated with various central nervous system pathologies. Dysfunctional *WWOX* induces tau and Aβ aggregation, leading to apoptosis. Additionally, *WWOX*'s role in brain development and pathology, such as AD, is well-documented (Stevenson-Hoare et al., 2023). This gene plays a critical role in inflammation and is associated with AD progression. Therefore, dysfunctional *WWOX*, such as loss or excessive *WWOX*, contributes to neurodegenerative diseases (AD, Parkinson's disease, among others) (Hsu et al., 2021). Hence, in our results, this gene predominantly appeared hypomethylated at most sites after exercise, although some sites exhibited hypermethylation.

The primary strength of the current study was to analyze methylation patterns using a chip capable of examining 850 thousand CpG sites, providing a comprehensive assessment of the alterations that combined physical training may induce in the methylation patterns of genes related to AD. No methods were employed to assess dietary patterns, and it is known that diet constitutes important environmental factors that can modify methylation patterns. However, participants were instructed not to make lifestyle changes during their involvement in the study, in addition to completing a 24-h dietary recall at the pre-collection stage and adhering to the same record during the post-intervention collection.

Finally, it is plausible that alterations in the methylation profile might not solely underlie the mechanisms inducing adaptations in physical performance but could represent an additional adaptation to the exercise program in a more established trained condition. In future studies, determining the gene expression associated with hypomethylated and hypermethylated sites will be crucial.

5. Conclusion

The combined physical training conducted over a 14-week period induced alterations in the methylation patterns of gene sites related to the development of AD in middle-aged and older women unaffected by neurodegenerative diseases. This epigenetic evidence highlights that physical exercise for 14 weeks was adequate to cause varying levels of methylation in genes responsible for the development of AD in women aged 50 to 70 years, acting as a protective factor for the development of the disease. Moreover, it facilitated improvements in functional capacity, physical performance, and blood pressure among these women.

Funders

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CRediT authorship contribution statement

Guilherme da Silva Rodrigues: Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Natália Yumi Noronha:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **Isabella Harumi Yonehara Noma:** Writing – review & editing, Writing – original draft, Methodology,

Table 2
Differentially Methylated Sites Across the 14 Weeks of Training.

Genes	CpG	CHR	Gene Region	Type	rs	β1 I x III	p-value
ABCA7	cg02817925	19	Body	island	rs150971192	0.009	0.020
ABCA7	cg17993394	19	Body	shelf	rs59237564	−0.025	0.008
ABCA7	cg26970077	19	Body	shore	rs542467691	−0.038	0.015
ABCA7	cg12064401	19	Body	island	rs576278275	−0.024	0.017
ABCA7	cg15681366	19	Body	shelf	rs561581862	−0.017	0.027
ABCA7	cg18846541	19	ExonBnd	shore	rs149365975	−0.017	0.030
ABCA7	cg26263477	19	TSS1500	island	rs184510773	−0.008	0.042
ACE	cg24189328	17	TSS1500	shore		−0.029	0.006
ADAM10	cg16123305	15	Body	opensea	rs113109705	−0.016	0.035
BDNF	cg12021170	11	TSS1500	opensea	rs184293959	−0.029	0.039
BDNF	cg08760147	11	Body	opensea	rs184548784	−0.022	0.042
BDNF	cg11241206	11	TSS1500	shore	rs56195957	0.01	0.046
CLU	cg08594681	8	1stExon	shelf		0.018	0.030
INPP5D	cg04975586	2	ExonBnd	opensea	rs201252871	−0.021	0.016
INPP5D	cg18097144	2	TSS1500	shore	rs188579739	−0.028	0.015
INPP5D	cg16151789	2	Body	opensea		−0.027	0.033
INPP5D	cg06831850	2	Body	opensea	rs574989226	−0.017	0.038
IQCK	cg17440572	16	Body	opensea	rs114245109	−0.024	0.014
IQCK	cg17595464	16	5'UTR	island	rs530998132	−0.007	0.017
IQCK	cg10032375	16	Body	opensea	rs540939870	−0.019	0.040
PTK2B	cg12242935	8	5'UTR	opensea	rs191315642	−0.014	0.045
SMARCA4	cg03921155	19	Body	opensea	rs568094122	−0.03	0.044
SORL1	cg25236143	11	Body	opensea	rs544790047	−0.027	0.011
SORL1	cg04707711	11	Body	opensea	rs569947455	0.021	0.038
SPI1	cg24234141	11	3'UTR	shore	rs531943594	0.024	0.046
SPI1	cg01539849	11	3'UTR	island		0.011	0.045
WWOX	cg05475027	16	Body	opensea	rs550214854	−0.022	0.018
WWOX	cg03648054	16	Body	opensea	rs527659014	−0.021	0.019
WWOX	cg03295251	16	Body	opensea	rs567996965	0.012	0.020
WWOX	cg02236679	16	Body	opensea	rs563057433	−0.026	0.022
WWOX	cg02546607	16	Body	opensea	rs373052053	0.024	0.022
WWOX	cg23054284	16	Body	opensea	rs148661660	−0.025	0.036
WWOX	cg14789524	16	Body	opensea	rs56073608	−0.027	0.037
WWOX	cg26229208	16	TSS1500	shore	rs141439937	−0.018	0.042
WWOX	cg02317358	16	Body	opensea	rs202052438	−0.019	0.045
WWOX	cg26581964	16	Body	opensea	rs145917497	−0.019	0.049

Note – CpG: Cytosine-phosphate-Guanine; CHR: Chromosome; TSS1500: Transcription Start Site 1500; TS200: Transcription Start Site 200; 5'UTR: 5' Untranslated Region; 3'UTR: 3' Unstranslated Region.

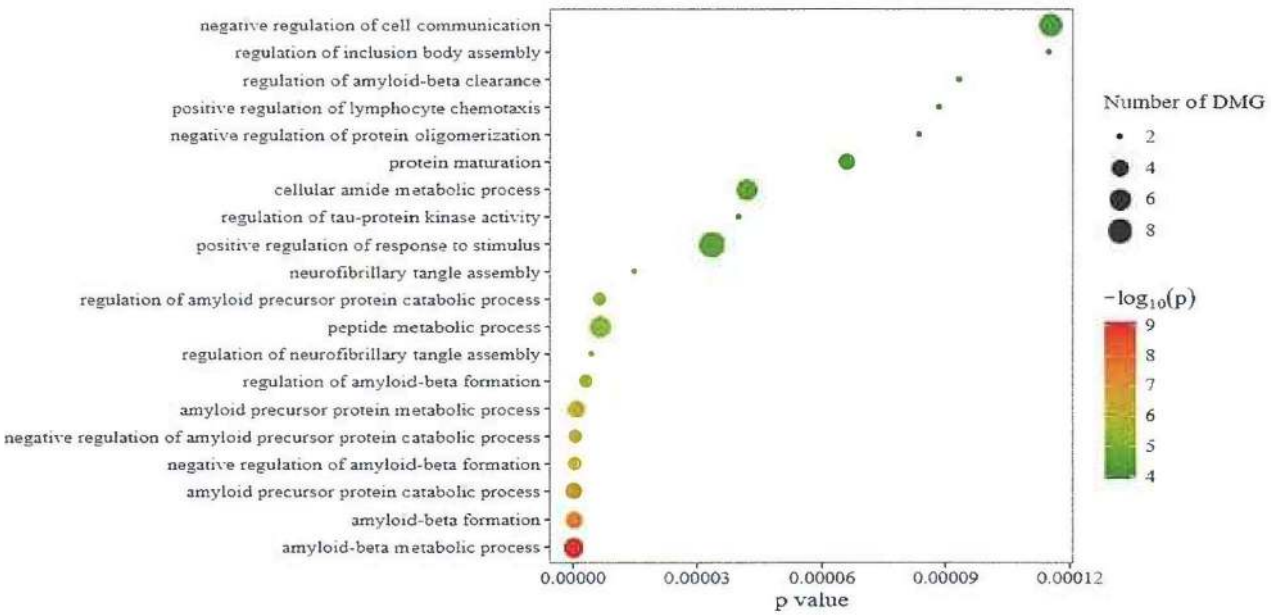

Fig. 5. Gene Ontology enrichment of probes that are differentially methylated after the 14 weeks of combined training.
Note - DMG: Number of Genes Differentially Methylated in the respective pathways.

Investigation, Formal analysis, Conceptualization. **João Gabriel Ribeiro de Lima:** Writing – review & editing, Writing – original draft, Validation, Conceptualization. **Andressa Crystine da Silva Sobrinho:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Marcela Augusta de Souza Pinhel:** Writing – review & editing, Writing – original draft, Validation, Conceptualization. **Mariana Luciano de Almeida:** Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Lígia Moriguchi Watanabe:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Conceptualization. **Carla Barbosa Nonino:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Carlos Roberto Bueno Júnior:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Data availability

The DNA methylation datasets presented in this study can be found online: https://doi.org/10.6084/m9.figshare.21763535. Other data are available upon request to the corresponding author.

References

Amati, L., Marzulli, G., Martulli, M., Chiloiro, M., Jirillo, E., 2010. Effects of a hypocaloric diet on obesity biomarkers: prevention of low-grade inflammation since childhood. Curr. Pharm. Des. 16 (7), 893–897.

Athanasopoulos, D., Karagiannis, G., Tsolaki, M., 2016 Sep. Recent findings in Alzheimer disease and nutrition focusing on epigenetics. Adv. Nutr. 7 (5), 917–927.

Barrón-Cabrera, E., Ramos-Lopez, O., González-Becerra, K., Riezu-Boj, J.I., Milagro, F.I., Martínez-López, E., et al., 2019. Epigenetic modifications as outcomes of exercise interventions related to specific metabolic alterations: a systematic review. Lifestyle Genom. 12 (1–6), 25–44.

Borg, Gunnar A.V., Noble, Bruce J., January 1974. Perceived exertion. Exerc. Sport Sci. Rev. 2 (1), 131–154.

Boyne, D.J., King, W.D., Brenner, D.R., McIntyre, J.B., Courneya, K.S., Friedenreich, C. M., 2018 Jun 28. Aerobic exercise and DNA methylation in postmenopausal women: an ancillary analysis of the Alberta Physical Activity and Breast Cancer Prevention (ALPHA) trial. PloS One 13 (6), e0198641.

Brandao, C.F.C., Nonino, C.B., de Carvalho, F.G., Nicoletti, C.F., Noronha, N.Y., San Martin, R., et al., 2020 Jan 16. The effects of short-term combined exercise training on telomere length in obese women: a prospective, interventional study. Sports Med - Open. 6 (1), 5.

Buchman, A.S., Boyle, P.A., Yu, L., Shah, R.C., Wilson, R.S., Bennett, D.A., 2012 Apr 24. Total daily physical activity and the risk of AD and cognitive decline in older adults. Neurology 78 (17), 1323–1329.

Bulla, A., De Witt, B., Ammerlaan, W., Betsou, F., Lescuyer, P., 2016 Feb. Blood DNA yield but not integrity or methylation is impacted after long-term storage. Biopreservation Biobanking. 14 (1), 29–38.

Cass, S.P., 2017 Jan. Alzheimer's disease and exercise: a literature review. Curr. Sports Med. Rep. 16 (1), 19–22.

Correia, I.M., da Silva, Rodrigues G., Noronha, N.Y., Watanabe, L.M., Luciano de Almeida, M., Sobrinho, A.C. da S., et al., 2023 Apr 10. Older postmenopausal women with lower lean mass have hypermethylated sites in the PI3K-Akt pathway. Front Physiol. 14, 1150821.

Crous-Bou, M., Minguillón, C., Gramunt, N., Molinuevo, J.L., 2017 Sep 12. Alzheimer's disease prevention: from risk factors to early intervention. Alzheimers Res. Ther. 9 (1), 71.

De Roeck, A., Van Broeckhoven, C., Sleegers, K., 2019 Aug. The role of ABCA7 in Alzheimer's disease: evidence from genomics, transcriptomics and methylomics. Acta Neuropathol (Berl). 138 (2), 201–220.

Duthey, Béatrice, 2013. Background paper 6.11: Alzheimer disease and other dementias. A public health approach to innovation, 6, pp. 1–74.

El-Hayek, Y.H., Wiley, R.E., Khoury, C.P., Daya, R.P., Ballard, C., Evans, A.R., et al., 2019 Jul 23. Tip of the iceberg: assessing the global socioeconomic costs of Alzheimer's disease and related dementias and strategic implications for stakeholders. J. Alzheimers Dis. 70 (2), 323–341.

Fernandes, J., Arida, R.M., Gomez-Pinilla, F., 2017 Sep. Physical exercise as an epigenetic modulator of brain plasticity and cognition. Neurosci. Biobehav. Rev. 80, 443–456.

Florindo, A.A., Latorre, M., do RD de O., 2003 Jun. Validation and reliability of the Baecke questionnaire for the evaluation of habitual physical activity in adult men. Rev. Bras. Med. Esporte 9 (3), 129–135.

Foster, P.P., Rosenblatt, K.P., Kuljiš, R.O., 2011 May 6. Exercise-induced cognitive plasticity, implications for mild cognitive impairment and Alzheimer's disease. Front. Neurol. 2, 28.

Fuso, A., 2013 Mar 1. The 'golden age' of DNA methylation in neurodegenerative diseases. Clin. Chem. Lab. Med. 51 (3), 523–534.

Hamer, M., Chida, Y., 2009 Jan. Physical activity and risk of neurodegenerative disease: a systematic review of prospective evidence. Psychol. Med. 39 (1), 3–11.

Higgins-Chen, A.T., Thrush, K.L., Levine, M.E., 2021 Aug. Aging biomarkers and the brain. Semin. Cell Dev. Biol. 116, 180–193.

Hou, Y., Chen, H., He, Q., Jiang, W., Luo, T., Duan, J., et al., 2013 Apr. Changes in methylation patterns of multiple genes from peripheral blood leucocytes of Alzheimer's disease patients. Acta Neuropsychiatr. 25 (2), 66–76.

Hsu, C.Y., Lee, K.T., Sun, T.Y., Sze, C.I., Huang, S.S., Hsu, L.J., et al., 2021 Jul 14. WWOX and its binding proteins in neurodegeneration. Cells 10 (7), 1781.

Izquierdo, M., Häkkinen, K., Gonzalez-Badillo, J., Ibáñez, J., Gorostiaga, E., 2002 Jul 1. Effects of long-term training specificity on maximal strength and power of the upper and lower extremities in athletes from different sports. Eur. J. Appl. Physiol. 87 (3), 264–271.

Jakovljevic, M., Westerman, R., Sharma, T., Lamnisos, D., 2021. Aging and Global Health. In: Kickbusch, I., Ganten, D., Moeti, M. (Eds.), Handbook of Global Health. Springer International Publishing, Cham.

Jia, R. Xia, Liang, J. Hong, Xu, Y., Wang, Y. Quan, 2019 Dec. Effects of physical activity and exercise on the cognitive function of patients with Alzheimer disease: a meta-analysis. BMC Geriatr. 19 (1), 181.

Kaliman, P., Párrizas, M., Lalanza, J.F., Camins, A., Escorihuela, R.M., Pallàs, M., 2011 Sep. Neurophysiological and epigenetic effects of physical exercise on the aging process. Ageing Res. Rev. 10 (4), 475–486.

Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y., Morishima, K., 2017 Jan 4. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res. 45 (D1), D353–D361.

Leandro MPG, Moura JLS de, Barros GWP, Silva Filho AP da, Farias AC de O, Carvalho PRC. Effect of the aerobic component of combined training on the blood pressure of hypertensive elderly women. Rev. Bras. Med. Esporte 2019 Dec;25(6):469–73.

Li, A., Barber, R.F., 2019 Feb 1. Multiple testing with the structure-adaptive Benjamini–Hochberg algorithm. J. R. Stat. Soc. Ser. B Stat Methodol. 81 (1), 45–74.

Li, M., Zou, D., Li, Z., Gao, R., Sang, J., Zhang, Y., et al., 2019 Jan 8. EWAS atlas: a curated knowledgebase of epigenome-wide association studies. Nucleic Acids Res. 47 (D1), D983–8.

Liang, J. Hong, Lu, L., Li, J. Yu, Qu, X. Yuan, Li, J., Qian, S., et al., 2020 Nov. Contributions of modifiable risk factors to dementia incidence: a Bayesian network analysis. J. Am. Med. Dir. Assoc. 21 (11), 1592–1599.e13.

Livingston, G., Huntley, J., Sommerlad, A., Ames, D., Ballard, C., Banerjee, S., et al., 2020 Aug. Dementia prevention, intervention, and care: 2020 report of the lancet commission. Lancet 396 (10248), 413–446.

Nicolia, V., Lucarelli, M., Fuso, A., 2015 Aug. Environment, epigenetics and neurodegeneration: focus on nutrition in Alzheimer's disease. Exp. Gerontol. 68, 8–12.

Noronha, N.Y., da Silva, Rodrigues G., de Souza Pinhel, M.A., Cazier, J.B., Watanabe, L. M., Menezes, A.N., et al., 2022 May 6. Sample preparation to bioinformatics analysis of DNA methylation: association strategy for obesity and related trait studies. J. Vis. Exp. 183, 62598.

Northey, J.M., Cherbuin, N., Pumpa, K.L., Smee, D.J., Rattray, B., 2018 Feb. Exercise interventions for cognitive function in adults older than 50: a systematic review with meta-analysis. Br. J. Sports Med. 52 (3), 154–160.

Rikli, R.E., Jones, C.J., 1999 Apr. Development and validation of a functional fitness test for community-residing older adults. J. Aging Phys. Act. 7 (2), 129–161.

Rodrigues, J.A.L., Santos, B.C., Medeiros, L.H., Gonçalves, T.C.P., Júnior, C.R.B., 2021 Jul. Effects of different periodization strategies of combined aerobic and strength training on heart rate variability in older women. J. Strength Cond. Res. 35 (7), 2033–2039.

Rodrigues, G. da S., Rodrigues, K.P., de Almeida, M.L., Sobrinho, A.C. da S., Noronha, N. Y., CJR, Benjamim, et al., 2023 Feb 2. Comparing fourteen weeks of multicomponent training versus combined training in physically inactive older women: a randomized trial. Int. J. Environ. Res. Public Health 20 (3), 2699.

Ruangthai, R., Phoemsapthawee, J., 2019 May. Combined exercise training improves blood pressure and antioxidant capacity in elderly individuals with hypertension. J. Exerc. Sci. Fit. 17 (2), 67–76.

Shireby, G., Dempster, E.L., Policicchio, S., Smith, R.G., Pishva, E., Chioza, B., et al., 2022 Sep 24. DNA methylation signatures of Alzheimer's disease neuropathology in the cortex are primarily driven by variation in non-neuronal cell-types. Nat. Commun. 13 (1), 5620.

Shokrzadeh, M., Mohammadpour, A., 2018. Evaluation of a modified salt-out method for DNA extraction from whole blood lymphocytes: a simple and economical method for gene polymorphism. Pharm Biomed Res 4 (2), 28–32.

da Silva, Rodrigues G., Noma, I.H.Y., Noronha, N.Y., Watanabe, L.M., da Silva Sobrinho, A.C., de Lima, J.G.R., et al., 2023 Jul 19. Eight weeks of physical training decreases 2 years of DNA methylation age of sedentary women. Res. Q. Exerc. Sport 1–11.

da Silva, Rodrigues G., Noronha, N.Y., Almeida, M.L., Sobrinho, A.C. da S., Watanabe, L. M., Pinhel, M.A. de S., et al., 2023 Mar 1. Exercise training modifies the whole blood DNA methylation profile in middle-aged and older women. J. Appl. Physiol. 134 (3), 610–621.

Stevenson-Hoare, J., Heslegrave, A., Leonenko, G., Fathalla, D., Bellou, E., Luckcuck, L., et al., 2023 Feb 13. Plasma biomarkers and genetics in the diagnosis and prediction of Alzheimer's disease. Brain 146 (2), 690–699.

Tian, Y., Morris, T.J., Webster, A.P., Yang, Z., Beck, S., Feber, A., et al., 2017 Dec 15. ChAMP: updated methylation analysis pipeline for Illumina BeadChips. Valencia A, editor. Bioinformatics 33 (24), 3982–3984.

Verheijen, J., Sleegers, K., 2018 Jun. Understanding Alzheimer disease at the Interface between genetics and transcriptomics. Trends Genet. 34 (6), 434–447.
Wang, X., Li, F., Zhu, H., Jiang, Z., Niu, G., Gao, Q., 2022. A hierarchical Bayesian latent class model for the diagnostic performance of mini-mental state examination and Montreal cognitive assessment in screening mild cognitive impairment due to Alzheimer's disease. J. Prev Alzheimers Dis. 9 (4), 589–600.
Wu, Y.L., Lin, Z.J., Li, C.C., Lin, X., Shan, S.K., Guo, B., et al., 2023 Mar 2. Epigenetic regulation in metabolic diseases: mechanisms and advances in clinical study. Signal Transduct. Target. Ther. 8 (1), 98.

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14-weeks combined exercise epigenetically modulated 118 genes of menopausal women with prediabetes

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Background: Pre-diabetes precedes Diabetes Mellitus (DM) disease and is a critical period for hyperglycemia treatment, especially for menopausal women, considering all metabolic alterations due to hormonal changes. Recently, the literature has demonstrated the role of physical exercise in epigenetic reprogramming to modulate the gene expression patterns of metabolic conditions, such as hyperglycemia, and prevent DM development. In the present study, we hypothesized that physical exercise training could modify the epigenetic patterns of women with poor glycemic control.

Methods: 48 post-menopause women aged 60.3 ± 4.5 years were divided according to their fasting blood glucose levels into two groups: Prediabetes Group, PG (n=24), and Normal Glucose Group, NGG (n=24). All participants performed 14 weeks of physical exercise three times a week. The Infinium

Methylation EPIC BeadChip measured the participants' Different Methylated Regions (DMRs).

Results: Before the intervention, the PG group had 12 DMRs compared to NGG. After the intervention, five DMRs remained different. Interestingly, when comparing the PG group before and after training, 118 DMRs were found. The enrichment analysis revealed that the genes were related to different biological functions such as energy metabolism, cell differentiation, and tumor suppression.

Conclusion: Physical exercise is a relevant alternative in treating hyperglycemia and preventing DM in post-menopause women with poor glycemic control.

KEYWORDS

physical exercise, DNA methylation, combined physical exercise, epigenomics, older women

Introduction

Diabetes Mellitus (DM) is characterized by chronic hyperglycemia resulting from deficiencies in insulin secretion, insulin action, or both. Type 2 Diabetes (T2D) results from a progressive loss of pancreatic islet function followed by long-term insulin resistance and elevated insulin secretion (1) Prediabetes is an intermediate condition that precedes TD2. These conditions could occur during menopause and are related to sex hormone levels and aging changes. Postmenopausal women tend to have fluctuations in the hormone's estrogen and progesterone, these changes affect how cells respond to insulin. The process of hormonal changes after menopause causes fluctuations in blood sugar levels in women (2). During menopause, women could also experience predictable symptoms, such as increased total cholesterol and triglycerides (TG), decreased high-density lipoprotein cholesterol (HDL-c), increased android fat distribution, and impaired vascular function (2). Preventive action to manage hyperglycemia should be appropriately implemented in the prediabetes stage since glycemic control is the common factor determining complications from DM, especially in menopause (2, 3).

In this context, studies have emphasized the importance of epigenetic regulation considering its role in activating or deactivating genes that impact metabolic outcomes (4). Studies using animal models demonstrated epigenetic variations associated with the susceptibility to the development of metabolic diseases, such as DM (5).

Another factor that could influence hyperglycemia and metabolic alterations is exposure to heavy metals in the environment. Some metals, such as lead (Pb^{208}), can increase oxidative stress-inducing metabolic changes related to DM (6). Correlations between heavy metals and glycosylated hemoglobin (HbA1c), an indicator of hyperglycemia and long-term alterations in glucose metabolism, have been found in cross-sectional studies in France and Saudi Arabia (7).

Exercise training can promote health and improve metabolism through different mechanisms. The impact of exercise training on epigenetic programming has been generating exciting results (8). Yahaya (9) demonstrated that myocyte enhancer factor 2 (*MEF2)* migrates to the nucleus during exercise after being phosphorylated, interacting with peroxisome proliferative active receptor gamma coactivator 1A *(PPARγC1A)*, and histone acetyltransferases (HATs).

Physical exercise can increase the production of reactive oxygen species, both in skeletal muscle and in immune cells, leading to the development of oxidative stress, lipid, protein, and DNA modifications (10). Therefore, physical exercise can cause changes in DNA methylation (9, 11). Studies show that the response to physical exercise depends on genetic code and on the epigenetic signature, which can cause hypo or hypermethylation, of genes involved in muscle metabolism (9, 12). One study showed that chronic physical exercise caused hypomethylation of the promoter region of the nuclear receptor factor, fatty acid transporter (SLC27A4) and GLUT4 in patients with obesity and diabetes (13). Many studies involving physical exercise and DNA methylation, as Sailani et al. (14), showed that DNA methylation was lower in 714 promoter regions in physically active men compared to inactive men. Furthermore, it suggests that physical exercise is associated with the DNA methylation pattern increasing insulin sensitivity (14).

These modifications resulted in the acetylation of type 4 glucose transporter (*GLUT4)*, improving the expression of the gene and insulin tolerance in the muscles (15). Exercise training can also ameliorate the effects of heavy metal accumulation by increasing

urinary elimination (16). Due to all these benefits, exercise training could prevent hyperglycemia and its complications. In the present study, we hypothesized that physical exercise training could modify the epigenetic patterns of women with poor glycemic control. Thus, we aimed to analyze if the 14-week combined physical exercise intervention could modify epigenetic variables and improve the glucose serum levels.

Materials and methods

Ethical aspects, study design, and participants

The present study was approved by the Ethics Committee for Research with Humans of the School of Physical Education and Sport of Ribeirão Preto, University of São Paulo (EEFERP-USP). The registration number is CAAE: 79582817.0.0000.5656. The study was also registered in the Brazilian Registry of Clinical Trials under RBR-3g38dx. Participants were recruited in the Physical Education Program for the Elderly at EEFERP – USP. The inclusion criteria were women between 50 and 70 years with a medical clearance certificate to perform physical exercise and who were sedentary at the baseline. The exclusion criteria included diagnostic limitations to physical training or physical test, current smokers, caffeine consumption in the last 24 hours before any blood draw, and more than 25% combined training absence.

Forty-eight post-menopause women participated in the study. They were divided into two groups according to the fasting blood glucose levels determined by the American Diabetes Society (2020): Pre-diabetes Group (PG), composed of 24 women with a fasting blood glucose level higher than 100mg/dL and the Normal Glucose Group (NGG), composed of 24 women with a fasting blood glucose level lower than 100mg/dL (9).

Assessments

The anthropometric evaluation was assessed before and after 14 weeks of combined training. The height was measured using a vertical stadiometer - Balmak - EST-223 and weight using the G-Tech digital scale - Balgl200, G-Tech. Subsequently, the body mass index was calculated using the formula (weight/(height*height)) (17). Waist and hip circumferences were measured following the recommendations of Malachias et al. (17). Participants' systolic and diastolic blood pressure was measured using an Automatic Digital Upper Arm Blood Pressure Monitor Meter (SBH, 2010 - OMRON®, model HEM-7113) (17).

The biochemical analyses included uric acid, total cholesterol, high-density lipoproteins (HDL-C), low-density lipoproteins cholesterol (LDL-C), triglycerides (TG), blood glucose, and insulin. We also calculated the HORMAR IR (Blood (mmol) x Insulin (Ul/mL) ÷ 22. 5).

To investigate the physical benefits of 14 weeks of combined training, the elbow flexion and extension, sitting and standing up from a chair, agility dynamics, and the six-minute walk test was adopted. Also, the 6MWT (m) methodology was used to measure aerobic capacity (18).

We also determined the total serum concentration of metals (Hg202, Al27, As75, Cd111, Pb208, Ni60, Li7) through inductively coupled plasma mass spectrometry (ICP-MS), following the protocol described by Batista et al. (19).

Combined training

Combined training was performed for 14 weeks, three times a week, lasting 60 minutes. The training was divided into strength exercises (30 minutes) and aerobic training (treadmill or bicycle, with a total duration of 30 minutes) (14).

The training periodization followed the first two weeks. The participants performed two sets of 15 to 17 maximum repetitions and 50% of the aerobic training in the reserve heart rate. The participants started to do 10 to 12 maximum repetitions in the remaining weeks, and aerobic training became 70% of the reserve heart rate. The participants-maintained values between three and six, a score presented on Borg's subjective effort scale. The score we adopted on a scale from 0 to 10 represents moderate intensity. For the selection of exercises for strength training, the need to train the main muscles was considered, totaling five exercises for the muscles of the upper limbs and three exercises for the muscles of the lower limbs. Our group previously detailed the protocol (14).

Large-scale DNA methylation array (Infinium-EPIC bead chips)

The DNA methylation was analyzed at the baseline and after the 14 weeks of training. Salting-out technique (20) was used to extract DNA from leukocytes. Biodrop spectrophotometry was used to assess DNA quality and concentration using. 1% agarose gel test was used to guarantee genomic DNA integrity. The run time was 90 minutes at 80W (21).

Following the manufacturer's recommendations, the bisulfite method was applied using the DNA EZ methylation kit (Zymo Research, CA, USA). Illumina BeadChip EPIC Infinium Methylation BeadChip 850k was used for the methylation experiment. All recommendations for the Infinium® HD Assay Methylation Protocol Guide were followed (22).

Bioinformatics analysis

The raw data files were exported from iScan. A Samplesheet file was created to associate the collected information with

raw data (23). The RStudio software (version 4.0.2) was used to process the methylation data. The data was organized as the participants' codes, age, anthropometric, physical, biochemical, and metal information. The Sentrix Position column informs which row and column each sample was located in. The Sentrix_ID column provides the corresponding code of the BeadChip (23). The Bioconductor ChAMP data package was used for data normalization through the MyNorm function (24).

Differentially methylated regions (DMRs) were analyzed using the champ.DMR() function to compare PG vs. NGG groups. The bumphunter method was used to aggregate all probes into small groups. The random permutation method was applied to estimate DMRs with $p < 0.05$. Based on text mining and literature review, all DMRs were functionally annotated (24).

After identifying the DMRs inter and intra-group, the singular value decomposition (SVD) analysis method was used to evaluate the number and nature of significant components, correlating with various components of interest (24). Methylation data were normalized *via* minfi's ssNoob (25). DNAmAge was calculated using the Methylclock package through the DNAmAge function (26). We also investigated the epigenetic clock of participants from both groups for intrinsic epigenetic age acceleration (IEAA) and extrinsic epigenetic age acceleration (EEAA). The epigenetic clock is a biomarker for age-related epigenetic changes as well as disease-specific changes (27). This clock has been recently developed by using specific DNA methylation sites to describe biological age at the level of the cell, tissue, and organ (28). The multi-tissue algorithm by Horvath generates age prediction, which highly correlates with chronological age above r=0.9 (29). In this study, DNAm Age was calculated using Horvath's epigenetic clock algorithm using 353 CpGs (3).

In SVD analysis, the champ algorithm first does SVD deconvolution on the beta dataset. Then, the Random Theory Matrix method in the "isva" package is used to calculate the numbers of the latent variable and the top components of the SVD result. This analysis is comparable to a Principal Components Analysis (24)

Statistical analysis

Two researchers verified the resulting data to maintain the values' reliability and accuracy. The Statistica 7.0 software (StatSoft Statistica®) was used to perform statistical comparisons. The Shapiro-Wilk test tested the normality. The Levene test assessed the equality of variance between groups. Then, repeated-measures ANOVA was performed, considering $p < 0.05$. Fisher's *post hoc* compared all means and controlled the error rate at the significance level. Then, a Spearman correlation was performed for the non-parametric data and a Pearson correlation for the parametric data to identify possible correlations. The Student's t-test compared means in the descriptive analysis.

Results

Effects of combined training in blood pressure, biochemical variables, and improvement of physical skills

The mean age of participants in the PG was 61.3 ± 5.2 years, while in the NGG was 59.9 ± 4.8 years (Table 1). The 14 weeks of combined training had an effect of time in systolic blood pressure ($F=24.304$; $p<0.001$), diastolic blood pressure ($F=6.180$; $p=0.016$), total cholesterol ($F=7.191$); $p=0.010$), HDL-C ($F=8.168$; $p=0.006$), TG ($F=11.176$; $p=0.001$), and glucose ($F=25.281$; $p<0.001$). No undesirable effects were noted, but we did not observe group-time effect or group-time interaction (Table 1).

We observed the effect of time on elbow flexion and extension ($F=17.028$; $p<0.001$) for upper limb strength; sitting and standing up from a chair ($F=20.264$; $p<0.001$) for lower limb; agility test ($F=51.248$; $p<0.001$); and six-minute walk test ($F=23.872$; $p<0.001$) for aerobic fitness. The effect of time in both groups demonstrated an improvement in physical skills after 14 weeks of combined training. No group-time effect or group-time interaction was observed (Table 2).

Combined training decreases metals in both groups

Analyzing metal levels and combined training together, we observed the effect of time decreasing mercury (Hg) ($F=5.028$; $p=0.030$) and lead (Pb) ($F=12.100$; $p=0.001$) in both groups. No effect or group-time interaction was observed (Table 3). There was a positive correlation between the arterial blood pressure and Pb^{208} in the PG_{pre} (PAS: $r=0.389$, $p=0.003$; PAD: $r=0.337$, $p=0.010$). After the intervention, the correlation was not significant. The epigenetic clock was not different between groups and did not change after the 14-week physical intervention (Figure 1).

Differentially Methylated Regions after the 14 weeks of exercise compared with groups

Before the physical exercise training, we found 12 DMRs between the PG_{pre} and the NGG_{pre} (Table 4). After the 14 weeks of exercise, five regions remained Differentially Methylated between the two groups (Table 5).

After the 14-week of exercise, we found 118 DMRs in the PG_{post} compared to the PG_{pre} (Table 6), and we performed a

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TABLE 1 Mean ± standard deviation of anthropometric, blood pressure, and biochemical variables pre and post-14 weeks of combined training (PG: n = 24; NGG: n = 24).

Variables	Group	PRE	POST	p (group)	p (time)	p (group x time)
Age (years)	PG	61.3±5.2		0.184		
	NGG	59.9±4.8				
Anthropometrics						
Stature (m)	PG	1.6±0.1		0.199		
	NGG	1.6±0.1				
BM (kg)	PG	74.2±12.3	74.2±11.6	0.229	0.858	0.848
	NGG	70.3±8.2	70.2±8.0			
BMI (kg/m2)	PG	30.7±4.2	30.1±4.2	0.223	0.188	0.892
	NGG	28.2±3.2	27.8±2.8			
WC (cm)	PG	95.2±11.1	98.3±12.6	0.278	0.728	0.081
	NGG	94.1±10.3	92.2±9.4			
HC (cm)	PG	108.5±8.8	106.2±10.0	0.166	0.386	0.139
	NGG	103.6±7.3	104.2±6.2			
Blood pressure						
SBP (mmHg)	PG	140.9±20.1	126.7±15.1	<0.001#	<0.001*	0.192
	NGG	123.7±9.6	115.5±9.0			
DBP (mmHg)	PG	82.0±12.3	77.7±8.5	0.003#	0.016*	0.48
	NGG	74.0±6.7	71.6±5.4			
Biochemistries						
Uric acid (mg/dL)	PG	4.3±1.2	4.5±1.3	0.234	0.187	0.928
	NGG	4.0±1,1	4.1±1.0			
Cholesterol (mg/dL)	PG	220.2±37.0	207.2±38.1	0.614	0.010*	0.909
	NGG	212.8±33.1	201.9±29.1			
Glucose (mg/dL)	PG	115.1±24.0	104.9±12.0	<0.001#	0.23	0.05
	NGG	92.7±4.6	95.4±7.4			
HDL (mg/dL)	PG	50.7±9.4	48.9±8.9	0.026#	0.006*	0.114
	NGG	59.9±14.1	53.3±11.7			
LDL (mg/dL)	PG	135.6±37.1	131.1±37.3	0.299	0.198	0.848
	NGG	127.4±30.6	119.9±40.8			
Triglycerides (mg/dL)	PG	152.9±49.1	131.6±36.0	0.073	0.001*	0.654
	NGG	128.3±50.5	112.1±38.0			
Insulin UI/ml	PG	14.1±11.9	14.9±10.0	0.052	0.108	0.694
	NGG	8.8±4.9	10.5±5.1			
HOMAR IR	PG	71.5±63.8	71.7±54.3	0.018#	0.316	0.338
	NGG	36.4±19.6	44.7±22.7			

BMI, body mass index; WC, waist circumference; HC, hip circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoproteins LDL, low-density lipoproteins; CM, centimeters; mmHg, millimeters of mercury; kg, kilogram; m, meters; PG, prediabetes group; NGG, normal glucose group. *statistical difference time; #statistical difference group; $p < 0.05$ for pre versus post (repeated measures two-way ANOVA was used).

Singular Value Decomposition Analysis (SVD) to verify if the covariables of interest could explain the DNA methylation patterns (Figure 2).

We also performed a Linear Regression of significant traits (Pb and glucose) and the DNA methylation targets of the PG group. We found three DMRs for Pb and 25 DMRs for glucose (Figure 3). *BRCA1* was hypomethylated after a 14-week physical exercise intervention and was associated with glucose in the PG group. There was also an interesting overlap between the DMRs related to Pb and glucose enriched in the gene *SLFN12*. Notably, this gene region was differently methylated in the PG_{pre} group than NGG_{pre} and associated with Pb in the PG. After the intervention, a decrease in Pb was observed, and the methylation patterns of *SLFN12* in PG_{post} became similar to NGG_{post}.,,

TABLE 2 Mean ± standard deviation of the PRE AND POST motor variables 14 weeks of combined training (PG: n 24; NGG: n 24).

Variables	Group	PRE	POST	p (group)	p (time)	p (group x time)
EFE (repetitions)	PG	18.5 ± 4.3	21.8 ± 4.4	0.705	<0.001*	0.929
	NGG	18.2 ± 3.7	21.3 ± 3.4			
SAS (repetitions)	PG	14.5 ± 4.5	18.4 ± 6.1	0.363	<0.001*	0.880
	NGG	13.7 ± 3.5	17.3 ± 4.2			
Agility (sec)	PG	26.6 ± 3.2	23.4 ± 3.6	0.253	<0.001*	0.285
	NGG	25.9 ± 2.3	21.7 ± 2.7			
6MWT (m)	PG	540.3 ± 67.1	572.4 ± 60.3	0.905	<0.001*	0.257
	NGG	532.3 ± 38.8	584.2 ± 60.7			

EFE, elbow flexion and extension; SAS, sit and stand; sec, seconds; m, meters; PG, prediabetes group; NGG, normal glucose group. *statistical difference time; $p < 0.05$ for pre versus post (repeated measures two-way ANOVA was used).

TABLE 3 Mean ± standard deviation of PRE and POST metal values 14 weeks of combined training (PG: n 24; NGG: n 24).

Variables (µg/L)	Group	PRE	POST	p (group)	p (time)	p (group x time)
Hg	PG	0.06 ± 0.1	0.03 ± 0.1	0.651	0.030*	0.651
	NGG	0.05 ± 0.1	0.02 ± 0.1			
Al	PG	184.3 ± 150.7	159.5.1 ± 154.8	0.46	0.476	0.907
	NGG	152.2 ± 177.5	135.0 ± 59.0			
As	PG	2.9 ± 0.4	2.9 ± 0.2	0.386	0.818	0.181
	NGG	2.7 ± 0.2	3.0 ± 0.0			
Cd	PG	0.2 ± 0.8	0.03 ± 0.0	0.391	0.212	0.452
	NGG	0.8 ± 0.0	0.02 ± 0.0			
Pb	PG	2.1 ± 1.6	1.1 ± 0.3	0.385	0.001*	0.33
	NGG	1.7 ± 0.8	1.1 ± 0.0			
Ni	PG	6.3 ± 1.0	5.3 ± 1.3	0.175	0.44	0.879
	NGG	4.4 ± 2.4	3.0 ± 0.8			
Li	PG	0.6 ± 0.3	0.5 ± 0.1	0.492	0.104	0.934
	NGG	0.6 ± 0.3	0.5 ± 0.2			

Hg, mercury; Al, aluminum; As, arsenic; Cd, cadmium; Pb, lead; Ni, nickel; Li, lithium; PG, prediabetes group; NGG, normal glucose group. *statistical difference time; $p < 0.05$ for pre versus post (repeated measures two-way ANOVA.

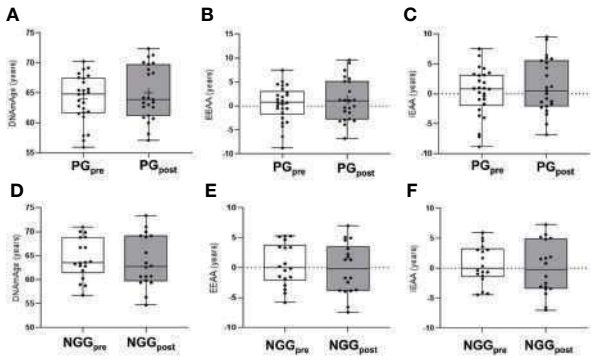

FIGURE 1
Differences in epigenetic age in PRE and POST comparisons 14 weeks of combined training. PG, prediabetes group; NGG, normal glucose group; EEAA, extrinsic epigenetic age acceleration; IEAA, intrinsic epigenetic age acceleration; Pre and pos-intervention comparison for the epigenetic clock in the PG group (panels **A–C**). Pre and post-intervention comparison for the epigenetic clock in the NGG group (panels **D–F**). (repeated measures two-way ANOVA was used). The presented values were not statically different between groups.

TABLE 4 Differentially Methylated Regions (DMRs) of the Prediabetes Group (PG) compared to the Normal Glucose Group (NGG) before 14 weeks of physical activity intervention.

NGG_{pre}	PG_{pre}	Gene	$\Delta\beta$	p-value
0.534	0.723	*IGR*	-0.19	<0.001
0.5	0.579	*RWDD3*	-0.08	0.013
0.25	0.317	*PM20D1*	-0.07	0.023
0.743	0.806	*WFIKKN2*	-0.06	0.015
0.747	0.784	*AURKC*	-0.04	0.026
0.199	0.231	*LOC441666*	-0.03	0.005
0.565	0.595	*HLA-DPB1*	-0.03	<0.001
0.104	0.131	*SLFN12*	-0.03	0.014
0.06	0.07	*CRYZ*	-0.01	0.007
0.045	0.035	*NPY*	0.01	0.004
0.059	0.049	*MIR596*	0.01	0.008
0.451	0.364	*DIP2C*	0.09	0.005

Discussion

Combined training demonstrated benefits in physical and biochemical variables, providing positive results to cardiovascular health

One of the aims of this study was to investigate the effect of combined training on epigenetic patterns in the prediabetes (PG) and normal glucose group (NGG). Combined training is an alternative program involving aerobic and strength exercises in the same training session (30). Its effects are widely recognized in the literature for the numerous benefits to the cardiorespiratory system, prevention of oxidative stress, and critical metabolic alterations in the glycemic balance (31). According to Bellazi et al. (12), the regulating factor of blood glucose concentration is achieved due to the power of action of the three variables: insulin, adequate meals, and physical exercise (32). Indeed, after 14 weeks of training, we found a decrease in glycemic values in the PG group, and in both groups, we observed decreased levels of total cholesterol and TG levels, indicating an improvement in metabolic indicators of cardiovascular health.

Moreover, the combined training improved both groups' systolic and diastolic blood pressure. A study by Schroeder et al. (13) observed that the combined training protocol was the only one that promoted significant changes in blood pressure values, with positive reductions in diastolic pressure (33). They demonstrated that the combined training had positive cumulative results in all cardiovascular outcomes (33).

Combined training also demonstrated benefits in functional capacity in our study, providing a time effect for the PG and NGG groups. These findings corroborated with Medeiros et al. (14), demonstrating an increase in maximal and functional strength after 12 weeks of training in 58 women aged 50-75 years (34).

Regarding heavy metal concentration, we observed a decrease in mercury and lead levels after 14 weeks of combined training. Another finding of our study was the positive correlation between lead and systolic and diastolic blood pressure values in the PG_{pre} group. Lead is widespread in the environment, and even low blood levels of this toxic metal may raise the risk of cardiovascular diseases, stroke, and chronic kidney disease. It is estimated that in adult populations lead exposures account for 5% of the population attributable risk for high blood pressure. Further, single toxicant exposures provide some evidence for developmental nephrotoxicity demonstrated by altered estimated glomerular filtration rate (eGFR) and other kidney biomarkers, increasing a risk for nephropathy in individuals with T2D (35). In a cross-sectional study carried

TABLE 5 Differentially Methylated Regions (DMRs) of the Prediabetes Group (PG) compared to the Normal Glucose Group (NGG) before 14 weeks of physical activity intervention.

NGG_{post}	PG_{post}	Gene	$\Delta\beta$	p-value
0.699	0.495	*IGR*	-0.2	<0.001
0.259	0.188	*LOC441666*	-0.07	0.012
0.078	0.064	*CRYZ*	-0.01	0.004
0.592	0.585	*HLA-DPB1*	-0.01	0.001
0.036	0.048	*NPY*	0.01	<0.001

TABLE 6 Differentially Methylated Regions (DMRs) from the PG_{post} compared to the PG_{pre} after 14-week physical intervention activity.

$\Delta\ \beta\ PG_{post}$ - PG_{pre}	Gene	p-value	$\Delta\ \beta\ PG_{post}$ - PG_{pre}	Gene	p-value
0.043	*CALD1*	0.004	-0.009	*HNRNPH1*	0.008
0.011	*MSI2*	0.024	-0.009	*ICK*	0.021
0.003	*RNF121*	0.024	-0.009	*AGL*	0.02
0	*SHFM1*	0.022	-0.009	*ANKRD11*	0.005
-0.001	*IDI1*	0.035	-0.009	*APH1A*	0.011
-0.001	*NUDT6*	0.024	-0.009	*ZSWIM7*	0.024
-0.001	*NDUFA4*	0.014	-0.009	IGR	0.02
-0.001	*APRT*	0.006	-0.009	*BCKDHA*	0.031
-0.001	*ATP6V1A*	0.01	-0.009	*NUP43*	0.028
-0.002	*WDR7*	0.01	-0.01	*RBPJ*	0.022
-0.002	*PLD3*	0.03	-0.01	*BDP1*	0.004
-0.003	*ZNHIT2*	0.02	-0.01	*COX8A*	0.011
-0.003	*ZNRD1*	0.019	-0.01	*GABPB2*	0.022
-0.003	*SLFN11*	0.013	-0.01	*TOM1L2*	0.026
-0.003	*FDPS*	0.008	-0.01	*PIGL*	0.016
-0.004	*FANCF*	0.005	-0.01	*HMGN4*	0.015
-0.004	*SNW1*	0.02	-0.01	*MOBKL3*	0.021
-0.004	*YIPF4*	0.008	-0.01	*HIST1H2BN*	0.004
-0.005	*ATP5A1*	0.012	-0.011	*CASP8AP2*	0.032
-0.005	*CHCHD8*	0.023	-0.011	*TAF15*	0.019
-0.005	*CEP97*	0.009	-0.011	*CENPK*	0.014
-0.005	*CCDC28A*	0.007	-0.012	*NECAP1*	0.007
-0.005	*PNPT1*	0.008	-0.012	*RANGAP1*	0.017
-0.005	*RBBP5*	0.011	-0.012	*EXOSC10*	0.013
-0.005	*NCRNA00188*	0.011	-0.012	*PSMB5*	0.009
-0.005	*GNAI3*	0.021	-0.012	*ZMYND11*	0.015
-0.005	*CWF19L1*	0.016	-0.013	*KAZALD1*	0.017
-0.006	*METTL3*	0.015	-0.013	*ERH*	0.023
-0.006	*ANAPC1*	0.028	-0.013	*FNTB*	0.007
-0.006	*LOC552889*	0.01	-0.013	*C7orf55*	0.024
-0.006	*NBR2/BRCA1*	0.01	-0.013	*GLIPR1L1*	0.008
-0.006	*CBY1*	0.004	-0.013	*GPATCH4*	0.012
-0.006	*C14orf101*	0.026	-0.013	*SRP14*	0.027
-0.007	*SLC4A1AP*	0.004	-0.014	*NRBF2*	0.022
-0.007	*AARS*	0.013	-0.014	*L3MBTL3*	0.027
-0.007	*C11orf65*	0.012	-0.014	*RND3*	0.032
-0.007	*DPY30*	0.025	-0.015	*ZNF684*	0.015
-0.007	*UBL7*	0.002	-0.015	*SNHG3/RCC1*	0.006
-0.007	IGR	0.015	-0.015	*MYOF*	0.002
-0.007	*BHLHE40*	0.029	-0.015	*TNFRSF10A*	0.017
-0.007	*COPZ1*	0.015	-0.015	*IDH3B*	0.024
-0.007	*H3F3B*	0.003	-0.015	*UFC1*	0.015
-0.007	*MCM2*	0.01	-0.017	*PCNP*	0.004
-0.007	*RPS27A*	0.003	-0.017	*MTMR12*	0.007
-0.008	IGR	0.007	-0.019	*RPL23A*	0.009
-0.008	*NCRNA00167*	0.005	-0.019	*C6orf52*	0.005
-0.008	*DBR1*	0.011	-0.019	*UFD1L*	0.011
-0.008	*C11orf60*	0.005	-0.02	*TNKS2*	0.011

(Continued)

TABLE 6 Continued

$\Delta\ \beta\ PG_{post}$ - PG_{pre}	Gene	p-value	$\Delta\ \beta\ PG_{post}$ - PG_{pre}	Gene	p-value
-0.008	*SLC25A19*	0.025	-0.02	*ILF2*	0.004
-0.008	*SIDT1*	0.006	-0.02	*COX5B*	0.01
-0.008	*LEO1*	0.018	-0.02	*GNB2L1*	0.011
-0.009	*MED28*	0.004	-0.02	*C4orf14*	0.012
-0.009	*VWA5A*	0.018	-0.022	*WDTC1*	0.004
-0.009	*NR1H2*	0.027	-0.022	*HIST1H4C*	0.011
-0.009	*PES1*	0.028	-0.022	*PFDN2*	0.011
-0.009	*WDR62*	0.011	-0.024	*SNORD27*	0.01
-0.009	*MOCS3/DMP1*	0.009	-0.028	*CMTM6*	0.003
-0.009	*LSM5*	0.005	-0.033	*CNIH4*	0.008
-0.009	*COPS4*	0.02	-0.048	*KLF14*	0.018

out by Asgary et al. (15), the authors demonstrated that lead, mercury, and cadmium levels were higher in patients with coronary artery disease (36).

Differentially methylated regions in the prediabetes group are related to inflammation, insulin signaling, and cancer and are associated with a higher risk of T2DM

Our findings demonstrated that genes in differentially methylated regions of the PG group were related to inflammation and insulin signaling. Physical activity has improved glycemic control in adults with type 2 diabetes (27). Moreover, it can induce metabolic adaptations and changes in DNA methylation due to energy requirements (14). These changes in DNA methylation allowed for increased insulin sensitivity, expression of genes involved in energy metabolism, myogenesis, contractile properties, and oxidative stress (14). In the Figure 4 is shown some metabolic pathways functionally enriched from the obtained DMRs.

Figure 5 shows hypermethylated pathways that are potentially altered after 14-week physical exercise intervention. Here we described specific hypermethylated genes' role in PG observed in our study. *HLA-DPB1* plays a central role in the immune system by presenting peptides derived from extracellular proteins. Also, it is linked with inflammation and systemic insulin resistance (37). *RWDD3* gene also plays an immune function. The isoforms RWDD3 positively regulate the

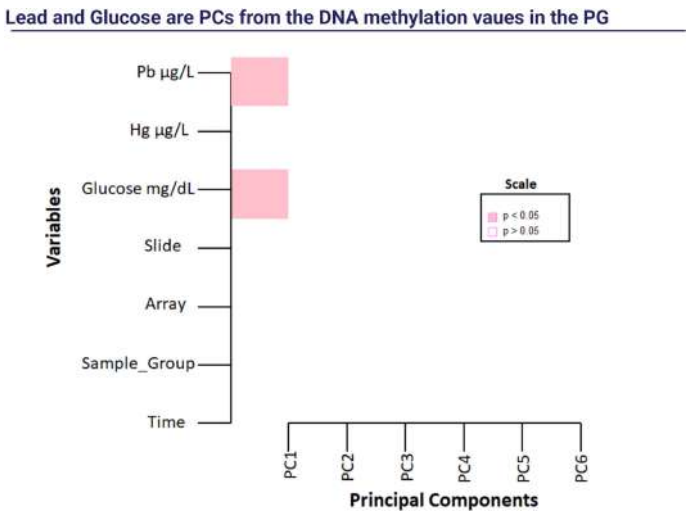

FIGURE 2
Singular Value Decomposition Analysis (SVD). Note: Pb: lead; Hg: mercury.

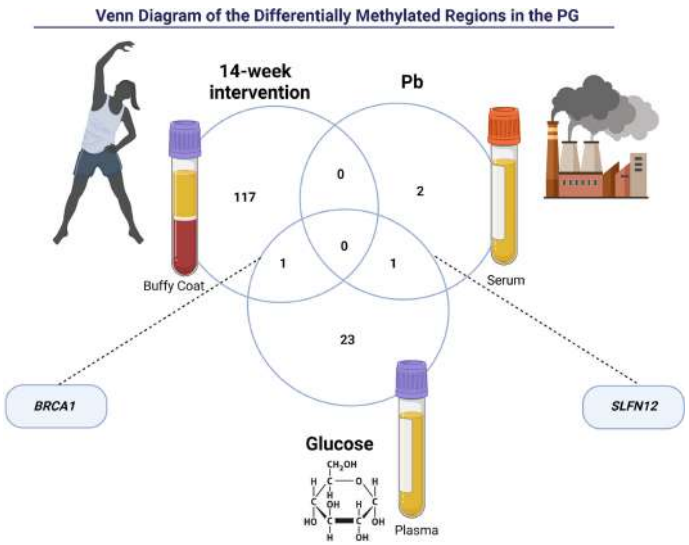

FIGURE 3
The intersection of Differently Methylated Regions of the prediabetes group (PG) between post-intervention, lead (Pb), and glucose. *BRCA1* gene is related to 14-week intervention and glucose. *SLFN12* gene is related to glucose and lead (Pb).

NF-kappa-β signaling pathway by enhancing the NF-kappa-β inhibitor, promoting its stabilization, and regulating the hypoxia-inducible factor-1 alpha (38). Other genes are found hypermethylated in PG group-related metabolism and genomic stability. The gene *TYW3/CRYZ* is related to levels of resistin. Previous studies reported associations between elevated circulating resistin levels and increased risk of cardiovascular disease (28). PM20D1 is an enzyme that condenses fatty acids and amino acids. The *PM20D1* locus shows genome-wide association studies (GWAS) with body mass index (BMI) and weaker associations with obesity-related conditions like type 2 diabetes and HDL-C levels (39). Aurora C kinases (AURKC) are essential kinases for cell division *via* regulating mitosis, especially the process of chromosomal segregation, associated with malignant cell transformation and genomic instability (16). PDE3A binding to SLFN12 results in cell killing and optimizing

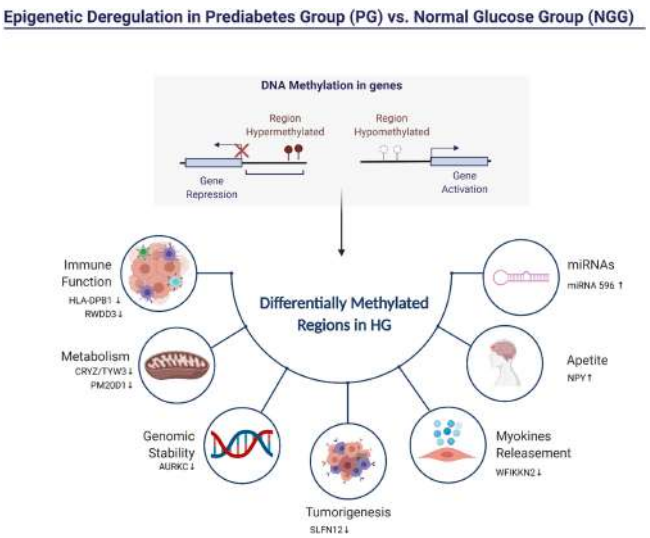

FIGURE 4
Representation of the pathways possibly influenced by differentially methylated regions (DMRs) in PG vs. NGG.

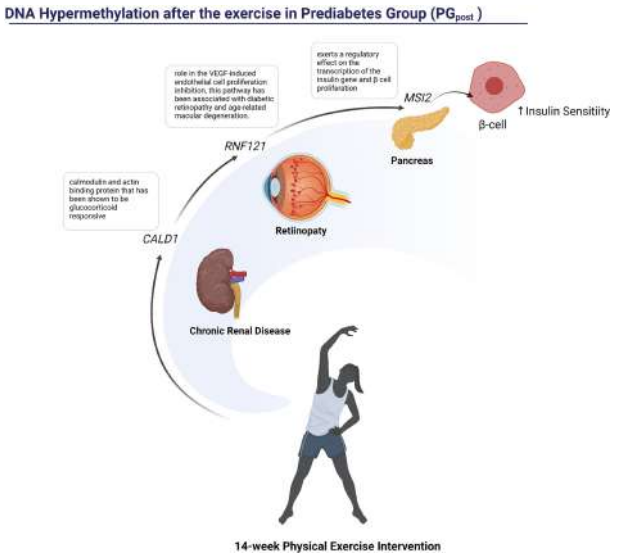

FIGURE 5
Representation of the pathways influenced possibly by hypermethylated regions in PG after the 14-week physical exercise intervention.

cancer therapeutics (40). WFIKKN2 is a follistatin domain-containing protein that binds GDF8/GDF11 proteins with high affinity, both of which have been implicated in DM development (41). The hypermethylation of these genes in individuals of the PG group could indicate a compromised metabolic function which could be associated with a higher risk of T2DM (42).

The epigenetic reprogramming of genes after a 14-week physical exercise intervention can promote many alterations at a cellular level that regulates cellular metabolism in human metabolic diseases such as diabetes, obesity, and cancer

After the 14-week physical exercise intervention, 118 DMRs were detected. Three of these genes were hypermethylated: *CALD1*, *RNF121*, and *MSI2*. The *CALD1* was previously related to diabetes nephropathy (43), while the role of RNF121 in the VEGF-induced endothelial cell proliferation inhibition has been associated with diabetic retinopathy and age-related macular degeneration (44). The *MSI2* hypomethylation has already been described as an epigenetic biomarker for hyperglycemic individuals by the Korean Genome Epidemiology Study (KoGES) (45).

The additional 115 genes were hypomethylated and involved in many biological functions (Figure 6). Regarding the effects on general metabolism, the FDPS (*Farnesyl pyrophosphate synthase*) is a crucial enzyme for several classes of essential metabolites, including sterols, dolichols, carotenoids, and ubiquinones. The BHLHE40 Class E basic helix-loop-helix protein 40 is also a target that may influence the general metabolism due to its role as a transcriptional repressor in

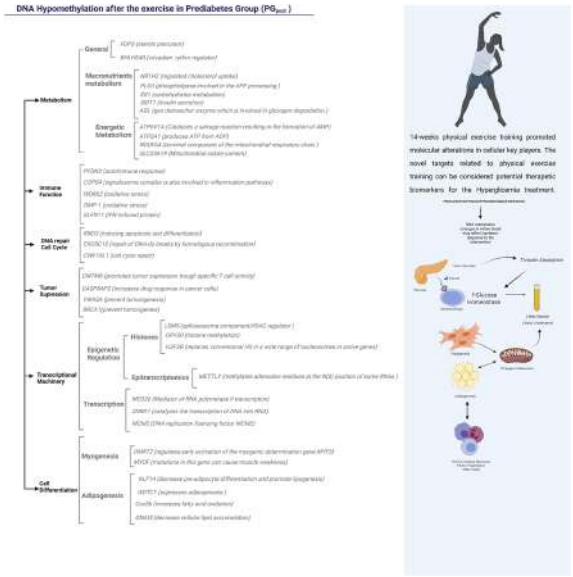

FIGURE 6
Representation of the functions possibly influenced by hypomethylated regions in PG after the 14-week physical exercise intervention. The Functional Annotation was done using the Genecards Database and literature text mining.

regulating the circadian rhythm and negatively regulating the activity of the clock genes and clock-controlled genes (46).

The mechanisms related to metabolism also included targets related to macronutrients, such as lipids and carbohydrates, which are important in the etiology of hyperglycemia (47). The NR1H2 (Oxysterols receptor LXR-beta; Nuclear receptor) gene was differentially methylated and can regulate cholesterol uptake through the ubiquitination of LDLR, VLDLR, and LRP8. Indeed, the PLD3 - Phospholipase D3 is an important key in lipids metabolism and may be involved in the Amyloid Precursor Protein (APP), already been related to Alzheimer´s Disease (AD). Three genes were differentially methylated in carbohydrate metabolism: IDI1, AGL, and SIDT1. ID1 has already been related to nephropathy, a condition connected to advanced stages of T2D (48). The AGL gene encodes the glycogen debrancher enzyme involved in glycogen degradation (49). The SIDT1 gene is associated with insulin secretion, and its expression was decreased in individuals with hyperglycemia (50).

Four differentially methylated genes were associated with energetic metabolism: ATP6V1A, ATP5A1, NDUFA4, and SLC25A19. The ATP6V1A is responsible for acidifying a variety of intracellular molecules, leading to HIF1A hydroxylation and catalyzing AMP formation (51). The ATP5A1 produces ATP from ADP (52). NDUFA4 is a cytochrome c oxidase subunit NDUFA4 and catalyzes oxygen reduction in water (53). The SLC25A19 mitochondrial thiamine pyrophosphate carrier is a transporter mediating thiamine pyrophosphate (ThPP) entrance into mitochondria (54).

The most interesting genes involved with the immune function were the COPS4 signalosome complex subunit 4, involved in phosphorylation of p53/TP53, c-jun/JUN, IkappaBalpha/NFKBIA, which leads to modulation of the inflammasome (55); theWDR62 and DMP-1 related to oxidative stress; and the SLFN11, interferon (IFN)-induced target (16).

The tumor suppression mechanisms, insulin resistance, and metabolic diseases have a common path (56). The CMTM6 depletion significantly decreases tumor-specific T cell activity (57). Indeed, the hypermethylation of two CpG sites upstream of the CASP8AP2 promoter influenced gene expression and treatment outcomes in lymphoblastic leukemia (58). In the present study, CASP8AP2 was hypomethylated after the intervention, suggesting that physical exercise training may decrease the risk of developing cancer in this population. The VWA5A plays a role in tumorigenesis as a tumor suppressor (59). BRCA1 is another important tumor suppressor, and studies have shown that individuals with hyperglycemia had this gene naturally more methylated than normoglycemic individuals (60).

Physical exercise can promote many alterations at a cellular level. Liu et al., 2020, demonstrated that miRNAs could epigenetically modulate the myogenesis and adipogenesis processes after a 12-week physical exercise intervention in older individuals (61). In the present study, important differentially methylated regions in the PG were related to myogenesis and adipogenesis after the intervention. The DMRT2 directly regulates early activation of the myogenic determination. It is required to initiate or maintain the proper organization of the sclerotome, dermomyotome, and myotome (by similarity). Mutations in the *MYOF* gene can cause muscle weakness, which is involved in membrane regeneration and repair. Reduced *KLF14* expression increases pre-adipocyte proliferation and decreases lipogenesis. In mice, the deletion of *KLF14* partially reproduces the human phenotype of insulin resistance, dyslipidemia, and T2D (62). The deregulation of the KLF14/PLK1 cascade plays a crucial role in thrombin-induced endothelial dysfunction in T2DM patients (63).

There is substantial epidemiological evidence that exposure to low levels of various environmental chemicals can influence the development of chronic metabolic diseases, including diabetes (64). It has been well demonstrated that lead shows potential genotoxicity to various research subjects under different conditions (65, 66). A study with human lymphoblastoid TK6 cells found that lead exposure results in DNA damage *via* promoting oxidative stress and the promoter methylation of DNA repair genes, such as *BRCA1* (67).

The *BRCA1* plays a critical role in DNA repair. Mutations in the gene that lead to loss of protein function can accumulate damaged DNA within the cell tumor-suppressor gene, which is strongly linked to an increased risk of breast cancer development (68). Recent studies demonstrated that BRCA1 is expressed in skeletal muscle and is a critical regulator of metabolic function in cultured human myotubes (69). Also, deletion of *BRCA1* expression in vascular cells results in the reduced catabolic breakdown of free fatty acids, and it can influence the metabolic phenotype of cultured cells and potentially the cells' ability to respond to nutrient overload conditions (70).

Schlafens (*SLFN*) are intriguing proteins that play different roles in regulating cell proliferation, cell differentiation, immune cell growth and maturation, and inhibiting viral replication (17, 71).. However, it is still poorly understood how SLFNs interfere with the cell cycle machinery. It is a particularly tempting target for further research, as this gene is known to be down-regulated during T cell activation and up-regulated by type I interferons (IFNs) (18). It has also been suggested in cancer therapy since its expression was positively correlated with the growth inhibitory action of topoisomerase inhibitors on human cancer cells (19).

Acute exercise seems to be associated with active demethylation pathways. The mechanism proposed for active demethylation involves ten-eleven translocation (TET) proteins, suggesting that metabolites produced during exercise could alter the activity of TET proteins (20).

This study's strengths were demonstrating the modulation of biochemical and physical parameters after a physical exercise intervention between different groups. In addition to verifying differently methylated regions in the PG group compared to the NGG group using a large-scale DNA methylation array (Infinium-EPIC beadchips) and the effect of combined training.

The limitations were that analyzes of RNA expressions of the differentially methylated genes were not performed.

Conclusions

The 14-week combined physical exercise intervention led to the modulation of biochemical and physical parameters and the epigenetic reprogramming of genes involved in many biological functions such as metabolism, cell differentiation, tumor suppression, and immune function. Moreover, exercise training decreases mercury and lead serum concentrations. In future studies, longer intervention could be proposed to check the sustained effects of long-term combined physical exercise practice.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number can be found below:GEO, GSE199700.

Ethics statement

The present study was approved by the ethics committee for Research with Human of the School of Physical Education and Sport of Ribeirão Preto, University of São Paulo (EEFERP-USP). The registration number is CAAE: 79582817.0.0000.5656. The study was also registered in the Brazilian Registry of Clinical Trials under RBR-3g38dx. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization: NN and GR; methodology: NN, IN, and GR; formal analysis: NN, GR, CS-L, AC, and KR; resources: CBu and CN; data curation: GR, NN, and IN; performed experiments: NN, GR, LW, MP, DM, WS, and MA; writing—original draft preparation: NN, GR, IN, TP, CBr, and KR; writing—review and editing: TP, NN, GR, IN, and CBr; visualization: All authors; supervision: FB, CBu, and CN; funding acquisition: FB, CBu, and CN. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Association, AD. Diagnosis and classification of diabetes mellitus. *Diabetes Care* (2009) 32:S62–7. doi: 10.2337/dc09-S062

2. Kaaja RJ. Metabolic syndrome and the menopause. *Menopause Int* (2008) 14 (1):21–5. doi: 10.1258/mi.2007.007032

3. Luc K, Schramm-Luc A, Guzik TJ, Mikolajczyk TP. Oxidative stress and inflammatory markers in prediabetes and diabetes. *J Physiol Pharmacol* (2019) 70:809–24. doi: 10.26402/jpp.2019.6.01

4. Muka T, Asllanaj E, Avazverdi N, Jaspers L, Stringa N, Milic J, et al. Age at natural menopause and risk of type 2 diabetes: A prospective cohort study. *Diabetologia* (2017) 60:1951–60. doi: 10.1007/s00125-017-4346-8

5. Kato M, Natarajan R. Epigenetics and epigenomics in diabetic kidney disease and metabolic memory. *Nat Rev Nephrol* (2019) 15:327–45. doi: 10.1038/s41581-019-0135-6

6. Rosen ED, Kaestner KH, Natarajan R, Patti M-E, Sallari R, Sander M, et al. Epigenetics and epigenomics: Implications for diabetes and obesity. *Diabetes* (2018) 67:1923–31. doi: 10.2337/db18-0537

7. Javaid A, Akbar I, Javed H, Khan U, Iftikhar H, Zahra D, et al. Role of heavy metals in diabetes: Mechanisms and treatment strategies. *Crit Rev Eukaryot Gene Expr* (2021) 31:65–80. doi: 10.1615/CritRevEukaryotGeneExpr.2021037971

8. Cai J, Li Y, Liu S, Liu Q, Xu M, Zhang J, et al. Associations between multiple heavy metals exposure and glycated hemoglobin in a Chinese population. *Chemosphere* (2021) 287:132159. doi: 10.1016/j.chemosphere.2021.132159

9. Yahaya T. Role of epigenetics in the pathogenesis and management of type 2 diabetes mellitus. *Translation: Univ Toledo J Med Sci* (2019) 6:20–8. doi: 10.46570/utjms.vol6-2019-319

10. Denham J. Exercise and epigenetic inheritance of disease risk. *Acta Physiol (Oxf)* (2018) 222:1–20. doi: 10.1111/apha.12881

11. Tang S, Yu X, Wu C. Comparison of the levels of five heavy metals in human urine and sweat after strenuous exercise by ICP-MS. *J Appl Mathematics Phys* (2016) 4:183–8. doi: 10.4236/jamp.2016.42022

12. Bellazzi R, Nucci G, Cobelli C. The subcutaneous route to insulin-dependent diabetes therapy. *IEEE Eng Med Biol Mag* (2001) 20:54–64. doi: 10.1109/51.897828

13. Schroeder EC, Franke WD, Sharp RL, Lee D-C. Comparative effectiveness of aerobic, resistance, and combined training on cardiovascular disease risk factors: A randomized controlled trial. *PloS One* (2019) 14:e0210292. doi: 10.1371/journal.pone.0210292

14. Medeiros LHL, Sandbakk SB, Bertazone TMA, Bueno Júnior CR. Comparison of periodization models of concurrent training in recreationally active postmenopausal women. *J Strength Cond Res* (2020) 36:977–83. doi: 10.1519/JSC.0000000000003559

15. Asgary S, Movahedian A, Keshvari M, Taleghani M, Sahebkar A, Sarrafzadegan N. Serum levels of lead, mercury and cadmium in relation to coronary artery disease in the elderly: A cross-sectional study. *Chemosphere* (2017) 180:540–4. doi: 10.1016/j.chemosphere.2017.03.069

16. Anguita-Ruiz A, Bustos-Aibar M, Plaza-Díaz J, Mendez-Gutierrez A, Alcalá-Fdez J, Aguilera CM, et al. Omics approaches in adipose tissue and skeletal muscle addressing the role of extracellular matrix in obesity and metabolic dysfunction. *Int J Mol Sci* (2021) 22:2756. doi: 10.3390/ijms22052756

17. Malachias MVB. 7th Brazilian guideline of arterial hypertension: Presentation. *Arq Bras Cardiol* (2016) 107:XV–XIX. doi: 10.5935/abc.20160140

18. Rikli RE, Jones CJ. Development and validation of a functional fitness test for community-residing older adults. *J Aging Phys Act* (1999) 7(2):129–61. doi: 10.1123/japa.7.2.129

19. Batista BL, Rodrigues JL, Nunes JA, Souza VC, de O, Barbosa F. Exploiting dynamic reaction cell inductively coupled plasma mass spectrometry (DRC-ICP-MS) for sequential determination of trace elements in blood using a dilute-and-Shoot procedure. *Anal Chim Acta* (2009) 639:13–8. doi: 10.1016/j.aca.2009.03.016

20. Cabo R, Hernes S, Slettan A, Haugen M, Ye S, Blomhoff R, et al. Effects of polymorphisms in endothelial nitric oxide synthase and folate metabolizing genes on the concentration of serum nitrate, folate, and plasma total homocysteine after folic acid supplementation: A double-blind crossover study. *Nutrition* (2015) 31:337–44. doi: 10.1016/j.nut.2014.08.009

21. De Santis MA, Giuliani MM, Giuzio L, De Vita P, Lovegrove A, Shewry PR, et al. Differences in gluten protein composition between old and modern durum wheat genotypes in relation to 20th century breeding in Italy. *Eur J Agron* (2017) 87:19–29. doi: 10.1016/j.eja.2017.04.003

22. Li H, Chen R, Cai J, Cui X, Huang N, Kan H. Short-term exposure to fine particulate air pollution and genome-wide DNA methylation: A randomized, double-blind, crossover trial. *Environ Int* (2018) 120:130–6. doi: 10.1016/j.envint.2018.07.041

23. Masser DR, Stanford DR, Freeman WM. Targeted DNA methylation analysis by next-generation sequencing. *J Vis Exp* (2015) 96:52488. doi: 10.3791/52488

24. Tian Y, Morris TJ, Webster AP, Yang Z, Beck S, Feber A, et al. ChAMP: Updated methylation analysis pipeline for illumina BeadChips. *Bioinformatics* (2017) 33:3982–4. doi: 10.1093/bioinformatics/btx513

25. Fortin J-P, Triche TJ, Hansen KD. Preprocessing, normalization and integration of the illumina HumanMethylationEPIC array with minfi. *Bioinformatics* (2017) 33:558–60. doi: 10.1093/bioinformatics/btw691

26. Pelegí-Sisó D, de Prado P, Ronkainen J, Bustamante M, González JR. Methylclock: A bioconductor package to estimate DNA methylation age. *Bioinformatics* (2021) 37:1759–60. doi: 10.1093/bioinformatics/btaa825

27. Carbia-Nagashima A, Gerez J, Perez-Castro C, Paez-Pereda M, Silberstein S, Stalla GK, et al. RSUME, a small RWD-containing protein, enhances SUMO conjugation and stabilizes HIF-1alpha during hypoxia. *Cell* (2007) 131:309–23. doi: 10.1016/j.cell.2007.07.044

28. Qi Q, Menzaghi C, Smith S, Liang L, de Rekeneire N, Garcia ME, et al. Genome-wide association analysis identifies TYW3/CRYZ and NDST4 loci associated with circulating resistin levels. *Hum Mol Genet* (2012) 21:4774–80. doi: 10.1093/hmg/dds300

29. Benson KK, Hu W, Weller AH, Bennett AH, Chen ER, Khetarpal SA, et al. Natural human genetic variation determines basal and inducible expression of PM20D1, an obesity-associated gene. *PNAS* (2019) 116:23232–42. doi: 10.1073/pnas.1913199116

30. Lewis TA, de Waal L, Wu X, Youngsaye W, Wengner A, Kopitz C, et al. Optimization of PDE3A modulators for SLFN12-dependent cancer cell killing. *ACS Med Chem Lett* (2019) 10:1537–42. doi: 10.1021/acsmedchemlett.9b00360

31. Gudmundsdottir V, Zaghlool SB, Emilsson V, Aspelund T, Ilkov M, Gudmundsson EF, et al. Circulating protein signatures and causal candidates for type 2 diabetes. *Diabetes* (2020) 69:1843–53. doi: 10.2337/db19-1070

32. Kondás K, Szláma G, Trexler M, Patthy L. Both WFIKKN1 and WFIKKN2 have high affinity for growth and differentiation factors 8 and 11. *J Biol Chem* (2008) 283:23677–84. doi: 10.1074/jbc.M803025200

33. Félétou M, Galizzi J-P, Levens NR. NPY receptors as drug targets for the central regulation of body weight. *CNS Neurol Disord Drug Targets* (2006) 5:263–74. doi: 10.2174/187152706777452236

34. Wang J, Chen L, Qiang P. The role of IGF2BP2, an M6A reader gene, in human metabolic diseases and cancers. *Cancer Cell Int* (2021) 21:99. doi: 10.1186/s12935-021-01799-x

35. Chen Y, Gong W, Dai W, Pan Z, Xu X, Jiang H. MiR-596 down regulates SOX4 expression and is a potential novel biomarker for gastric cancer. *Trans Cancer Res* (2020) 9:1294–302. doi: 10.21037/tcr.2020.02.02

36. Wang Z, Wang Z, Zhou Z, Ren Y. Crucial genes associated with diabetic nephropathy explored by microarray analysis. *BMC Nephrol* (2016) 17:128. doi: 10.1186/s12882-016-0343-2

37. Maghsoudlou A, Meyer R, Rezazadeh K, Arafa E, Pudney J, Hartsough EJ, et al. RNF121 inhibits angiogenic growth factor signaling by restricting cell surface expression of VEGFR-2. *Traffic* (2016) 17:289–300. doi: 10.1111/tra.12353

38. Ahmed SAH, Ansari SA, Mensah-Brown EPK, Emerald BS. The role of DNA methylation in the pathogenesis of type 2 diabetes mellitus. *Clin Epigenet* (2020) 12:104. doi: 10.1186/s13148-020-00896-4

39. Sato F, Muragaki Y, Zhang Y. DEC1 negatively regulates AMPK activity *via* LKB1. *Biochem Biophys Res Commun* (2015) 467:711–6. doi: 10.1016/j.bbrc.2015.10.077

40. Chiu C-J, Taylor A. Dietary hyperglycemia, glycemic index and metabolic retinal diseases. *Prog Retin Eye Res* (2011) 30:18–53. doi: 10.1016/j.preteyeres.2010.09.001

41. Schisler JC, Charles PC, Parker JS, Hilliard EG, Mapara S, Meredith D, et al. Stable patterns of gene expression regulating carbohydrate metabolism determined by geographic ancestry. *PloS One* (2009) 4:e8183. doi: 10.1371/journal.pone.0008183

42. Perveen S, Gupta N, Kumar M, Kaur P, Chowdhury MR, Kabra M. Spectrum of amyloglucosidase mutations in Asian Indian patients with glycogen storage disease type III. *Am J Med Genet A* (2020) 182:1190–200. doi: 10.1002/ajmg.a.61547

43. Ottosson-Laakso E, Krus U, Storm P, Prasad RB, Oskolkov N, Ahlqvist E, et al. Glucose-induced changes in gene expression in human pancreatic islets: Causes or consequences of chronic hyperglycemia. *Diabetes* (2017) 66:3013–28. doi: 10.2337/db17-0311

44. Alzamora R, Al-Bataineh MM, Liu W, Gong F, Li H, Thali RF, et al. AMP-activated protein kinase regulates the vacuolar h+-ATPase *via* direct phosphorylation of the a subunit (ATP6V1A) in the kidney. *Am J Physiology-Renal Physiol* (2013) 305:F943–56. doi: 10.1152/ajprenal.00303.2013

45. Chomova M, Balazova M, Muchova J. Diabetes-induced abnormalities of mitochondrial function in rat brain cortex: The effect of n-3 fatty acid diet. *Mol Cell Biochem* (2017) 435:109–31. doi: 10.1007/s11010-017-3061-6

46. Pitceathly RDS, Rahman S, Wedatilake Y, Polke JM, Cirak S, Foley AR, et al. NDUFA4 mutations underlie dysfunction of a cytochrome c oxidase subunit linked to human neurological disease. *Cell Rep* (2013) 3:1795–805. doi: 10.1016/j.celrep.2013.05.005

47. Kang J, Samuels DC. The evidence that the DNC (SLC25A19) is not the mitochondrial deoxyribonucleotide carrier. *Mitochondrion* (2008) 8:103–8. doi: 10.1016/j.mito.2008.01.001

48. Bech-Otschir D, Kraft R, Huang X, Henklein P, Kapelari B, Pollmann C, et al. COP9 signalosome-specific phosphorylation targets P53 to degradation by the ubiquitin system. *EMBO J* (2001) 20:1630–9. doi: 10.1093/emboj/20.7.1630

49. Kaul K, Misri S, Ramaswamy B, Ganju RK. Contribution of the tumor and obese microenvironment to triple negative breast cancer. *Cancer Lett* (2021) 509:115–20. doi: 10.1016/j.canlet.2021.03.024

50. Burr ML, Sparbier CE, Chan Y-C, Williamson JC, Woods K, Beavis PA, et al. CMTM6 maintains the expression of PD-L1 and regulates anti-tumour immunity. *Nature* (2017) 549:101–5. doi: 10.1038/nature23643

51. Li Z-G, Jiao Y, Li W-J, Deng G-R, Cui L, Gao C, et al. Hypermethylation of two CpG sites upstream of CASP8AP2 promoter influences gene expression and treatment outcome in childhood acute lymphoblastic leukemia. *Leuk Res* (2013) 37:1287–93. doi: 10.1016/j.leukres.2013.07.018

52. Anghel SI, Correa-Rocha R, Correa-Rochal R, Budinska E, Boligan KF, Boliganl KF, et al. Breast cancer suppressor candidate-1 (BCSC-1) is a melanoma tumor suppressor that down regulates MITF. *Pigment Cell Melanoma Res* (2012) 25:482–7. doi: 10.1111/j.1755-148X.2012.01018.x

53. Karachanak-Yankova S, Dimova R, Nikolova D, Nesheva D, Koprinarova M, Maslyankov S, et al. Epigenetic alterations in patients with type 2 diabetes mellitus. *Balkan J Med Genet* (2016) 18:15–24. doi: 10.1515/bjmg-2015-0081

54. Liu H-W, Cheng H-C, Tsai S-H, Sun W-H. Effect of progressive resistance training on circulating adipogenesis-, myogenesis-, and inflammation-related MicroRNAs in healthy older adults: An exploratory study. *GER* (2020) 66:562–70. doi: 10.1159/000510148

55. Small KS, Todorčević M, Civelek M, El-Sayed Moustafa JS, Wang X, Simon MM, et al. Regulatory variants at KLF14 influence type 2 diabetes risk *via* a female-specific effect on adipocyte size and body composition. *Nat Genet* (2018) 50:572–80. doi: 10.1038/s41588-018-0088-x

56. Hao J-S, Zhu C-J, Yan B-Y, Yan C-Y, Ling R. Stimulation of KLF14/PLK1 pathway by thrombin signaling potentiates endothelial dysfunction in type 2 diabetes mellitus. *BioMed Pharmacother* (2018) 99:859–66. doi: 10.1016/j.biopha.2018.01.151

57. Zhu H, Ge K, Lu J, Jia C. Downregulation of GNAI3 promotes the pathogenesis of Methionine/Choline-deficient diet-induced nonalcoholic fatty liver disease. *Gut Liver* (2020) 14:492–9. doi: 10.5009/gnl19115

58. Jones OA, Maguire ML, Griffin JL. Environmental pollution and diabetes: A neglected association. *Lancet* (2008) 371:287–8. doi: 10.1016/S0140-6736(08)60147-6

59. Pasha Shaik A, Sankar S, Reddy SC, Das PG, Jamil K. Lead-induced genotoxicity in lymphocytes from peripheral blood samples of humans: *In vitro* studies. *Drug Chem Toxicol* (2006) 29:111–24. doi: 10.1080/01480540500408739

60. Nersesyan A, Kundi M, Waldherr M, Setayesh T, Mišík M, Wultsch G, et al. Results of micronucleus assays with individuals who are occupationally and environmentally exposed to mercury, lead and cadmium. *Mutat Res Rev Mutat Res* (2016) 770:119–39. doi: 10.1016/j.mrrev.2016.04.002

61. García-Lestón J, Méndez J, Pásaro E, Laffon B. Genotoxic effects of lead: An updated review. *Environ Int* (2010) 36:623–36. doi: 10.1016/j.envint.2010.04.011

62. Liu X, Wu J, Shi W, Shi W, Liu H, Wu X. Lead induces genotoxicity *via* oxidative stress and promoter methylation of DNA repair genes in human lymphoblastoid TK6 cells. *Med Sci Monit* (2018) 24:4295–304. doi: 10.12659/MSM.908425

63. Roy R, Chun J, Powell SN. BRCA1 and BRCA2: Different roles in a common pathway of genome protection. *Nat Rev Cancer* (2012) 12:68–78. doi: 10.1038/nrc3181

64. Jackson KC, Tarpey MD, Valencia AP, Iñigo MR, Pratt SJ, Patteson DJ, et al. Induced cre-mediated knockdown of Brca1 in skeletal muscle reduces mitochondrial respiration and prevents glucose intolerance in adult mice on a high-fat diet. *FASEB J* (2018) 32:3070–84. doi: 10.1096/fj.201700464R

65. Jackson KC, Gidlund E-K, Norrbom J, Valencia AP, Thomson DM, Schuh RA, et al. BRCA1 is a novel regulator of metabolic function in skeletal muscle. *J Lipid Res* (2014) 55:668–80. doi: 10.1194/jlr.M043851

66. Privat M, Radosevic-Robin N, Aubel C, Cayre A, Penault-Llorca F, Marceau G, et al. BRCA1 induces major energetic metabolism reprogramming in breast cancer cells. *PloS One* (2014) 9:e102438. doi: 10.1371/journal.pone.0102438

67. Vomhof-DeKrey EE, Lee J, Lansing J, Brown C, Darland D, Basson MD. Schlafen 3 knockout mice display gender-specific differences in weight gain, food efficiency, and expression of markers of intestinal epithelial differentiation, metabolism, and immune cell function. *PloS One* (2019) 14:e0219267. doi: 10.1371/journal.pone.0219267

68. Kovalenko PL, Basson MD. Schlafen 12 expression modulates prostate cancer cell differentiation. *J Surg Res* (2014) 190:177–84. doi: 10.1016/j.jss.2014.03.069

69. Katsoulidis E, Carayol N, Woodard J, Konieczna I, Majchrzak-Kita B, Jordan A, et al. Role of schlafen 2 (SLFN2) in the generation of interferon α-induced growth inhibitory responses. *J Biol Chem* (2009) 284:25051–64. doi: 10.1074/jbc.M109.030445

70. Li D, Chen J, Ai Y, Gu X, Li L, Che D, et al. Estrogen-related hormones induce apoptosis by stabilizing schlafen-12 protein turnover. *Mol Cell* (2019) 75:1103–1116.e9. doi: 10.1016/j.molcel.2019.06.040

71. Zoppoli G, Regairaz M, Leo E, Reinhold WC, Varma S, Ballestrero A, et al. Putative DNA/RNA helicase schlafen-11 (SLFN11) sensitizes cancer cells to DNA-damaging agents. *Proc Natl Acad Sci U.S.A.* (2012) 109:15030–5. doi: 10.1073/pnas.1205943109

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UNIVERSIDADE DE SÃO PAULO
Faculdade de Ciências Farmacêuticas
Departamento de Análises Clínicas e Toxicológicas

Sao Paulo, Brazil – October 27, 2025.

Dear Sir/Madam:

My name is Ana Campa, I am a Full Ph.D. Professor at the Faculty of Pharmaceutical Sciences of the University of Sao Paulo (USP) and a member of the Sao Paulo State Academy of Sciences. Over the course of my career, I have served as Head of the Department of Clinical and Toxicological Analyses and participated in numerous academic and governmental committees, including CAPES, CNPq, FAPESP, and the Ministry of Education. I have also supervised more than 45 master's dissertations and doctoral theses, contributing to the training of highly qualified professionals in the biomedical sciences. My research focuses on Biochemistry and Cell Biology, with a particular emphasis on the role of tryptophan metabolites in modulating immune system cells and tumor development.

It is with great enthusiasm that I write this letter in support of the application of Dr. Isabella Harumi Yonehara Noma for classification as an individual of extraordinary ability under the EB-1 category. I had the opportunity to work closely with Dr. Noma when she assisted in teaching clinical biochemistry at the University of Sao Paulo, when I was able to observe her dedication, professionalism, and the high level of competence she demonstrated in her role. Her ability to mentor and guide students was exemplary, and she played a key role in enhancing their learning experience.
In addition, I have been following Dr. Noma's research as her contributions are of great importance to the scientific community. One of her most relevant investigations was titled *"Peroxiredoxin-2 represses NRAS-mutated melanoma cells invasion by modulating EMT markers"*. In this study, Dr. Noma demonstrated that PRDX2 acts to inhibit epithelial-mesenchymal transition in NRAS-mutated melanoma cells, identifying a potential therapeutic target for reducing melanoma invasiveness. This finding is technically significant because EMT is a major mechanism of metastasis, and few studies had explored PRDX2 in this context, also demonstrating the originality of her work.
She also investigated the interaction of high-risk HPV and *Ureaplasma parvum* co-infections with cervical cancer development. This study highlighted the importance of considering viral and bacterial co-infections in cancer risk assessment, which guides preventive healthcare strategies emphasizing importance of cervical screening to find precancerous cervical cell changes and presence of HPV. Cervical cancer found at an early stage is usually easier to treat, and her study strengthens the recommendations of the National Institutes of Health (NIH),

Dr. Noma's research extends to epigenetic studies connecting lifestyle factors with genetic modifications, such as "14-Week exercise training modifies the DNA methylation levels at gene sites in non-Alzheimer's disease women aged 50 to 70 years" or "14-weeks combined exercise epigenetically modulated 118 genes of menopausal women with prediabetes" highlighted importance of epigenetic modifications in diseases like Alzheimer's or diabetes

Her scientific record includes over 58 citations to date, 15 peer-reviewed publications, 13 abstracts in conference proceedings, 28 oral presentations, and 2 published book chapters. This demonstrates the high impact of the innovative scientific contributions across oncology and cellular and molecular biology.

Av. Prof. Lineu Prestes, nº 580, Bloco 17 - Cidade Universitária - CEP 05508-000 - São Paulo - SP
Fone: (011) 2648-1314 - Fax (011) 2648-0684

Dr. Noma has actively collaborated on important studies addressing diabetes, COVID-19, and obesity, contributing valuable insights that are crucial for national healthcare strategies. Her interdisciplinary approach not only advances scientific knowledge but also informs public health policies aimed at improving health outcomes across populations. Given these impactful contributions across multiple areas of research, her application for the EB-1 Visa is strongly supported due to her high potential to influence significant health issues through innovative research in the United States.
If you need further information, feel free to contact me.
Sincerely,

Ana Campa.

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2-Methoxyestradiol-3,17-*O*,*O*-bis-sulfamate (STX140) Inhibits Proliferation and Invasion via Senescence Pathway Induction in Human BRAFi-Resistant Melanoma … YA Franco, MO de Moraes Jr, LAC Carvalho, W Dohle, RO da Silva, ... International Journal of Molecular Sciences 24 (14), 11314	1	2023
Trend change estimation for interrupted time series with heteroscedastic and autocorrelated errors: application in syphilis occurrences in Brazil EM de Souza, D Sodré, IHY Noma, CA Tanoshi, RB Pedroso Acta Scientiarum. Technology 44, e59513	1	2022
Análise da morbidade hospitalar por leishmaniose cutânea no estado do paraná, Brasil, 2008-2018 KLT Ganaza, CA Tanoshi, IHY Noma, RB Pedroso, MDB Carvalho, ...	1	2018
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Isabella Harumi Yonehara Noma

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Association between the relative abundance of phyla actinobacteria, vitamin C consumption, and DNA methylation of genes linked to immune response pathways NY Noronha, IHY Noma, R Fernandes Ferreira, GS Rodrigues, LS Martins, ... Frontiers in nutrition 11, 1373499	2	2024
Economic impact of hospitalizations due to leishmaniasis in southern Brazil KLTG Domingues, CA Tanoshi, IHY Noma, MD de Barros Carvalho, ... Research, Society and Development 11 (8), e39211831139-e39211831139	2	2022
2-Methoxyestradiol-3,17-*O*,*O*-bis-sulfamate (STX140) Inhibits Proliferation and Invasion via Senescence Pathway Induction in Human BRAFi-Resistant Melanoma … YA Franco, MO de Moraes Jr, LAC Carvalho, W Dohle, RO da Silva, ... International Journal of Molecular Sciences 24 (14), 11314	1	2023
Trend change estimation for interrupted time series with heteroscedastic and autocorrelated errors: application in syphilis occurrences in Brazil EM de Souza, D Sodré, IHY Noma, CA Tanoshi, RB Pedroso Acta Scientiarum. Technology 44, e59513	1	2022
Análise da morbidade hospitalar por leishmaniose cutânea no estado do paraná, Brasil, 2008-2018 KLT Ganaza, CA Tanoshi, IHY Noma, RB Pedroso, MDB Carvalho, ...	1	2018
DNA methylation signatures of arsenic exposure and obesity NY Noronha, GS Rodrigues, LM Watanabe, IHY Noma, JB Cazier, ... Environmental Epigenetics, dvaf019		2025
Unveiling PAI-1 as a new player in chemoresistant melanoma in aged human skin reconstructed in vitro RO da Silva, LA Carvalho, DE Camarena, JR Silva, IH Noma, MO Moraes, ... Cancer Research 84 (6_Supplement), 5410-5410		2024
Avaliação do efeito da conoidina-A no potencial de proliferação e invasão de células de melanoma NRAS-mutado ST Souza, IHY Noma, SS Maria-Engler Resumos		2023
Peroxirredoxina 2 na modulação do fenótipo invasivo em melanoma com mutação em NRAS IHY Noma		2023

TITLE	CITED BY	YEAR

Universidade de São Paulo

Gliotoxin and its potential cytotoxicity against NRAS-mutated melanoma 2022
IHY Noma, LAC Carvalho, SS Maria-Engler
Livro de Resumos

SURVEILLANCE OF CARBAPENEM-RESISTANT Enterobacteriaceae IN
DC MOREIRA, ABS ECKER, IHY NOMA, NH FEDRIGO, FV FABRI, ...
UNIVERSIDADE ESTADUAL DE MARINGÁ, PARANÁ, PR (AVENIDA COLOMBO, 5790, BLOCO I …

E. Evidence that the beneficiary is an author of scholarly articles in the field of science published in professional media

Isabella Harumi Yonehara Noma

URL to access this CV: http://lattes.cnpq.br/6549549395123599
Lattes ID: **6549549395123599**
Last updated on 07/31/2025

Postdoctoral Researcher at the H. Lee Moffitt Cancer Center. Doctor of Science from the School of Pharmaceutical Sciences (FCF) (CAPES score: 7) at the University of São Paulo (USP), with a focus on metastatic melanoma. We investigated the complexity of the microenvironment in the melanoma invasion process using three-dimensional reconstructed skin models at the Skin Pathophysiology Laboratory-USP. Bachelor's degree in Biomedicine from the State University of Maringá (2017), with undergraduate research projects in the areas of Basic Microbiology and Clinical Immunology (UEM). Master's degree in Health Sciences, working at the Clinical Cytology and Sexually Transmitted Diseases Laboratory, with a focus on the detection of HPV and other associated bacteria using molecular biology techniques. **(Text provided by the author)**

Personal Information

Name

Isabella Harumi Yonehara Noma

Name in bibliographic citations

NOMA, I. H. Y.;NOMA, ISABELLA;YONEHARA NOMA, ISABELLA HARUMI;HARUMI YONEHARA NOMA, ISABELLA;NOMA, ISABELLA HARUMI YONEHARA

Lattes iD

http://lattes.cnpq.br/6549549395123599

Orcid iD

? https://orcid.org/0000-0002-7413-1316

Country of Nationality

Brazil

Academic Background/Degree

2020 - 2023

Ph.D. in Pharmacy (Pathophysiology and Toxicology).
University of São Paulo, USP, Brazil.
Title: Peroxiredoxin 2 in the modulation of the invasive phenotype in NRAS-mutant melanoma, Year of completion: 2023.
Advisor: Silvya Stuchi Maria-Engler.
Fellow of: National Council for Scientific and Technological Development, CNPq, Brazil.

2018 - 2020

Master's degree in Health Sciences.
State University of Maringá, UEM, Brazil.
Title: Prevalence of Ureaplasma and

Mycoplasma in women of childbearing age from Maringá, Year of Completion: 2020.
Advisor: Marcia Edilaine Lopes Consolaro.
Fellow of: Coordination for the Improvement of Higher Education Personnel, CAPES, Brazil.
Areas of activity: Scientific research and development.

2023 - 2025

MBA in Data Science and Analytics, Distance Learning. (Course Load: 425h).
University of São Paulo, Luiz de Queiroz College of Agriculture, USP ESALQ, Brazil.
Title: Clustering of Cutaneous and Acral Melanoma: Impact of genetic alterations on immune response.
Advisor: William Barbosa.

2019 - 2020

Specialization in Biomedical Engineering with an emphasis in Clinical Engineering. (Course Load: 360h).
Estácio de Sá University, UNESA, Brazil.
Title: GEOSPATIAL ANALYSIS OF DIAGNOSTIC IMAGING EQUIPMENT ASSOCIATED WITH SOCIODEMOGRAPHIC CHARACTERISTICS IN BRAZIL.

2014 - 2017

Bachelor's degree in Biomedicine.
State University of Maringá, UEM, Brazil.
Title: Evaluation of the nociceptive response of mice infected with Leishmania (Leishmania) amazonensis and its relationship with nitric oxide.
Advisor: Sandra Mara Alessi Aristides.
Scholarship holder of: Coordination for the Improvement of Higher Education Personnel, CAPES, Brazil.

2011 - 2013

High School. (Secondary education).
Santa Cruz School, CSC, Brazil.

Postdoctoral Research

2023 - 2024

Postdoctoral Research.
H. Lee Moffitt Cancer Center and Research Institute, HLMCCRI, United States.
Fellow of: National Council for Scientific and Technological Development, CNPq, Brazil.
Major area: Health Sciences

Complementary Education

2020 - 2020

University extension course in DES-UEM Distance Courses – Applied Biostatistics. (Course load: 34h).
State University of Maringá, UEM, Brazil.

2020 - 2020

Health in Data: How to Work with the DataSUS Systems. (Course load: 2h).
State University of Maringá, UEM, Brazil.

2020 - 2020

Introduction to Machine Learning. (Course load: 2h).
State University of Maringá, UEM, Brazil.

2020 - 2020

French Language A2 - Paraná fala Francês (Paraná Speaks Ffrench – Language program)- Module 2 Online. (Couser load: 30h).
State University of Maringá, UEM, Brazil.

2020 - 2020

1st International Digital Congress on 3D Biofabrication and Bioprinting. (Course load: 8h).
Araraquara University, UNIARA, Brazil.

2020 - 2020

1st Basic Oncology Course of LAOMA.
(Course load: 10h).
Araraquara University, UNIARA, Brazil.

2020 - 2020

Understanding Cancer Metastasis. (Course load: 4h).
Johns Hopkins University, JHU, United States.

2020 - 2020

Introduction to the Biology of Cancer. (Course load: 8h).
Johns Hopkins University, JHU, United States.

2020 - 2020

Cell Culture and Applications in the Study of Chronic and Infectious Diseases. (Course load: 15h).
Federal University of Paraná, UFPR, Brazil.

2020 - 2020

XI Board Review in Medical Oncology: Update, Discussion, and Innovation and II. (Course load: 40h).
Einstein's Teaching and Research Institute, IIEPAE, Brazil.

2020 - 2020

Systematic Review: Essential Elements for Reliable Evidence. (Carga horária: 2h). State University of Maringá, UEM, Brazil.

2020 - 2020

Oral Communication and Academic Activities- Online Module. (Course load: 35h).
University of São Paulo, USP, Brazil.

2019 - 2019

University extension course in the 1st Vacation Course in Immunology and Immunogenetics. (Course load:

40h).
State University of Maringá, UEM, Brazil.

2019 - 2019

University extension course in UEM at Expoingá. (Course load: 5h).
State University of Maringá, UEM, Brazil.

2019 - 2019

University extension course in Active Learning. (Carga horária: 4h).
State University of Maringá, UEM, Brazil.

2019 - 2019

University extension course in Paraná fala francês A1- level 2. (Course load: 60h).
State University of Maringá, UEM, Brazil.

2019 - 2019

Conversation in French laguage. (Course load: 8h).
State University of Maringá, UEM, Brazil.

2019 - 2019

Discovering Phages. (Course load: 15h).
State University of Maringá, UEM, Brazil.

2019 - 2019

Extracellular Vesicles Involved in Chronic and Infectious Diseases. (Course load: 4h).
State University of Maringá, UEM, Brazil.

2019 - 2019

Primers: who are they and how to outline them?.
State University of Londrina, UEL, Brazil.

2018 - 2018

University extension course in Paraná fala Francês. (Course load: 60h).
State University of Maringá, UEM, Brazil.

2018 - 2018

University extension course in Paraná fala Inglês (Paraná Speaks Englsih). (Course load: 30h).
State University of Maringá, UEM, Brazil.

2016 - 2016

Antibiotic as Promotors of Antibiotic Resistance. (Course load: 2h).
Graduate Program in Biosciences and Pathophysiology, PBF, Brazil.

2014 - 2016

Japanese language. (Course load: 300h). State University of Maringá, UEM, Brazil.

2015 - 2015

In Situ Hepatic Perfusion. (Course load: 4h).
State University of Maringá, UEM, Brazil.

2015 - 2015

Study and Guidance for the Japanese Language Proficiency Examination N.05. (Course load: 12h).
State University of Maringá, UEM, Brazil.

2014 - 2014

Assisted Reproduction. (Course load: 4h).
State University of Londrina, UEL, Brazil.

Professional Experience

H. Lee Moffitt Cancer Center and Research Institute, HLMCCRI, United States.

Institutional Affiliation

2025 - Current

Employment Type: Employee under the Brazilian Labor Code (CLT), Position: Postdoctoral Researcher, Workload: 40, Work Schedule: Exclusive dedication.

Institutional Affiliation

2023 - 2024

Employment Type: Fellow, Position: Postdoctoral Researcher, Workload: 40

State University of Maringá, UEM, Brazil.

Institutional Affiliation

2018 - 2020

Employment Type: Fellow, Position: Graduate Student, Workload: 40, Work Schedule: Exclusive dedication.

Other information

Master's Degree in the Health Sciences Program - Fellow

Institutional Affiliation

2017 - 2017

Employment Type: Intern, Position: Mandatory Internship in Immunogenetics, Workload: 40

Institutional Affiliation

2017 - 2017

Employment Type: Intern, Position: Mandatory Internship in Clinical Analysis, Workload: 40

Institutional Affiliation

2014 - 2017

Employment Type: Teaching Project, Position: Laboratory Practice, Workload: 4

Institutional Affiliation

2015 - 2016

Employment Type: Fellow, Position: Microbiology Teaching Assistant, Workload: 20

University of São Paulo, USP, Brazil.

Institutional Affiliation

2022 - 2022

Employment Type: Fellow, Position: Teaching Intern, Workload: 6

Research projects

2023 - 2024

The role of HDAC8 in UVR-driven melanoma initiation

Description: Exposure to ultraviolet radiation (UVR) is the major risk factor for melanoma development and leads to complex phenotypic responses in melanocytes, including dendrite formation, enhanced melanogenesis, and cell cycle arrest. Previous studies identified the type I histone deacetylase,HDAC8, as an epigenetic regulator of melanoma cells induced following exposure to UVR,hypoxia, and BRAF inhibitor therapy. Our studies suggest that HDAC8 expression is upregulated in melanocytes that experience high levels of UVR-induced damage. No strategies yet exist to prevent the accumulation of sun-damaged melanocytes in the skin, representing a significant barrier to effective melanoma prevention. In this new proposal, we aimed to investigate whether UVR-induced HDAC8 activation allows some melanocytes to evade cell death, enabling cells with pathogenic mutations to survive and potentially develop into melanoma. We utilized a unique mouse melanoma model driven by mutant BRAF and heterozygous PTEN loss, in which HDAC8expression can be conditionally regulated in skin melanocytes, to elucidate the

role of epigenetic reprogramming in UVR-induced melanoma initiation. Additionally, we employed siRNA mediated overexpression of HDAC8 in cell lines. Our findings demonstrated that HDAC8overexpression following UV irradiation increases the survival of UVR-damaged melanocytes and melanoma cells, mediated by the downregulation of p53 and acetylated p53. This research provides a novel perspective on how UVR-induced epigenetic reprogramming targeting HDAC8contributes to the persistence of melanocytes and melanoma cells harboring pathogenic mutations, allowing them to grow and evade apoptosis..
Status: Completed; Type: Research.

Participants: Isabella Harumi Yonehara Noma - Participant / Silvya Stuchi Maria-Engler - Coordinator / SMALLEY, KEIRAN S. M. - Participant.

2023 - Current

METASTATIC POTENTIAL OF PEROXIREDOXIN 2 IN NRAS-MUTANT MELANOMAS

Description: Description: Cutaneous melanoma is a curable neoplasm when diagnosed at early stages, but it is associated with a worse disease course in advanced stages. Melanoma lesions in sun-exposed sites, associated with a high mutational burden and aberrant epigenetic regulation, contribute to the development and progression of the disease. This is the type of skin cancer with the highest metastatic potential, which leads to its high mortality rate. The main mutations occur in the MAPK pathway: 50% of patients have alterations in BRAF, 15-20% in NRAS, and the remainder in NF1 or are triple wild-type. Targeted therapy revolutionized the treatment of melanoma with the use of BRAF and MEK inhibitors; however, intrinsic and acquired resistance leads to treatment failure. In this context, NRAS mutations characterize an even more aggressive tumor with no specific therapeutic options, associating this mutation with a poorer prognosis. Intratumoral heterogeneity occurs as a consequence of the phenotypic plasticity of the cells, which assume and transition through different transcriptional states controlled by MITF levels, making the cells more proliferative or invasive. This event is associated with metastasis and therapy failure. Therefore, alternative pathways should be explored as potential options for the treatment of patients with NRAS mutations. Given that one of the factors limiting the efficacy of targeted therapy is resistance to oxidative stress, the control of redox homeostasis is an interesting starting point. Hydrogen peroxide plays a central role in redox signaling and is involved in all hallmarks of cancer, from proliferation to metabolism, angiogenesis, and immune system evasion. In addition, hydrogen peroxide is capable of altering MITF levels. In this context, molecules that regulate its activity are of substantial interest. Peroxiredoxins (PRDXs) are the main hydrogen peroxide-consuming enzymes in cells due to their high expression and catalytic capacity. Several studies report the role of antioxidant enzymes in redox homeostasis and tumorigenesis. In addition to the exceptional role of PRDXs in removing the oxidant from the environment, when they become oxidized and

inactive, they allow hydrogen peroxide to react directly or indirectly with other targets, thereby regulating the cell's redox signaling. Due to their central role in controlling hydrogen peroxide, PRDXs are directly involved in different types of cancer. In fact, the PRDX2 isoform is epigenetically silenced in some cancers, and our group recently demonstrated that it is also downregulated in patients with melanoma metastasis, reinforcing its involvement in the invasion process. In this context, it has been shown that PRDX2 is capable of inducing the stability of adherens junctions in BRAF-mutant melanomas. However, there are still no studies relating PRDXs to NRAS mutation; thus, we observed that NRAS-mutant melanoma cell lines showed different proliferative and invasive profiles, corresponding to differences in PRDX2 expression. Thus, our proposal is to investigate the role of PRDX2 in the phenotypic plasticity of NRAS-mutant melanomas. To this end, the silencing of this protein will be studied through gene editing (shRNA) and by a pharmacological PRDX inhibitor (Conoidin A), in addition to the effect of its restoration through the use of gliotoxin, a PRDX mimetic. Functional parameters such as proliferation, colony-forming ability, migration, and invasion in 3D skin models will be evaluated. Such observations reinforce our hypothesis that PRDX2 may be a novel target in the search for more specific therapies for NRAS-mutant melanomas.
Status: In progress; Type: Research.
Students involved: Doctoral students: (3) .

Participants: Isabella Harumi Yonehara Noma - Coordinator / Silvya Stuchi Maria-Engler - Participant / Larissa Anastácio da Costa Carvalho - Participant.

2016 - 2017

Variability of β-lhamases present in Gram-negative bacilli isolated during the hospitalization of patients in a Teaching Hospital

Project certified by Coordinator Maria Cristina Bronharo Tognim on 11/11/2019.

Description: Considering the importance of Gram-negative bacteria (GNB) in hospitals, especially in intensive care units, due to their antimicrobial resistance, and also considering that patients may be sources or reservoirs of these microorganisms, this project aims to assess the presence of different GNB that may colonize or infect patients during their hospital stay. As methodology, we will use the selection of patients who were registered in an Excel database from the project "Epidemiological Surveillance of Multidrug-Resistant Bacteria at the University Hospital of Maringá". Patients will be selected by the initials of their names, and only patients who present microbiological data on GNB isolates from colonization and infection will be included. All microorganisms will be retrieved from the freezer collection, and resistance to carbapenems will be confirmed by the agar dilution method. For all isolates, tests will be performed to detect β-lactam resistance

markers by PCR for ESBLs (bla CTX, bla SHV e bla TEM), as well as for oxacillinase-type carbapenemases and metallo-β-lactamases. The isolates will be molecularly characterized using the ERIC technique. For some cases, the use of antimicrobials in the patients' medical records will be evaluated. The aim is to use the findings to better understand the dynamics of the microorganism and its control.
Status: Completed; Type: Research.
Students involved: Undergraduate: (2) / Doctoral: (1).

Participants: Isabella Harumi Yonehara Noma - Participant / Alessandra Barrochelli da Silva Ecker - Participant / Déborah de Castro Moreira - participant / Maria Cristina Bronharo Tognim - Coordinator.

2016 - 2016

RELATIONSHIP BETWEEN NOCICEPTION AND NITRIC OXIDE PRODUCTION IN ANIMALS INFECTED WITH LEISHMANIA (LEISHMANIA) AMAZONENSIS

Project certified by Coordinator Thais Gomes Verzignassi Silveira on 03/02/2021.

Description: American tegumentary leishmaniasis, considered a neglected disease, is endemic in many countries, including Brazil. The disease is characterized by ulcers and may heal spontaneously or present severe and mutilating metastases in the mucosa. The lesions generally progress to ulcerations in the absence of pain, suggesting that the infection interferes with pain perception. In this project, we evaluated the pain threshold using increasing doses of the parasite with an electronic aesthesiometer, as well as paw edema in lesions induced by parasite inoculation, measured weekly with the aid of a plethysmometer and a caliper. The results showed an increase in edema as the parasite concentrations increased, as well as an increase in pain intensity. We also evaluated nitric oxide production in the paw lesions of animals inoculated with the parasite; however, the preliminary results were negative. In conclusion, we found that edema formation is accompanied by increased pain; however, nitric oxide production does not appear to be related to pain or edema.
Status: Completed; Type: Research.
Students involved: Undergraduate: (2) / Academic master's: (1).

Participants: Isabella Harumi Yonehara Noma - Participant / Paula Karoline Sfordi da Silva - Participant / Valéria Medrado Pereira - Participant / Sandra Mara Alessi Aristides - Participant / Thais Gomes Verzignassi Silveira - Coordinator.

Outreach projects

2016 - 2017

Dr. Genetics: Online Informational Content to Assist with Human Genetic Testing

Description: The dissemination of information through the internet has become increasingly rapid and accessible to the population as a whole. At the beginning of its use, access to it was, for the most part, limited to technology enthusiasts and professionals in the field; however, over the years, both access and the content available have come to be largely produced by laypeople on the subject. Brazilians represent the largest share worldwide in terms of average time spent browsing, enabling a major exchange of information, including in the field of health. Although very useful for access to information, many specialists emphasize that much of the information made available is inadequate or incomplete. In Brazil, there is no regulation or certification of websites containing health information. Therefore, it becomes essential to make information available to the general public, information provided by universities and grounded in scientific knowledge, which can thus help strengthen physician-patient trust and reduce the anxiety and discomfort resulting from illnesses. The objective of this project is to develop a website with information about genetic tests, including cytogenetic and molecular tests, in the various areas of genetics, including genetic counseling and oncology, among others. In addition to supplementary information, such as interpretation, where they can be performed, and their costs both through the Unified Health System and through health insurance. The website will be promoted through visits and posters at health clinics in Maringá, Maringá Municipal Hospital, and Maringá University Hospital. The information made available will be based on books, indexed articles, and supporting laboratories and hospitals. Students in the fields of Biomedicine and Medicine participating in the project will gain knowledge about genetic tests and will have the opportunity to transmit this knowledge to the general population, since the demand for such tests is increasing, whether for diagnostic assistance, prognosis, or even the establishment of therapeutic approaches, taking into account the promising development of new targeted molecular drugs.
Status: Completed; Type: Outreach.
Students Involved: Undergraduate: (7) .

Participants: Isabella Harumi Yonehara Noma - Participant / Tamires Leite Valverde - Participant / Veronica Elisa Pimenta Vicentini - Participant / Luiz Felipe MSB Blanco - Participant / Lucas B Gremaschi - Participant / Patrícia Alexandra da Silva Saavedra Romão - Participant / Natalia Mestre Braz - Participant / Eliane Papa Ambrosio Albuquerque - Coordinator.

2016 - 2016

Student integration in the execution of volunteer activities, aiming at greater dissemination and awareness among the Maringá community about little-known diseases that manifest in childhood, progressive muscular dystrophies with emphasis on Muscular Dystrophy.

Project certified by Coordinator Maria Ida Bonini Ravanelli Speziali on 19/10/2022.

Description: It is believed that the vast majority of society is unaware of the occurrence of some diseases that affect children. Muscular dystrophies fit perfectly within the group of little-known diseases. Although they are considered rare, their incidence strongly contradicts this premise. Through this work, the aim is, through the integration of students from the various areas of the State University of Maringá, to carry out activities that promote greater knowledge within society about diseases that affect children. In this way, the first year of this project, which intends to address one disease per year, will direct its activities toward muscular dystrophies, thus allowing society to mobilize by participating in events committed to improving the quality of life of patients affected by Duchenne muscular dystrophy, chosen as the slogan of this project because it is the most frequent as well as the most lethal among all muscular dystrophies.
Status: Completed; Type: Outreach.
Students Involved: Undergraduate: (13) .

Participants: Isabella Harumi Yonehara Noma - Participant / Paula Karoline Sfordi da Silva - Participant / Tamires Leite Valverde - Participant / Patrícia Alexandra da Silva Saavedra Romão - Participant / Natalia Mestre Braz - Participant / Lais de Sena Marques - Participant / Maria Ida Bonini Ravanelli Speziali - Coordinator / Fabiana Galvão da Motta Lima - Participant / Francyhellen Franco Montanha - Participant / Gabriela Fernanda Martinez - Participant / Maria Naomi Yamanaka - Participant / Sara Fabricio dos Santos - Participant / Aarão Fabrício dos Santos - Participant / Leonardo de Oliveira Figueiredo - Participant.

Areas of Expertise

1.

Major Area: Health Sciences / Area: Pharmacy / Subarea: Clinical Cytology.

2.

Major Area: Health Sciences / Area: Pharmacy / Subarea: Pathophysiology and Laboratory Diagnosis.

Languages

English

Understands Well, Speaks Fairly, Reads Well, Writes Fairly.

Awards and Honors

2022

Honorable Mention for the study entitled "Targeting Peroxiredoxin-2 as a potential treatment for NRAS-mutated metastatic melanoma", University of São Paulo.

2022

SMR PARTICIPATION AWARD, Society for Melanoma Research.

Publications

Bibliographic Production

Citations

Google Scholar

Total number of studies: 19 Total number of citations: 74 Date: 02/10/2025

https://scholar.google.com/citations?user=gtlvO4YAAAAJ&hl=pt-BR

Full Articles Published in Journals

Sort by

Chronological Order

1.

NORONHA,, NATÁLIA YUMI ; RODRIGUES, GUILHERME DA SILVA ; WATANABE, LÍGIA MORIGUCHI ; **NOMA, ISABELLA HARUMI YONEHARA** ; CAZIER, JEAN-BAPTISTE ; SAE-LEE, CHANACHAI ; CHITTA, PITAKSIN ; PEREIRA, VANESSA APARECIDA BATISTA ; PINHEL, MARCELA AUGUSTA DE SOUZA ; DIANI, LUISA MARIA ; BARBOSA, FERNANDO ; PLÖSCH, TORSTEN ; NONINO, CARLA BARBOSA . DNA methylation signatures of arsenic exposure and obesity. Environmental Epigenetics JCR , v. -, p. -, 2025.

2.

DA SILVA RODRIGUES, GUILHERME ; NORONHA, NATÁLIA YUMI ; **NOMA, ISABELLA HARUMI YONEHARA** ; DE LIMA, JOÃO GABRIEL RIBEIRO ; DA SILVA SOBRINHO, ANDRESSA CRYSTINE ; DE SOUZA PINHEL, MARCELA AUGUSTA ; DE ALMEIDA, MARIANA LUCIANO ; WATANABE, LÍGIA MORIGUCHI ; NONINO, CARLA BARBOSA ; JÚNIOR, CARLOS ROBERTO BUENO . 14-Week exercise training modifies the DNA methylation levels at gene sites in non-Alzheimer's disease women aged 50 to 70 years. EXPERIMENTAL GERONTOLOGY JCR , v. 186, p. 112362, 2024. **Citations:** WEB OF SCIENCE™ 2 | SCOPUS 2

3.

NORONHA, NATÁLIA YUMI ; **NOMA, ISABELLA HARUMI YONEHARA** ; FERNANDES FERREIRA, RAFAEL ; RODRIGUES, GUILHERME DA SILVA ; MARTINS, LUZANIA DOS SANTOS ; WATANABE, LÍGIA MORIGUCHI ; PINHEL, MARCELA AUGUSTA DE SOUZA ; MELLO SCHINEIDER, ISABELLE ; DIANI, LUISA MARIA ; CARLOS, DANIELA ; NONINO, CARLA BARBOSA . Association between the relative abundance of phyla and bacteria, vitamin C consumption,

and DNA methylation of genes linked to immune response pathways. FRONTIERS IN NUTRITION JCR , v. 11, p. -, 2024. **Citations:** WEB OF SCIENCE ™ 2 | SCOPUS 1

4.

NOMA, ISABELLA HARUMI YONEHARA; CARVALHO, LARISSA ANASTACIO DA COSTA ; CAMARENA, DENISSE ESTHER MALLAUPOMA ; SILVA, RENAIRA OLIVEIRA ; MORAES JUNIOR, MANOEL OLIVEIRA DE ; DE SOUZA, SOPHIA TAVARES ; NEWTON-BISHOP, JULIA ; NSENGIMANA, JEREMIE ; MARIA-ENGLER, SILVYA STUCHI . Peroxiredoxin-2 represses NRAS-mutated melanoma cells invasion by modulating EMT markers. BIOMEDICINE & PHARMACOTHERAPY JCR , v. 177, p. 116953, 2024. **Citations:** WEB OF SCIENCE ™ 2

5.

CARVALHO, LARISSA ANASTACIO DA COSTA ; **NOMA, ISABELLA HARUMI YONEHARA** ; UEHARA, ADRIANA HIROMI ; SIENA, ADAMO DAVI DIOGENES ; OSAKI, LUCIANA HARUMI ; MORI, MATEUS PRATES ; PINTO, NADJA CRISTHINA DE SOUZA ; FREITAS, VANESSA MORAIS ; JUNIOR, WILSON ARAUJO SILVA ; SMALLEY, KEIRAN S. M. ; MARIA-ENGLER, SILVYA STUCHI . Modeling Melanoma Heterogeneity In Vitro: Redox, Resistance and Pigmentation Profiles. ANTIOXIDANTS JCR , v. 13, p. 555, 2024. **Citations:** WEB OF SCIENCE ™ 2 | SCOPUS 2

6.

FRANCO, YLANA ADAMI ; DE MORAES, MANOEL OLIVEIRA ; CARVALHO, LARISSA A. C. ; DOHLE, WOLFGANG ; DA SILVA, RENAIRA OLIVEIRA ; **NOMA, ISABELLA HARUMI YONEHARA** ; LIMA, KELI ; POTTER, BARRY V. L. ; MACHADO-NETO, JOÃO A. ; MARIA-ENGLER, SILVYA STUCHI . 2-Methoxyestradiol-3,17-O,O-bis-sulfamate (STX140) Inhibits Proliferation and Invasion via Senescence Pathway Induction in Human BRAFi-Resistant Melanoma Cells. INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES JCR , v. 24, p. 11314, 2023. **Citations:** WEB OF SCIENCE ™ 1 | SCOPUS 1

7.

DA SILVA RODRIGUES, GUILHERME ; **NOMA, ISABELLA HARUMI YONEHARA** ; NORONHA, NATALIA YUMI ; WATANABE, LIGIA MORIGUCHI ; DA SILVA SOBRINHO, ANDRESSA CRYSTINE ; DE LIMA, JOÃO GABRIEL RIBEIRO ; SAE-LEE, CHANACHAI ; BENJAMIM, CICERO JONAS RODRIGUES ; NONINO, CARLA BARBOSA ; BUENO, CARLOS ROBERTO . Eight Weeks of Physical Training Decreases 2 Years of DNA Methylation Age of Sedentary Women. RESEARCH QUARTERLY FOR EXERCISE AND SPORT JCR , v. -, p. 1-11, 2023. **Citations:** WEB OF SCIENCE ™ 2 | SCOPUS 2

8.

WATANABE, LÍGIA MORIGUCHI ; PEREIRA, VANESSA APARECIDA BATISTA ; NORONHA, NATALIA YUMI ; DE SOUZA PINHEL, MARCELA AUGUSTA ; WOLF, LETICIA SANTANA ; DE OLIVEIRA, CRISTIANA CORTES ; PLAÇA, JESSICA RODRIGUES ; **NOMA, ISABELLA HARUMI YONEHARA** ; DA SILVA RODRIGUES, GUILHERME ; DE SOUZA, VANESSA CRISTINA OLIVEIRA ; JUNIOR, FERNANDO BARBOSA ; NONINO, CARLA BARBOSA . The influence of serum selenium in differential epigenetic and transcriptional regulation of CPT1B gene in women with obesity. JOURNAL OF TRACE ELEMENTS IN MEDICINE AND BIOLOGY JCR , v. -, p. 127376, 2023. **Citations:** WEB OF SCIENCE ™ 3 | SCOPUS 2

9.

YUMI NORONHA, NATÁLIA ; DA SILVA RODRIGUES, GUILHERME ; **HARUMI YONEHARA NOMA, ISABELLA** ; FERNANDA CUNHA BRANDAO, CAMILA ; PEREIRA RODRIGUES, KARINE ; COLELLO BRUNO, ALEXANDRE ; SAE-LEE, CHANACHAI ; MORIGUCHI WATANABE, LIGIA ; AUGUSTA DE SOUZA PINHEL, MARCELA ; MELLO SCHINEIDER, ISABELLE ; LUCIANO DE ALMEIDA, MARIANA ; BARBOSA JUNIOR, FERNANDO ; ARAUJO MORAIS, DÉBORAH ; TAVARES DE SOUSA JUNIOR, WELLINGTON ; PLÖSCH, TORSTEN ; ROBERTO BUENO JUNIOR, CARLOS ; BARBOSA NONINO, CARLA . 14-weeks combined exercise epigenetically modulated 118 genes of menopausal women with prediabetes. Frontiers in Endocrinology JCR, v. 13, p. 895489, 2022. **Citations:** WEB OF SCIENCE ™ 14 | SCOPUS 11

10.

DOMINGUES, KARLA LARISSA TRASSI GANAZA ; TANOSHI, CÍNTHIA AKEMI ; **NOMA, ISABELLA HARUMI YONEHARA** ; CARVALHO, MARIA DALVA DE BARROS ; PELLOSO, SANDRA MARISA ; CONSOLARO, MARCIA EDILAINE LOPES ; ARISTIDES, SANDRA MARA ALESSI ; DEMARCHI, IZABEL GALHARDO ; PEDROSO, RAISSA BOCCHI ; LONARDONI, MARIA VALDRINEZ CAMPANA . Economic impact of hospitalizations due to leishmaniasis in southern Brazil. RESEARCH, SOCIETY AND DEVELOPMENT, v. 11, p. e39211831139, 2022.

11.

NORONHA, NATÁLIA YUMI ; DA SILVA RODRIGUES, GUILHERME ; DE SOUZA PINHEL, MARCELA AUGUSTA ; CAZIER, JEAN-BAPTISTE ; WATANABE, LÍGIA MORIGUCHI ; MENEZES, ALBERT NOBRE ; BUENO, CARLOS ROBERTO ; NICOLETTI, CAROLINA FERREIRA ; DE OLIVEIRA, BRUNO AFFONSO PARENTI ; SCHINEIDER, ISABELLE MELLO ; **YONEHARA NOMA, ISABELLA HARUMI** ; DIAS ALCARÁS, IGOR CAETANO ; BARBOSA, FERNANDO ; BARBOSA NONINO, CARLA . Sample Preparation to Bioinformatics Analysis of DNA Methylation: Association Strategy for Obesity and Related Trait Studies. Jove-Journal of Visualized Experiments JCR, v. 183, p. e62598, 2022. **Citations:**
WEB OF SCIENCE ™ 8 | SCOPUS 7

12.

NOMA, ISABELLA; SHINOBU-MESQUITA, CRISTIANE ; SUEHIRO, TAMY ; MORELLI, FABRICIO ; DE SOUZA, MARIA ; DAMKE, EDILSON ; DA SILVA, VÂNIA ; CONSOLARO, MARCIA . Association of Righ-Risk Human Papillomavirus and Ureaplasma parvum Co-Infections with Increased Risk of Low-Grade Squamous Intraepithelial Cervical Lesions. Asian Pacific Journal of Cancer Prevention, v. 22, p. 1239-1246, 2021. **Citations:** SCOPUS 8

13.

SANTOS E SILVA, JUAN CARLO ; VASCONCELOS, AMANDA PEREIRA ; **YONEHARA NOMA, ISABELLA HARUMI** ; NORONHA, NATÁLIA YUMI ; AQUINO, RODRIGO ; GIDDALURU, JEEVAN ; DURÃO, LUIZ ; COSTA-MARTINS, ANDRÉ GUILHERME ; SCHUCH, VIVIANE ; MORAES-VIEIRA, PEDRO M. ; NAKAYA, HELDER I. . Gene Signatures of Autopsy Lungs from Obese Patients with COVID-19. CLINICAL NUTRITION ESPEN JCR , v. 43, p. 986, 2021. **Citations:**
WEB OF SCIENCE ™
8 | SCOPUS 8

1.

TANOSHI, C. A. ; **NOMA, I. H. Y.** ; GANAZA, K. L. T. ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. ; ARISTIDES, S. M. A. . HOSPITAL MORBIDITY EXPENDITURES DUE TO VISCERAL LEISHMANIASIS IN A REGION OF SOUTHERN BRAZIL. In: 5th International Congress of the CCS, 3rd International Congress on Obesity Treatment and 7th PCS Meeting. State University of Maringá, 2018, Maringá. Proceedings of the 5th International Congress of the CCS, 3rd International Congress on Obesity Treatment and 7th PCS Meeting, 2018. p. 5.

2.

GANAZA, K. L. T. ; TANOSHI, C. A. ; **NOMA, I. H. Y.** ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; CONSOLARO, M. E. L. ; ARISTIDES, S. M. A. ; DEMARCHI, I. G. ; LONARDONI, M. V. C. . ANALYSIS OF HOSPITAL MORBIDITY DUE TO CUTANEOUS LEISHMANIASIS IN THE STATE OF PARANÁ, BRAZIL, 2008-2018. In: 5th International Congress of the CCS, 3rd International Congress on Obesity Treatment and 7th PCS Meeting. State University of Maringá, 2018, Maringá. Proceedings of the 5th International Congress of the CCS, 3rd International Congress on Obesity Treatment and 7th PCS Meeting, 2018.

3.

NOMA, I. H. Y.; GANAZA, K. L. T. ; TANOSHI, C. A. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; ARISTIDES, S. M. A. ; DEMARCHI, I. G. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. . MUCOCUTANEOUS LEISHMANIASIS IN PARANÁ: EVALUATION OF HOSPITAL MORBIDITY FROM 2008 TO 2018. In: 5th International Congress of the CCS, 3rd International Congress on Obesity Treatment and 7th PCS Meeting. State University of Maringá, 2018, Maringá. Proceedings of the 5th International Congress of the CCS, 3rd International Congress on Obesity Treatment and 7th PCS Meeting, 2018. p. 6.

4.

TANOSHI, C. A. ; GANAZA, K. L. T. ; **NOMA, I. H. Y.** ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; CONSOLARO, M. E. L. ; LONARDONI, M. V. C. ; ARISTIDES, S. M. A. . The Prevalence of a Neglected Disease: Epidemiology and Morbidity of Leishmaniases in Paraná. In: V International Meeting of Biosciences and Physiopathology and 9th Symposium on Biosciences Applied to Pharmacy., 2018, Maringá. Proceedings of the 9th Academic Week of Biomedicine, 2018.

5.

NOMA, I. H. Y.; ECKER, A. B. S. ; MOREIRA, D. C. ; SILVA, L. C. ; PEREIRA, P. M. ; SZCZEREPA, M. M. A. ; TOGNIM, M. C. B. . VARIABILITY OF β-LACTAMASES PRESENT IN GRAM-NEGATIVE BACILLI ISOLATED DURING PATIENTS' STAY IN A TEACHING HOSPITAL. In: 26th Annual Scientific Initiation Meeting and 6th Annual Junior Scientific Initiation Meeting, 2017, Maringá. Proceedings of the XXVI EAIC VI EAIC Jr., 2017. p. 67.

6.

ALBUQUERQUE, E. P. A. ; MONTANHA, F. F. ; **NOMA, I. H. Y.** ; GREMASCHI, L. B. ; BLANCO, L. F. M. ; BRAZ, N. M. ; ROMAO, P. A. S.

S. ; VALVERDE, T. L. ; VICENTINI, V. E. P. . Dr. Genetics: Information in the Service of the Community. In: UEM Outreach and Culture Forum: University Outreach, Commitment and Social Transformation, 2016, Maringá. Proceedings of the 14th UEM Outreach and Culture Forum: University Outreach: Commitment and Social Transformation, 2016. v. 23. p. 13-16.

7.

NOMA, I. H. Y.; PEREIRA, V. M. ; SANUKI, M. S. ; BORGHI, S. M. ; VERRI JUNIOR, W. A. ; SILVA, P. K. S. ; ARISTIDES, S. M. A. . RELATIONSHIP BETWEEN NOCICEPTION AND NITRIC OXIDE PRODUCTION IN ANIMALS INFECTED WITH LEISHMANIA (LEISHMANIA) AMAZONENSIS. In: 25th Annual Scientific Initiation Meeting and 5th Annual Junior Scientific Initiation Meeting, 2016, Maringá. Proceedings of the XXV EAIC V EAIC Jr., 2016. p. 62.

8.

NOMA, I. H. Y.; WATANABE, A. S. M. ; SILVA, M. R. T. ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . Evaluation of the Pathogenicity and Virulence Factors of Candida albicans in the Experimental Candidemia Model. In: 3rd Academic Week of Biomedicine ? UEM, 2015, Maringá. PROCEEDINGS OF THE THIRD ACADEMIC WEEK OF BIOMEDICINE , 2015.

9.

WATANABE, A. S. M. ; SILVA, M. R. T. ; **NOMA, I. H. Y.** ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . EVALUATION OF THE VIRULENCE FACTORS OF CANDIDA TROPICALIS AFTER EXPERIMENTAL CANDIDEMIA. In: 3rd Academic Week of Biomedicine ? UEM, 2015, Maringá. PROCEEDINGS OF THE THIRD ACADEMIC WEEK OF BIOMEDICINE , 2015.

10.

SILVA, M. R. T. ; WATANABE, A. S. M. ; **NOMA, I. H. Y.** ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . EVALUATION OF THE PATHOGENICITY PROFILE OF CANDIDA ALBICANS AFTER SUBSEQUENT CONTACTS WITH THE HOST. In: 3rd Academic Week of Biomedicine ? UEM, 2015, Maringá. PROCEEDINGS OF THE THIRD ACADEMIC WEEK OF BIOMEDICINE , 2015.

Abstracts Published in Conference Proceedings

1.

RODRIGUES, R. F. ; SILVA, L. L. ; TANOSHI, C. A. ; **NOMA, I. H. Y.** ; SILVA, G. E. C. . ANALYSIS OF THE EPIDEMIOLOGICAL PROFILE OF ACCIDENTS INVOLVING VENOMOUS ANIMALS IN THE STATE OF PARANA: DURING THE PERIOD FROM 2015 TO 2019. In: VII International Meeting of Biosciences and Physiopathology and 11th Symposium on Biosciences Applied to Pharmacy, 2020, Maringá. Proceedings of the VII International Meeting of Biosciences and Physiopathology and 11th Symposium on Biosciences Applied to Pharmacy. Maringá: Isabella Harumi Yonehara Noma, 2020. v. 1.

2.

NOMA, I. H. Y.; TANOSHI, C. A. ; SANTOS, D. S. ; RODRIGUES, R. F. ; SILVA, L. L. ; CONSOLARO, M. E. L. ; SOUZA, E. M. ; PELLOSO, S. M.

; PEDROSO, R. B. . THE PREVALENCE OF SYPHILIS IN PRENATAL CARE WOMEN IN PARANA STATE, BRAZIL. In: III INTERNATIONAL SYMPOSIUM OF EXPERIMENTAL PATHOLOGY, 2019, Londrina. Proceedings of the III International Symposium os Experimental Pathology. Londrina : UEL, 2019: Eduardo José de Almeida Araújo ...[et al.], 2019. p. 48.

3.

TANOSHI, C. A. ; **NOMA, I. H. Y.** ; RODRIGUES, R. F. ; SILVA, L. L. ; ROSSONI, D. F. ; PEDROSO, R. B. ; ARISTIDES, S. M. A. . CHALLENGES TO HUMAN LEISHMANIASIS: DISCUSSION OF THE DISEASE BASED ON ITS BEHAVIOR. In: III INTERNATIONAL SYMPOSIUM OF EXPERIMENTAL PATHOLOGY, 2019, Londrina. Proceedings of the
III International Symposium os Experimental Pathology, 2019. p. 40.

4.

NOMA, I. H. Y.; SUEHIRO, T. T. ; MORELLI, F. ; SOUZA, M. V. F. ; MESQUITA, C. S. S. ; SILVA, V. R. S. ; CONSOLARO, M. E. L. . Co-infections between Human Papillomavirus (HPV) and genital mycoplasmas in asymptomatic women. In: VI International Meeting of Biosciences and Physiopathology ? 10th Symposium on Biosciences Applied to Pharmacy, 2019, Ivatuba. Proceedings of the VI International Meeting of Biosciences & Physiopathology. Maringá, 2019. p. 227.

5.

NOMA, I. H. Y.; GANAZA, K. L. T. ; TANOSHI, C. A. ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; ARISTIDES, S. M. A. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. . PUBLIC EXPENDITURES ON HOSPITAL MORBIDITY DUE TO LEISHMANIASIS IN PARANA, BRAZIL, BRAZIL. In: V International Meeting of Biosciences and Physiopathology e 9th Symposium on Biosciences Applied to Pharmacy., 2018, Maringá. Proceedings of the de Simpósio do Programa de Biociências e Fisiopatologia- UEM, 2018. v. 1. p. 175.

6.

NOMA, I. H. Y.; ECKER, A. B. S. ; SZCZEREPA, M. M. A. ; TOGNIM, M. C. B. . Dissemination of enterobacteria among patients during hospitalization in the intensive care unit of a teaching hospital in southern Brazill. In: IV International Meeting of Biosciences and Physiopathology, 2017, Maringá. 7th Symposium on Biosciences Applied to Pharmacy, 2017.

7.

MOREIRA, D. C. ; ECKER, A. B. S. ; **NOMA, I. H. Y.** ; FEDRIGO, N. H. ; FABRI, F. V. ; COSTA, B. B. ; ZERBINATI, H. T. ; MENEZES, A. V. ; FERNANDES, A. S. ; TOGNIM, M. C. B. . SURVEILLANCE OF CARBAPENEM-RESISTANT ENTEROBACTERIACEAE IN PATIENTS DURING HOSPITALIZATION IN AN ADULT INTENSIVE CARE UNIT. In: 29th BRAZILIAN CONGRESS OF MICROBIOLOGY - CBM, 2017, Foz do Iguaçu. 29th Brazilian Congress of Microbiology 2017, 2017.

8.

SILVA, L. C. ; PEREIRA, P. M. ; DURANTE, P. C. ; MATTOS, M. S. F. ; ZERBINATI, H. T. ; **NOMA, I. H. Y.** ; FERNANDES, A. S. ; CARDOSO, C. L. ; TOGNIM, M. C. B. ; NISHIYAMA, S. A. B. . PRELIMINARY EVALUATION OF THE FREQUENCY OF ACINETOBACTER BAUMANNI ISOLATE AT THE BUCAL AND NASAL CAVITY FROM UNIVERSITY STUDENTS. In: 29th Brazilian Congress of Microbiology, 2017, Foz do Iguaçu. 29th BRAZILIAN CONGRESS OF MICROBIOLOGY 2017, 2017.

9.

FEDRIGO, N. H. ; MATTA, P. E. G. ; PEREIRA, P. M. ; **NOMA, I. H. Y.** ; TOGNIM, M. C. B. . Activity of fosfomycin against Acinetobacter baumannii and performance of susceptibility testing methods. In: 7th Symposium on Biosciences Applied to Pharmacy and III International Meeting of Biosciences and Physiopathology, 2016, Maringá. Proceedings of the 7th Symposium on Biosciences Applied to Pharmacy, 2016.

10.

NOMA, I. H. Y.; PEREIRA, V. M. ; SILVA, P. K. S. ; SANUKI, M. S. ; SILVEIRA, T. G. V. ; ARISTIDES, S. M. A. . RELATIONSHIP BETWEEN NOCICEPTION AND NITRIC OXIDE PRODUCTION IN ANIMALS INFECTED WITH Leishmania (Leishmania) amazonensis. In: 4th Academic Week of Biomedicine - UEM, 2016, Maringá. Proceedings of the 4th Week of Biomedicine, 2016.

11.

MATTA, P. E. G. ; ECKER, A. B. S. ; ZARPELLON, M. N. ; MOREIRA, D. C. ; **NOMA, I. H. Y.** ; TAMURA, N. K. ; GARCIA, L. B. ; TOGNIM, M. C. B. . PREVALENCE OF RESISTANCE TO CARBAPENEMS IN THE MAIN GRAM-NEGATIVE BACILLI ISOLATED IN A TEACHING HOSPITAL. In: 14th Brazilian Congress on Infection Control and Hospital Epidemiology, 2014, Curitiba. Journal of Infection Control, 2014. v. 3. p. 139.

12.

VIANA, G. F. ; ZAGO, M. C. B. ; SAALFELD, S. M. S. ; **NOMA, I. H. Y.** ; MATTA, P. E. G. ; MITSUGUI, C. S. ; ABREU FILHO, B. A. ; TOGNIM, M. C. B. . CHANGES IN THE MODE OF DISSEMINATION OF Acinetobacter baumannii ISOLATES IN A UNIVERSITY HOSPITAL. In: 14th Brazilian Congress on Infection Control and Hospital Epidemiology, 2014, Curitiba. Journal of Infection Control, 2014. v. 3. p. 148.

13.

ZAGO, M. C. B. ; VIANA, G. F. ; SAALFELD, S. M. S. ; **NOMA, I. H. Y.** ; FARIAS, A. P. M. ; HELBEL, C. ; TOGNIM, M. C. B. . DISSEMINATION OF Acinetobacter baumannii IN A TERTIARY HOSPITAL IN THE NORTHERN REGION OF PARANA. In: 14th BRAZILIAN CONGRESS ON INFECTION CONTROL AND HOSPITAL EPIDEMIOLOGY, 2014, Curitiba. DISSEMINATION OF Acinetobacter baumannii IN A TERTIARY HOSPITAL IN THE NORTHERN REGION OF PARANA, 2014. v. 3. p. 135.

Paper Presentations

1.

NOMA, I. H. Y.; CRUZ, E. ; DUTRA, A. C. ; NEGRI, M. F. N. . Geospatial Analysis of Diagnostic Imaging Equipment in Brazil. 2020. (Paper Presentation/Conference or Lecture).

2.

CRUZ, E. ; **NOMA, I. H. Y.** ; DUTRA, A. C. ; NEGRI, M. F. N. . Life-Sustaining Equipment: A Demographic Geospace Analysis in National Territory. 2020. (Paper Presentation/Conference or Lecture).

3.

RODRIGUES, R. F. ; SILVA, L. L. ; TANOSHI, C. A. ; **NOMA, I. H. Y.** ; SILVA, G. E. C. . ANALYSIS OF THE EPIDEMIOLOGICAL PROFILE OF SCORPIONISM IN THE SOUTHERN STATES OF BRAZIL: A STUDY REGARDING THE PERIOD FROM 2015 TO 2019. 2020. (Paper Presentation/Other).

4.

NOMA, I. H. Y.; SUEHIRO, T. T. ; MORELLI, F. ; SOUZA, M. V. F. ; MESQUITA, C. S. S. ; SILVA, V. R. S. ; CONSOLARO, M. E. L. . Co-infections between Human Papillomavirus (HPV) and genital mycoplasmas in asymptomatic women. (Paper Presentation/Symposium).

5.

TANOSHI, C. A. ; **NOMA, I. H. Y.** ; RODRIGUES, R. F. ; SILVA, L. L. ; PEDROSO, R. B. ; ARISTIDES, S. M. A. . Challenges to human leishmaniasis: discussion of the disease based on its behavior. 2019. (Paper Presentation/Symposium).

6.

NOMA, I. H. Y.; TANOSHI, C. A. ; RODRIGUES, R. F. ; SILVA, L. L. ; CONSOLARO, M. E. L. ; PEDROSO, R. B. . The prevalence of syphilis in prenatal care women in Paraná State, Brazil. 2019. (Paper Presentation/Symposium).

7.

NOMA, I. H. Y.; GANAZA, K. L. T. ; TANOSHI, C. A. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; ARISTIDES, S. M. A. ; DEMARCHI, I. G. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. . Mucocutaneous leishmaniasis in Paraná, evaluation of hospital morbidity from 2008 to 2018.. 2018. (Paper Presentation/Congress).

8.

GANAZA, K. L. T. ; TANOSHI, C. A. ; **NOMA, I. H. Y.** ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; CONSOLARO, M. E. L. ; ARISTIDES, S. M. A. ; DEMARCHI, I. G. ; LONARDONI, M. V. C. . Analysis of hospital morbidity due to cutaneous leishmaniasis in the state of Paraná, Brazil, 2008-2018. 2018. (Paper Presentation/Congress).

9.

TANOSHI, C. A. ; **NOMA, I. H. Y.** ; GANAZA, K. L. T. ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. ; ARISTIDES, S. M. A. . HOSPITAL MORBIDITY EXPENDITURES DUE TO VISCERAL LEISHMANIASIS IN A REGION OF SOUTHERN BRAZIL.. 2018. (Paper Presentation/Congress).

10.

TANOSHI, C. A. ; GANAZA, K. L. T. ; **NOMA, I. H. Y.** ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; CONSOLARO, M. E. L. ; LONARDONI, M. V. C. ; ARISTIDES, S. M. A. . The prevalence of a neglected infectious disease: epidemiology and morbidity of leishmaniases in Paraná. 2018. 2018. (Paper Presentation/Symposium).

11.

NOMA, I. H. Y.; GANAZA, K. L. T. ; TANOSHI, C. A. ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; ARISTIDES, S. M. A. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. . PUBLIC EXPENDITURES ON HOSPITAL MORBIDITY DUE TO LEISHMANIASIS IN PARANÁ, BRAZIL. 2018. (Paper Presentation/Symposium).

12.

ECKER, A. B. S. ; MOREIRA, D. C. ; **NOMA, I. H. Y.** ; VIANA, G. F. ; MITSUGUI, C. S. ; PEREIRA, P. M. ; TOGNIM, M. C. B. . CLONAL VARIABILITY AND INVESTIGATION OF BETA-LACTAMASE GENES IN ACINETOBACTER BAUMANNII ISOLATES OBTAINED DURING PATIENTS' STAY IN THE INTENSIVE CARE UNIT.
2017. (Paper Presentation/Congress).

13.

PEREIRA, P. M. ; MATTA, P. E. G. ; **NOMA, I. H. Y.** ; SILVA, L. C. ; FARIAS, A. P. M. ; ZARPELLON, M. N. ; TOGNIM, M. C. B. . ACTIVITY OF FOSFOMYCIN AGAINST ACINETOBACTER BAUMANNII ISOLATES: EVALUATION OF DIFFERENT METHODOLOGIES. 2017.
(Paper Presentation/Congress).

14.

NOMA, I. H. Y.; ECKER, A. B. S. ; MOREIRA, D. C. ; SILVA, L. C. ; PEREIRA, P. M. ; SZCZEREPA, M. M. A. ; TOGNIM, M. C. B. . VARIABILITY OF β-LACTAMASES PRESENT IN GRAM-NEGATIVE BACILLI ISOLATED DURING PATIENTS' STAY IN A TEACHING HOSPITAL. 2017. (Paper Presentation/Other).

15.

NOMA, I. H. Y.; ECKER, A. B. S. ; SZCZEREPA, M. M. A. ; TOGNIM, M. C. B. . Dissemination of enterobacteria among patients during hospitalization in the intensive care unit of a teaching hospital in southern Brazill.. 2017. (Paper Presentation/Symposium).

16.

SILVA, L. C. ; PEREIRA, P. M. ; DURANTE, P. C. ; MATTOS, M. S. F. ; ZERBINATI, H. T. ; **NOMA, I. H. Y.** ; FERNANDES, A. S. ; CARDOSO, C. L. ; TOGNIM, M. C. B. ; NISHIYAMA, S. A. B. . PRELIMINARY EVALUATION OF THE FREQUENCY OF ACINETOBACTER BAUMANNI ISOLATE AT THE BUCAL AND NASAL CAVITY FROM UNIVERSITY STUDENTS. 2017. (Paper Presentation/Congress).

17.

MOREIRA, D. C. ; ECKER, A. B. S. ; **NOMA, I. H. Y.** ; FEDRIGO, N. H. ; FABRI, F. V. ; COSTA, B. B. ; ZERBINATI, H. T. ; MENEZES, A. V. ; FERNANDES, A. S. ; TOGNIM, M. C. B. . SURVEILLANCE OF CARBAPENEM-RESISTANT ENTEROBACTERIACEAE IN PATIENTS DURING HOSPITALIZATION IN AN ADULT INTENSIVE CARE UNIT.. 2017. (Paper Presentation/Congress).

18.

ROMAO, P. A. S. S. ; ALBUQUERQUE, E. P. A. ; MONTANHA, F. F. ; **NOMA, I. H. Y.** ; GREMASCHI, L. B. ; BLANCO, L. F. M. ; BRAZ, N. M. ; VALVERDE, T. L. ; VICENTINI, V. E. P. . Dr. Genetics: Online informational content to support genetic testing. 2016. (Paper Presentation/Other).

19.

NOMA, I. H. Y.; PEREIRA, V. M. ; SANUKI, M. S. ; BORGHI, S. M. ; VERRI JUNIOR, W. A. ; SILVA, P. K. S. ; ARISTIDES, S. M. A. . RELATIONSHIP BETWEEN NOCICEPTION AND NITRIC OXIDE PRODUCTION IN ANIMALS INFECTED WITH LEISHMANIA (LEISHMANIA) AMAZONENSIS. 2016. (Paper Presentation/Other).

20.

FEDRIGO, N. H. ; MATTA, P. E. G. ; PEREIRA, V. M. ; **NOMA, I. H. Y.** ; TOGNIM, M. C. B. . Activity of fosfomycin against Acinetobacter baumannii and performance of susceptibility testing methods. 2016. (Paper Presentation/Symposium).

21.

NOMA, I. H. Y.; WATANABE, A. S. M. ; SILVA, M. R. T. ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . Evaluation of the pathogenicity and virulence factors of Candida albicans in the experimental candidemia model. 2015. (Paper Presentation/Other).

22.

LODI, F. G. ; ZAGO, M. C. B. ; MATTA, P. E. G. ; **NOMA, I. H. Y.** ; TAMURA, N. K. ; AOKI, E. E. ; TOGNIM, M. C. B. . Temporal evolution of the minimum inhibitory concentration for daptomycin in gram-positive cocci. 2015. (Paper Presentation/Congress).

23.

FEDRIGO, N. H. ; TREVIZOLI, T. M. B. ; **NOMA, I. H. Y.** ; MITSUGUI, C. S. ; AOKI, E. E. ; TAMANINI, J. B. ; TOGNIM, M. C. B. . Temporal analysis of resistance to carbapenems in non-fermenting gram-negative bacilli. 2015. (Paper Presentation/Congress).

24.

WATANABE, A. S. M. ; SILVA, M. R. T. ; **NOMA, I. H. Y.** ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . EVALUATION OF THE VIRULENCE FACTORS OF CANDIDA TROPICALIS AFTER EXPERIMENTAL CANDIDEMIA. 2015.(Paper Presentation/Other).

25.

SILVA, M. R. T. ; WATANABE, A. S. M. ; **NOMA, I. H. Y.** ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . EVALUATION OF THE PATHOGENICITY PROFILE OF CANDIDA ALBICANS AFTER SUBSEQUENT CONTACTS WITH THE HOST. 2015. (Paper Presentation/Other).

26.

MATTA, P. E. G. ; ECKER, A. B. S. ; ZARPELLON, M. N. ; MOREIRA, D. C. ; **NOMA, I. H. Y.** ; TAMURA, N. K. ; GARCIA, L. B. ; TOGNIM, M. C. B. . PREVALENCE OF RESISTANCE TO CARBAPENEMS IN THE MAIN GRAM-NEGATIVE BACILLI ISOLATED IN A TEACHING HOSPITAL. 2014. (Paper Presentation/Congress).

27.

VIANA, G. F. ; ZAGO, M. C. B. ; SAALFELD, S. M. S. ; **NOMA, I. H. Y.** ; MATTA, P. E. G. ; MITSUGUI, C. S. ; ABREU FILHO, B. A. ; TOGNIM, M. C. B. . CHANGES IN THE MODE OF DISSEMINATION OF Acinetobacter baumannii ISOLATES IN A UNIVERSITY HOSPITAL. 2014. (Paper Presentation/Congress).

28.

ZAGO, M. C. B. ; VIANA, G. F. ; SAALFELD, S. M. S. ; **NOMA, I. H. Y.** ; FARIAS, A. P. M. ; HELBEL, C. ; TOGNIM, M. C. B. DISSEMINATION OF Acinetobacter baumannii IN A TERTIARY HOSPITAL IN THE NORTHERN REGION OF PARANA. 2014. (Paper Presentation/Congress).

Technical Production

Social media, websites and blogs

1.

NOMA, I. H. Y.. IS IT POSSIBLE TO RECREATE AGED SKIN IN VITRO?. 2020; Topic: Scientific Outreach of the Skin Biology and Melanoma Laboratory. (Social media).

Participation in events, congresses, exhibitions, and fairs

1.

19th International Congress of the Society for Melanoma Research. PRDX2 as a possible phenotype switcher in NRAS-mutated melanomas. 2022. (Congress).

2.

Symposium in Commemoration of the 50th Anniversary of the Graduate Program in Pharmacy and Pathophysiology and Toxicologia.Targeting Peroxiredoxin 2 as a potential treatment for NRAS mutated metastatic melanoma. 2022. (Symposium).

3.

XXII Brazilian Congress of Toxicology. Gliotoxin and its potential cytotoxicity against NRAS mutated-melanoma. 2022. (Congress).

4.

The importance of the study of in vitro dermal irritation/corrosion in toxicological evaluation. 2021. (Other).

5.

Host-Pathogen Interaction Meeting. 2021. (Meeting).

6.

1st Symposium on Challenges in Molecular Oncology. 2021. (Symposium).

7.

Brazilian Congress in Biomedical Engineering (CBEB2020). GEOSPATIAL ANALYSIS OF DIAGNOSTIC IMAGING EQUIPMENT IN BRAZIL. 2020. (Congress).

8.

FisioTox Lecture Series. 2020. (Seminar).

9.

Unifesp Virtual Academic Congress 2020 - Science and University: Transformations for Society. 2020. (Congress).

10.

2nd Meeting on Genetics, Hemotherapy, and Hematological Pathologies. 2020. (Meeting).

11.

Increasing CRISPR editing efficiency with novel guide RNA methods. 2020. (Seminar).

12.

HPV Infection - Infecto in Series. 2020. (Seminar).

13.

Diagnostic and Therapeutic Perspectives Using Technology CRISPR. 2020. (Other).

14.

ONCOLOGY PILLS: Principles of hormone therapy in oncological treatment. 2020. (Seminar).

15.

ONCOLOGY PILLS: Principles of Oncological Treatment and Mechanisms of Chemotherapy. 2020. (Seminar).

16.

ONCOLOGY PILLS: Targeted Therapy and Immunotherapy in Modern Oncology. 2020. (Seminar).

17.

ONCOLOGY PILLS - Clinical cases in oncology: application of basic concepts. 2020. (Seminar).

18.

VII International Meeting of Biosciences and Physiopathology and 11th Symposium on Biosciences Applied to Pharmacy. 2020. (Symposium).

19.

Varilux-UEM French Film Festival 2019. 2019. (Exposition).

20.

III INTERNATIONAL SYMPOSIUM OF EXPERIMENTAL PATHOLOGY.THE PREVALENCE OF SYPHILIS IN PRENATAL CARE WOMEN IN PARANA STATE, BRAZIL. 2019. (Symposium).

21.

VI International Meeting of Biosciences and Physiopathology ? 10th Symposium on Biosciences Applied to Pharmacy.Co-infections between Human Papillomavirus (HPV) and genital mycoplasmas in asymptomatic women. 2019. (Meeting).

22.

8th Annual Meeting on Technological Initiation and Innovation. Session Coordinator. 2018. (Meeting).

23.

2nd Brazil-United States Workshop on Cervical Cancer. 2018. (Other).

24.

SUS and Brazilian Public Health: current scenario and perspectives. 2018. (Other).

25.

Shall We Talk About Rare Diseases?. 2018. (Other).

26.

5th International Congress of the CCS, 3rd International Congress on Obesity Treatment and 7th PCS Meeting. State University of Maringá. Mucocutaneous leishmaniasis in Paraná, evaluation of hospital morbidity from 2008 to 2018. 2018. (Congress).

27.

V International Meeting of Biosciences and Physiopathology e 9th Symposium on Biosciences Applied to Pharmacy..PUBLIC EXPENDITURES ON HOSPITAL MORBIDITY DUE TO LEISHMANIASIS IN PARANA, BRAZIL, BRAZIL.
2018. (Symposium).

28.

26th Annual Scientific Initiation Meeting and 6th Annual Junior Scientific Initiation Meeting. VARIABILITY OF β-LACTAMASES PRESENT IN GRAM-NEGATIVE BACILLI ISOLATED DURING PATIENTS' STAY IN A TEACHING HOSPITAL. 2017.
(Meeting).

29.

1st Workshop on Medical Genetics. 2017. (Other).

30.

25th Annual Scientific Initiation Meeting and 5th Annual Junior Scientific Initiation Meeting. RELATIONSHIP BETWEEN NOCICEPTION AND NITRIC OXIDE PRODUCTION IN ANIMALS INFECTED WITH LEISHMANIA (LEISHMANIA) AMAZONENSIS. 2016. (Meeting).

31.

1st Regional Symposium on Integrative and Exercise Physiology. 2016. (Symposium).

32.

4th Academic Week of Biomedicine - UEM. Relationship between nociception and nitric oxide production in animals infected with Leishmania (Leishmania) amazonensis. 2016. (Other).

33.

PROSED. Socio-educational Project of the 3rd Academic Week of Biomedicine - UEM. 2016. (Oficina).

34.

Health Conexion. 2015. (Other).

35.

3rd Academic Week of Biomedicine ? UEM. Evaluation of the Pathogenicity and Virulence Factors of Candida albicans in the Experimental Candidemia Model. 2015. (Other).

36.

4th Paraná Congress of Biomedical Sciences and 1st Pro-Health Workshop. 2014. (Congress).

37.

3rd Film and Scientific Outreach Exhibition in Biosciences. 2014. (Other).

38.

2nd Academic Week of Biomedicine - UEM. 2014. (Other).

39.

1st Student Discussion Forum. Alternative Energy Sources (Biodiesel and Biogas). 2011. (Other).

Organization of events, congresses, exhibitions, and fairs

1.

NOMA, ISABELLA HARUMI YONEHARA. 2nd FisioTox Lecture Series of the Graduate Program in Pharmacy (Pathophysiology and Toxicology). 2021. (Other).

2.

NOMA, I. H. Y.. VII International Meeting of Biosciences and Physiopathology and 11th Symposium on Biosciences Applied to Pharmacy. 2020. (Other).

3.

NOMA, I. H. Y.. VI International Meeting of Biosciences and Physiopathology ? 10th Symposium on Biosciences Applied to Pharmacy. 2019. (Other).

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I, André Vinícius Inacio Penna Mello, telephone number 415 425-2508, mailing address P.O. Box 90487, San Diego, CA 92169, certify that the professional translation of this document from Portuguese to English has been performed by myself, a qualified translator fluent in both languages, and that the following is an accurate and complete translation of the document.

Date: April 24, 2026.

Isabella Harumi Yonehara Noma

Endereço para acessar este CV: http://lattes.cnpq.br/6549549395123599
ID Lattes: **6549549395123599**
Última atualização do currículo em 31/07/2025

Pós-doutoranda pelo H. Lee Moffitt Cancer Center. Doutora em Ciências pela Faculdade de Ciências Farmacêuticas (FCF) (Capes-7) da USP com foco em melanomas metastáticos. Exploramos a complexidade do microambiente no processo de invasão do melanoma por meio de modelos tridimensionais de pele reconstruída no Laboratório de Fisiopatologia da pele-USP. Graduada em Biomedicina pela Universidade Estadual de Maringá (2017), com projetos de iniciação científica na área de Microbiologia Básica e Imunologia Clínica (UEM). Mestre pelo programa de Ciências da Saúde, atuando no Laboratório de Citologia Clínica e Doenças Sexualmente Transmissíveis com foco em detecção de HPV e outras bactérias associadas pela técnica de biologia molecular. **(Texto informado pelo autor)**

Identificação

Nome

Isabella Harumi Yonehara Noma

Nome em citações bibliográficas

NOMA, I. H. Y.;NOMA, ISABELLA;YONEHARA NOMA, ISABELLA HARUMI;HARUMI YONEHARA NOMA, ISABELLA;NOMA, ISABELLA HARUMI YONEHARA

Lattes iD

http://lattes.cnpq.br/6549549395123599

Orcid iD

? https://orcid.org/0000-0002-7413-1316

País de Nacionalidade

Brasil

Formação acadêmica/titulação

2020 - 2023

Doutorado em Farmácia (Fisiopatologia e Toxicologia).
Universidade de São Paulo, USP, Brasil.
Título: Peroxirredoxina 2 na modulação do fenótipo invasivo em melanoma com mutação em NRAS, Ano de obtenção: 2023.
Orientador: Silvya Stuchi Maria-Engler.
Bolsista do(a): Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq, Brasil.

2018 - 2020

Mestrado em Ciências da Saúde.
Universidade Estadual de Maringá, UEM, Brasil.
Título: Prevalê

de Ureaplasmas e

Micoplasmas em mulheres Maringaenses em de idade fértil, Ano de Obtenção: 2020.
Orientador: Marcia Edilaine Lopes Consolaro.
Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES, Brasil.
Setores de atividade: Pesquisa e desenvolvimento científico.

2023 - 2025

Especialização em MBA em Data Science e Analytics EaD. (Carga Horária: 425h).
Universidade de São Paulo, Escola Superior de Agricultura Luiz de Queiroz, USP ESALQ, Brasil.
Título: Clusterização de melanoma cutâneo e acral: Impacto das alterações genéticas na resposta imune.
Orientador: William Barbosa.

2019 - 2020

Especialização em Engenharia Biomédica com ênfase em engenharia clínica. (Carga Horária: 360h).
Universidade Estácio de Sá, UNESA, Brasil.
Título: ANÁLISE GEOESPACIAL DE EQUIPAMENTOS DE DIAGNÓSTICO POR IMAGEM ASSOCIADO ÀS CARACTERÍSTICAS SOCIODEMOGRÁFICAS NO BRASIL.

2014 - 2017

Graduação em Biomedicina.
Universidade Estadual de Maringá, UEM, Brasil.
Título: Avaliação da resposta nociceptiva de camundongos infectados por Leishmania (Leishmania) amazonensis e a sua relação com o óxido nítrico.
Orientador: Sandra Mara Alessi Aristides.
Bolsista do(a): Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, CAPES, Brasil.

2011 - 2013

Ensino Médio (2º grau).
Colégio Santa Cruz, CSC, Brasil.

Pós-doutorado

2023 - 2024

Pós-Doutorado.
H. Lee Moffitt Cancer Center and Research Institute, HLMCCRI, Estados Unidos.
Bolsista do(a): Conselho Nacional de Desenvolvimento Científico e Tecnológico, CNPq, Brasil.
Grande área: Ciências da Saúde

Formação Complementar

2020 - 2020

Extensão universitária em Cusos a distância DES-UEM - Bioestatística Aplicada. (Carga horária: 34h).
Universidade Estadual de Maringá, UEM, Brasil.

2020 - 2020

Saúde em dados: Como trabalhar com os sistemas do DataSUS. (Carga horária: 2h).
Universidade Estadual de Maringá, UEM, Brasil.

2020 - 2020

Introdução à Machine Learning. (Carga horária: 2h).
Universidade Estadual de Maringá, UEM, Brasil.

2020 - 2020

Língua Francesa A2- Paraná fala Francês-Modulo 2 Online. (Carga horária: 30h).
Universidade Estadual de Maringá, UEM, Brasil.

2020 - 2020

1st International Digital Congress on 3D Biofabrication and Bioprinting. (Carga horária: 8h).
Universidade de Araraquara, UNIARA, Brasil.

2020 - 2020

I CURSO DE ONCOLOGIA BÁSICA DA LAOMA. (Carga horária: 10h).
Universidade de Araraquara, UNIARA, Brasil.

2020 - 2020

Understanding Cancer Metastasis. (Carga horária: 4h).
Johns Hopkins University, JHU, Estados Unidos.

2020 - 2020

Introduction to the Biology of Cancer. (Carga horária: 8h).
Johns Hopkins University, JHU, Estados Unidos.

2020 - 2020

Cultivo celular e aplicações no estudo de doenças crônicas e infecciosas. (Carga horária: 15h).
Universidade Federal do Paraná, UFPR, Brasil.

2020 - 2020

XI Board Review in Medical Oncology: Atualização, Discussão e Inovação e II. (Carga horária: 40h).
Instituto Israelita de Ensino e Pesquisa Albert Einstein, IIEPAE, Brasil.

2020 - 2020

Revisão sistemática ? Elementos essenciais para evidências confiáveis. (Carga horária: 2h).
Universidade Estadual de Maringá, UEM, Brasil.

2020 - 2020

Oral Communication and Academic Activities-Modalidade Online. (Carga horária: 35h).
Universidade de São Paulo, USP, Brasil.

2019 - 2019

Extensão universitária em I Curso de Férias em Imunologia e Imunogenética. (Carga horária:

40h).
Universidade Estadual de Maringá, UEM, Brasil.

2019 - 2019

Extensão universitária em UEM na Expoingá. (Carga horária: 5h).
Universidade Estadual de Maringá, UEM, Brasil.

2019 - 2019

Extensão universitária em Active Learning. (Carga horária: 4h).
Universidade Estadual de Maringá, UEM, Brasil.

2019 - 2019

Extensão universitária em Paraná fala francês A1- nível 2. (Carga horária: 60h).
Universidade Estadual de Maringá, UEM, Brasil.

2019 - 2019

Conversation en langue française. (Carga horária: 8h).
Universidade Estadual de Maringá, UEM, Brasil.

2019 - 2019

Descobrindo fagos. (Carga horária: 15h).
Universidade Estadual de Maringá, UEM, Brasil.

2019 - 2019

Vesículas extracelulares envolvidas em doenças crônicas e infecciosas. (Carga horária: 4h).
Universidade Estadual de Maringá, UEM, Brasil.

2019 - 2019

Primers: who are they and how to outline them?.
Universidade Estadual de Londrina, UEL, Brasil.

2018 - 2018

Extensão universitária em Paraná fala Francês. (Carga horária: 60h).
Universidade Estadual de Maringá, UEM, Brasil.

2018 - 2018

Extensão universitária em Paraná fala Inglês. (Carga horária: 30h).
Universidade Estadual de Maringá, UEM, Brasil.

2016 - 2016

Antibiotic as Promotors of Antibiotic Resistence.. (Carga horária: 2h).
Programa de Pós-Graduação em Biociências e Fisiopatologia, PBF, Brasil.

2014 - 2016

Língua japonesa. (Carga horária: 300h).
Universidade Estadual de Maringá, UEM, Brasil.

2015 - 2015

Perfusão Hepática in situ.. (Carga horária: 4h).
Universidade Estadual de Maringá, UEM, Brasil.

2015 - 2015

Estudos e Orientações para Exame de Proeficiência em Língua Japonesa N.05. (Carga horária: 12h).
Universidade Estadual de Maringá, UEM, Brasil.

2014 - 2014

Reprodução Assistida.. (Carga horária: 4h).
Universidade Estadual de Londrina, UEL, Brasil.

Atuação Profissional

H. Lee Moffitt Cancer Center and Research Institute, HLMCCRI, Estados Unidos.

Vínculo institucional

2025 - Atual

Vínculo: Celetista, Enquadramento Funcional: Pós doutorando, Carga horária: 40, Regime: Dedicação exclusiva.

Vínculo institucional

2023 - 2024

Vínculo: Bolsista, Enquadramento Funcional: Pós doutorando, Carga horária: 40

Universidade Estadual de Maringá, UEM, Brasil.

Vínculo institucional

2018 - 2020

Vínculo: Bolsista, Enquadramento Funcional: Estudante de pós-graduação, Carga horária: 40, Regime: Dedicação exclusiva.

Outras informações

Mestrado no Programa de Ciências da Saúde - Bolsista

Vínculo institucional

2017 - 2017

Vínculo: Estagiario, Enquadramento Funcional: Estágio obrigatório em imunogenética, Carga horária: 40

Vínculo institucional

2017 - 2017

Vínculo: Estagiario, Enquadramento Funcional: Estágio obrigatório em análises clínicas, Carga horária: 40

Vínculo institucional

2014 - 2017

Vínculo: Projeto Ensino, Enquadramento Funcional: Práticas em laboratório, Carga horária: 4

Vínculo institucional

2015 - 2016

Vínculo: Bolsista, Enquadramento Funcional: Monitor de microbiologia, Carga horária: 20

Universidade de São Paulo, USP, Brasil.

Vínculo institucional

2022 - 2022

Vínculo: Bolsista, Enquadramento Funcional: Estagiário em Docência, Carga horária: 6

Projetos de pesquisa

2023 - 2024

The role of HDAC8 in UVR-driven melanoma initiation

Descrição: Exposure to ultraviolet radiation (UVR) is the major risk factor for melanoma development andleads to complex phenotypic responses in melanocytes, including dendrite formation, enhancedmelanogenesis, and cell cycle arrest. Previous studies identified the type I histone deacetylase,HDAC8, as an epigenetic regulator of melanoma cells induced following exposure to UVR,hypoxia, and BRAF inhibitor therapy. Our studies suggest that HDAC8 expression is upregulatedin melanocytes that experience high levels of UVR-induced damage. No strategies yet exist toprevent the accumulation of sun-damaged melanocytes in the skin, representing a significantbarrier to effective melanoma prevention. In this new proposal, we aimed to investigate whetherUVR-induced HDAC8 activation allows some melanocytes to evade cell death, enabling cells withpathogenic mutations to survive and potentially develop into melanoma. We utilized a uniquemouse melanoma model driven by mutant BRAF and heterozygous PTEN loss, in which HDAC8expression can be conditionally regulated in skin melanocytes, to elucidate the

role of epigeneticreprogramming in UVR-induced melanoma initiation. Additionally, we employed siRNAmediatedoverexpression of HDAC8 in cell lines. Our findings demonstrated that HDAC8overexpression following UV irradiation increases the survival of UVR-damaged melanocytes andmelanoma cells, mediated by the downregulation of p53 and acetylated p53. This research providesa novel perspective on how UVR-induced epigenetic reprogramming targeting HDAC8contributes to the persistence of melanocytes and melanoma cells harboring pathogenic mutations,allowing them to grow and evade apoptosis..
Situação: Concluído; Natureza: Pesquisa.

Integrantes: Isabella Harumi Yonehara Noma - Integrante / Silvya Stuchi Maria-Engler - Coordenador / SMALLEY, KEIRAN S. M. - Integrante.

2023 - Atual

POTENCIAL METASTÁTICO DA PEROXIRREDOXINA 2 EM MELANOMAS NRAS-MUTADOS

Descrição: Descrição: O melanoma cutâneo é uma neoplasia curável quando diagnosticada em estágios iniciais, mas está associada a uma pior evolução na doença avançada. Lesões de melanoma em locais expostos ao sol e associadas a uma alta carga mutacional e regulação epigenética aberrante, contribuem para o desenvolvimento e progressão da doença. Este é o tipo de câncer de pele com maior potencial metastático, o que leva a sua alta taxa de mortalidade. As principais mutações ocorrem na via das MAPKs: 50% dos pacientes apresentam alterações em BRAF, 15-20% em NRAS e os demais em NF1 ou triplo selvagem. A terapia alvo-dirigida revolucionou o tratamento do melanoma com o uso de inibidores de BRAF e MEK, porém a resistência intrínseca e adquirida acarreta na falha do tratamento. Nesse sentido, mutações em NRAS caracterizam um tumor ainda mais agressivo e sem opções terapêuticas específicas, relacionando esta mutação a um pior prognóstico. A heterogeneidade intratumoral ocorre como consequência da plasticidade fenotípica das células, que assumem e transitam por diferentes estados transcricionais controlados pelos níveis de MITF, tornando as células mais proliferativas ou invasivas. Tal evento está relacionado à metástase e falha da terapia. Dessa forma, vias alternativas devem ser exploradas como potenciais opções para o tratamento de pacientes com mutações em NRAS. Tendo em vista que um dos fatores que limitam a eficácia da terapia alvo-dirigida é a resistência ao estresse oxidativo, o controle da homeostase redox é um interessante ponto de partida. O peróxido de hidrogênio desempenha um papel central na sinalização redox, estando envolvido em todos os hallmarks do câncer, desde proliferação a metabolismo, angiogênese e escape do sistema imune. Além disso, o peróxido de hidrogênio é capaz de alterar os níveis de MITF. Nesse contexto, moléculas que controlem sua ação apresentam substancial interesse. As peroxiredoxinas (PRDXs) são as principais enzimas consumidoras de peróxido de hidrogênio nas células devido a sua alta expressão e capacidade catalítica. Diversos estudos relatam a ação de enzimas antioxidantes na homeostase redox e na tumorigênese. Além do papel excepcional das PRDXs removendo o oxidante do meio, ao se tornarem oxidadas e inativas, elas permitem

que o peróxido de hidrogênio reaja direta ou indiretamente com outros alvos, regulando assim a sinalização redox da célula. Devido a sua função central no controle do peróxido de hidrogênio, as PRDXs estão envolvidas diretamente em diferentes tipos de câncer. De fato, a isoforma PRDX2 está epigeneticamente silenciada em alguns canceres e nosso grupo demonstrou recentemente que ela também está diminuída em pacientes com metástase de melanoma, reforçando seu envolvimento com o processo de invasão. Nesse sentido, foi demonstrado que a PRDX2 é capaz de induzir a estabilidade de junções aderentes em melanomas com mutação em BRAF. Porém ainda não existem estudos que relacionem PRDXs a mutação em NRAS, sendo assim, observamos que linhagens de melanomas com mutação em NRAS mostraram diferentes perfis proliferativos e invasivos, correspondente à diferença de expressão de PRDX2. Assim, nossa proposta é investigar o papel da PRDX2 na plasticidade fenotípica de melanomas com mutação em NRAS. Para isso, será estudado o silenciamento dessa proteína por edição gênica (shRNA) e por um inibidor farmacológico de PRDXs (Conoidina A), além do efeito da sua restauração pelo uso da gliotoxina, um mimético de PRDX. Serão avaliados os parâmetros funcionais como proliferação, capacidade de formação de colônias, migração e invasão em modelos de pele 3D. Tais observações reforçam nossa hipótese de que a PRDX2 pode ser um novo alvo na busca de medicamentos mais específicos para melanomas com mutação em NRAS..
Situação: Em andamento; Natureza: Pesquisa.
Alunos envolvidos: Doutorado: (3) .

Integrantes: Isabella Harumi Yonehara Noma - Coordenador / Silvya Stuchi Maria-Engler - Integrante / Larissa Anastácio da Costa Carvalho - Integrante.

2016 - 2017

Variabilidade de B-lactamase presentes em bacilos Gram negativos isolados durante a permanência de pacientes em Hospital Ensino

Projeto certificado pelo(a) coordenador(a) Maria Cristina Bronharo Tognim em 11/11/2019.

Descrição: Considerando a importância das bactérias Gram Negativas (BGN) nos hospitais, principalmente em UTI, devido à sua resistência antimicrobiana e ainda considerando que os pacientes podem ser fontes ou reservatórios destes microrganismos, este projeto tem como objetivo avaliar a presença dos diferentes BGN que podem colonizar ou infectar os pacientes durante sua permanência hospitalar. Como metodologia utilizaremos a seleção de pacientes que estavam cadastrados em banco de dados do Excel provenientes do projeto ?Vigilância Epidemiológica de Bactérias Multirresistentes no Hospital Universitário de Maringá?. Os pacientes serão selecionados pelas iniciais dos nomes, onde só serão incluídos pacientes que apresentarem dados microbiológicos de isolados em colonização e infecção por BGN. Todos os microrganismos serão recuperados da coleção do freezer, será confirmada a resistência aos carbapenêmicos pelo método de ágar diluição todos os isolados serão

realizados testes para detecção de marcadores de resistência aos β lactâmicos por PCR para ESBL (bla CTX, bla SHV e bla TEM), para carbapenêmicos do tipo Oxacilinases e Metalo β lactamases. Os isolados serão caracterizados molecularmente pela técnica de ERIC. Para alguns casos serão avaliados o uso de antimicrobianos nos prontuários dos pacientes. Pretende-se com os achados compreender melhor a dinâmica do microrganismo e seu controle..
Situação: Concluído; Natureza: Pesquisa.
Alunos envolvidos: Graduação: (2) / Doutorado: (1) .

Integrantes: Isabella Harumi Yonehara Noma - Integrante / Alessandra Barrochelli da Silva Ecker - Integrante / Déborah de Castro Moreira - Integrante / Maria Cristina Bronharo Tognim - Coordenador.

2016 - 2016

RELAÇÃO DA NOCICEPÇÃO COM A PRODUÇÃO DE ÓXIDO NÍTRICO EM ANIMAIS INFECTADOS COM LEISHMANIA (LEISHMANIA) AMAZONENSIS

Projeto certificado pelo(a) coordenador(a) Thais Gomes Verzignassi Silveira em 02/03/2021.

Descrição: A leishmaniose tegumentar americana, considerada negligenciada é endêmica em muitos países, inclusive no Brasil. A doença é caracterizada por úlceras e pode ser curada espontaneamente ou apresentar metástases graves e multilantes na mucosa. As lesões geralmente progridem para ulcerações na ausência de dor, sugerindo que a infecção interfere com a percepção de dor. Nesse projeto avaliamos o limiar de dor com doses crescentes do parasito utilizando o anestesiômetro eletrônico e o edema de pata nas lesões induzidas pela inoculação do parasito medidos semanalmente com ajuda de um pletismômetro e com um paquímetro, os resultados mostraram um aumento no edema conforme o aumento das concentrações do parasito e também aumento na intensidade da dor. Avaliamos também a produção de óxido nítrico nas lesões da pata dos animais inoculada com o parasito, porém os resultados preliminares se mostraram negativos. Como conclusão avaliamos que a formação de edema é acompanhada pelo aumento de dor, porém a produção de óxido nítrico não estaria relacionada com a dor e o edema..
Situação: Concluído; Natureza: Pesquisa.
Alunos envolvidos: Graduação: (2) / Mestrado acadêmico: (1) .

Integrantes: Isabella Harumi Yonehara Noma - Integrante / Paula Karoline Sfordi da Silva - Integrante / Valéria Medrado Pereira - Integrante / Sandra Mara Alessi Aristides - Integrante / Thais Gomes Verzignassi Silveira - Coordenador.

Projetos de extensão

2016 - 2017

Dr genética: conteúdo informacional online no auxílio a exames genéticos humanos

Descrição: A dissipação de informação por meio da internet tem se tornado cada vez mais rápida e acessível à população como um todo. No inicio da sua utilização seu acesso era feito em sua grande maioria por amantes de tecnologias e profissionais da área, contudo, com o passar dos anos tanto o acesso como o conteudo disponiveis são feito em grande parte por leigos no assunto. Os brasileiros representam a maior fatia a nivel mundial no quesito tempo médio gasto navegando, possibilitando grande troca de informações incluido na área da saúde . Apesar de muito ùtil no acesso à informação muitos especialistas salientam que muita dessa informação disponibilizada são inadequadas ou incompletas, . No Brasil não há regulamentação ou certificação dos sites com informações sobre saúde.. Desta forma, torna-se essencial a disponibilidade de informação ao público em geral, informação essa oferecida por universidades, embasada em conhecimentos científicos, podendo assim auxiliar na confiança médico-paciente e na diminuição da ansiedade e desconforto decorrentes das enfermidades. O objetivo deste projeto é desenvolver um site com informações sobre exames genéticos, sejam citogenéticos e moleculares, nas diversas áreas da genética, incluindo aconselhamento genético e oncologia, entre outros. Além de informações adicionais, como interpretação, locais em que podem ser feitos e seus custos tanto através do Sistema Unico de Saúde como por Plano de saúde. A divulgação do site será feita através de visitas e cartazes nos postos de saúde em Maringá, Hospital Municipal de Maringá, Hospital Universitário de Maringá. As informações disponibilizadas terão como fonte livros, artigos indexados, laboratórios e hospitais de apoio. Os alunos das áreas de Biomedicina e Medicina participantes do projeto terão acesso ao conhecimento sobre exames geneticos e terão a oportunidade de transmitir esse conhecimento a população em geral, uma vez que a demanda de exames é crescente, sejam de auxílio diagnóstico, prognóstico ou ainda no estabelecimento de condutas terapêuticas, levando-se em conta o desenvolvimento promissor de novas drogas alvo-moleculares.
Situação: Concluído; Natureza: Extensão.
Alunos envolvidos: Graduação: (7) .

Integrantes: Isabella Harumi Yonehara Noma - Integrante / Tamires Leite Valverde - Integrante / Veronica Elisa Pimenta Vicentini - Integrante / Luiz Felipe MSB Blanco - Integrante / Lucas B Gremaschi - Integrante / Patrícia Alexandra da Silva Saavedra Romão - Integrante / Natalia Mestre Braz - Integrante / Eliane Papa Ambrosio Albuquerque - Coordenador.

2016 - 2016

Integração estudantil na execução de ações voluntárias, visando uma maior divulgação e conscientização da comunidade maringaense sobre doenças pouco conhecidas que se manifestam na infância, distrofias musculares progressivas com ênfase na Dist. Muscular

Projeto certificado pelo(a) coordenador(a) Maria Ida Bo[illegible] Ravanelli Speziali em

19/10/2022.

Descrição: Acredita-se que a sociedade em sua grande maioria, desconhece a ocorrência de algumas doenças que acometem crianças. As distrofias musculares se encaixam perfeitamente no grupo de doenças desconhecidas. Apesar de consideradas como raras a incidência das mesmas contraria fortemente essa premissa. Com este trabalho pretende-se através da integração de alunos das diversas áreas da Universidade Estadual de Maringá a realização de ações que promovam junto da sociedade um maior conhecimento sobre doenças que acometem crianças. Desta maneira, o primeiro ano de realização deste projeto, que pretende abordar uma doença por ano, estará direcionando suas ações para as distrofias musculares, permitindo assim que a sociedade se mobilize, participando de eventos que se comprometem com a melhoria da qualidade de vida de pacientes acometidos com distrofia muscular de Duchenne escolhida como slogan deste projeto por ser a distrofia mais frequente bem como a mais letal dentre todas as distrofias..
Situação: Concluído; Natureza: Extensão.
Alunos envolvidos: Graduação: (13) .

Integrantes: Isabella Harumi Yonehara Noma - Integrante / Paula Karoline Sfordi da Silva - Integrante / Tamires Leite Valverde - Integrante / Patrícia Alexandra da Silva Saavedra Romão - Integrante / Natalia Mestre Braz - Integrante / Lais de Sena Marques - Integrante / Maria Ida Bonini Ravanelli Speziali - Coordenador / Fabiana Galvão da Motta Lima - Integrante / Francyhellen Franco Montanha - Integrante / Gabriela Fernanda Martinez - Integrante / Maria Naomi Yamanaka - Integrante / Sara Fabricio dos Santos - Integrante / Aarão Fabrício dos Santos - Integrante / Leonardo de Oliveira Figueiredo - Integrante.

Áreas de atuação

1.

Grande área: Ciências da Saúde / Área: Farmácia / Subárea: Citologia Clínica.

2.

Grande área: Ciências da Saúde / Área: Farmácia / Subárea: Fisiopatologia e diagnóstico laboratorial.

Idiomas

Inglês

Compreende Bem, Fala Razoavelmente, Lê Bem, Escreve Razoavelmente.

Prêmios e títulos

2022

Menção honrosa do trabalho intitulado ? Targeting Peroxiredoxin-2 as a potential treatment for NRAS-mutated metastatic melanoma?, Universidade de São Paulo.

2022

SMR PARTICIPATION AWARD, Society for Melanoma Research.

Produções

Produção bibliográfica

Citações

Google Scholar

Total de trabalhos: 19 Total de citações: 74 Data: 10/02/2025

https://scholar.google.com/citations?user=gtlvO4YAAAAJ&hl=pt-BR

Artigos completos publicados em periódicos

Ordenar por

Ordem Cronológica

1.

NORONHA, NATÁLIA YUMI ; RODRIGUES, GUILHERME DA SILVA ; WATANABE, LÍGIA MORIGUCHI ; **NOMA, ISABELLA HARUMI YONEHARA** ; CAZIER, JEAN-BAPTISTE ; SAE-LEE, CHANACHAI ; CHITTA, PITAKSIN ; PEREIRA, VANESSA APARECIDA BATISTA ; PINHEL, MARCELA AUGUSTA DE SOUZA ; DIANI, LUISA MARIA ; BARBOSA, FERNANDO ; PLÖSCH, TORSTEN ; NONINO, CARLA BARBOSA . DNA methylation signatures of arsenic exposure and obesity. Environmental Epigenetics JCR, v. -, p. -, 2025.

2.

DA SILVA RODRIGUES, GUILHERME ; NORONHA, NATÁLIA YUMI ; **NOMA, ISABELLA HARUMI YONEHARA** ; DE LIMA, JOÃO GABRIEL RIBEIRO ; DA SILVA SOBRINHO, ANDRESSA CRYSTINE ; DE SOUZA PINHEL, MARCELA AUGUSTA ; DE ALMEIDA, MARIANA LUCIANO ; WATANABE, LÍGIA MORIGUCHI ; NONINO, CARLA BARBOSA ; JUNIOR, CARLOS ROBERTO BUENO . 14-Week exercise training modifies the DNA methylation levels at gene sites in non-Alzheimer's disease women aged 50 to 70 years. EXPERIMENTAL GERONTOLOGY JCR, v. 186, p. 112362, 2024. **Citações:** WEB OF SCIENCE 2 | SCOPUS 2

3.

NORONHA, NATÁLIA YUMI ; **NOMA, ISABELLA HARUMI YONEHARA** ; FERNANDES FERREIRA, RAFAEL ; RODRIGUES, GUILHERME DA SILVA ; MARTINS, LUZANIA DOS SANTOS ; WATANABE, LÍGIA MORIGUCHI ; PINHEL, MARCELA AUGUSTA DE SOUZA ; MELLO SCHINEIDER, ISABELLE ; DIANI, LUISA MARIA ; CARLOS, DANIELA ; NONINO, CARLA BARBOSA . Association between the relative abundance of phyla and obacteria, vitamin C consumption,

and DNA methylation of genes linked to immune response pathways. FRONTIERS IN NUTRITION JCR, v. 11, p. -, 2024. **Citações:** WEB OF SCIENCE ™ 2 | SCOPUS 1

4.

NOMA, ISABELLA HARUMI YONEHARA; CARVALHO, LARISSA ANASTACIO DA COSTA ; CAMARENA, DENISSE ESTHER MALLAUPOMA ; SILVA, RENAIRA OLIVEIRA ; MORAES JUNIOR, MANOEL OLIVEIRA DE ; DE SOUZA, SOPHIA TAVARES ; NEWTON-BISHOP, JULIA ; NSENGIMANA, JEREMIE ; MARIA-ENGLER, SILVYA STUCHI . Peroxiredoxin-2 represses NRAS-mutated melanoma cells invasion by modulating EMT markers. BIOMEDICINE & PHARMACOTHERAPY JCR, v. 177, p. 116953, 2024. **Citações:** WEB OF SCIENCE ™ 2

5.

CARVALHO, LARISSA ANASTACIO DA COSTA ; **NOMA, ISABELLA HARUMI YONEHARA** ; UEHARA, ADRIANA HIROMI ; SIENA, ADAMO DAVI DIOGENES ; OSAKI, LUCIANA HARUMI ; MORI, MATEUS PRATES ; PINTO, NADJA CRISTHINA DE SOUZA ; FREITAS, VANESSA MORAIS ; JUNIOR, WILSON ARAUJO SILVA ; SMALLEY, KEIRAN S. M. ; MARIA-ENGLER, SILVYA STUCHI . Modeling Melanoma Heterogeneity In Vitro: Redox, Resistance and Pigmentation Profiles. ANTIOXIDANTS JCR, v. 13, p. 555, 2024. **Citações:** WEB OF SCIENCE ™ 2 | SCOPUS 2

6.

FRANCO, YLANA ADAMI ; DE MORAES, MANOEL OLIVEIRA ; CARVALHO, LARISSA A. C. ; DOHLE, WOLFGANG ; DA SILVA, RENAIRA OLIVEIRA ; **NOMA, ISABELLA HARUMI YONEHARA** ; LIMA, KELI ; POTTER, BARRY V. L. ; MACHADO-NETO, JOÃO A. ; MARIA-ENGLER, SILVYA STUCHI . 2-Methoxyestradiol-3,17-O,O-bis-sulfamate (STX140) Inhibits Proliferation and Invasion via Senescence Pathway Induction in Human BRAFi-Resistant Melanoma Cells. INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES JCR, v. 24, p. 11314, 2023. **Citações:** WEB OF SCIENCE ™ 1 | SCOPUS 1

7.

DA SILVA RODRIGUES, GUILHERME ; **NOMA, ISABELLA HARUMI YONEHARA** ; NORONHA, NATÁLIA YUMI ; WATANABE, LÍGIA MORIGUCHI ; DA SILVA SOBRINHO, ANDRESSA CRYSTINE ; DE LIMA, JOÃO GABRIEL RIBEIRO ; SAE-LEE, CHANACHAI ; BENJAMIM, CICERO JONAS RODRIGUES ; NONINO, CARLA BARBOSA ; BUENO, CARLOS ROBERTO . Eight Weeks of Physical Training Decreases 2 Years of DNA Methylation Age of Sedentary Women. RESEARCH QUARTERLY FOR EXERCISE AND SPORT JCR, v. -, p. 1-11, 2023. **Citações:** WEB OF SCIENCE ™ 2 | SCOPUS 2

8.

WATANABE, LÍGIA MORIGUCHI ; PEREIRA, VANESSA APARECIDA BATISTA ; NORONHA, NATALIA YUMI ; DE SOUZA PINHEL, MARCELA AUGUSTA ; WOLF, LETICIA SANTANA ; DE OLIVEIRA, CRISTIANA CORTES ; PLAÇA, JESSICA RODRIGUES ; **NOMA, ISABELLA HARUMI YONEHARA** ; DA SILVA RODRIGUES, GUILHERME ; DE SOUZA, VANESSA CRISTINA OLIVEIRA ; JUNIOR, FERNANDO BARBOSA ; NONINO, CARLA BARBOSA . The influence of serum selenium in differential epigenetic and transcriptional regulation of CPT1B gene in women with obesity. JOURNAL OF TRACE ELEMENTS IN MEDICINE AND BIOLOGY JCR, v. -, p. 127376, 2023. **Citações:** WEB OF SCIENCE ™ 3 | SCOPUS 2

9.

YUMI NORONHA, NATÁLIA ; DA SILVA RODRIGUES, GUILHERME ; **HARUMI YONEHARA NOMA, ISABELLA** ; FERNANDA CUNHA BRANDAO, CAMILA ; PEREIRA RODRIGUES, KARINE ; COLELLO BRUNO, ALEXANDRE ; SAE-LEE, CHANACHAI ; MORIGUCHI WATANABE, LIGIA ; AUGUSTA DE SOUZA PINHEL, MARCELA ; MELLO SCHINEIDER, ISABELLE ; LUCIANO DE ALMEIDA, MARIANA ; BARBOSA JUNIOR, FERNANDO ; ARAUJO MORAIS, DEBORAH ; TAVARES DE SOUSA JUNIOR, WELLINGTON ; PLÖSCH, TORSTEN ; ROBERTO BUENO JUNIOR, CARLOS ; BARBOSA NONINO, CARLA . 14-weeks combined exercise epigenetically modulated 118 genes of menopausal women with prediabetes. Frontiers in Endocrinology JCR, v. 13, p. 895489, 2022. **Citações:** WEB OF SCIENCE ℠ 14 | SCOPUS 11

10.

DOMINGUES, KARLA LARISSA TRASSI GANAZA ; TANOSHI, CÍNTHIA AKEMI ; **NOMA, ISABELLA HARUMI YONEHARA** ; CARVALHO, MARIA DALVA DE BARROS ; PELLOSO, SANDRA MARISA ; CONSOLARO, MARCIA EDILAINE LOPES ; ARISTIDES, SANDRA MARA ALESSI ; DEMARCHI, IZABEL GALHARDO ; PEDROSO, RAISSA BOCCHI ; LONARDONI, MARIA VALDRINEZ CAMPANA . Economic impact of hospitalizations due to leishmaniasis in southern Brazil. RESEARCH, SOCIETY AND DEVELOPMENT, v. 11, p. e39211831139, 2022.

11.

NORONHA, NATÁLIA YUMI ; DA SILVA RODRIGUES, GUILHERME ; DE SOUZA PINHEL, MARCELA AUGUSTA ; CAZIER, JEAN-BAPTISTE ; WATANABE, LIGIA MORIGUCHI ; MENEZES, ALBERT NOBRE ; BUENO, CARLOS ROBERTO ; NICOLETTI, CAROLINA FERREIRA ; DE OLIVEIRA, BRUNO AFFONSO PARENTI ; SCHINEIDER, ISABELLE MELLO ; **YONEHARA NOMA, ISABELLA HARUMI** ; DIAS ALCARAS, IGOR CAETANO ; BARBOSA, FERNANDO ; BARBOSA NONINO, CARLA . Sample Preparation to Bioinformatics Analysis of DNA Methylation: Association Strategy for Obesity and Related Trait Studies. Jove-Journal of Visualized Experiments JCR, v. 183, p. e62598, 2022. **Citações:** WEB OF SCIENCE ℠ 8 | SCOPUS 7

12.

NOMA, ISABELLA; SHINOBU-MESQUITA, CRISTIANE ; SUEHIRO, TAMY ; MORELLI, FABRICIO ; DE SOUZA, MARIA ; DAMKE, EDILSON ; DA SILVA, VÂNIA ; CONSOLARO, MARCIA . Association of Righ-Risk Human Papillomavirus and Ureaplasma parvum Co-Infections with Increased Risk of Low-Grade Squamous Intraepithelial Cervical Lesions. Asian Pacific Journal of Cancer Prevention, v. 22, p. 1239-1246, 2021. **Citações:** SCOPUS 8

13.

SANTOS E SILVA, JUAN CARLO ; VASCONCELOS, AMANDA PEREIRA ; **YONEHARA NOMA, ISABELLA HARUMI** ; NORONHA, NATÁLIA YUMI ; AQUINO, RODRIGO ; GIDDALURU, JEEVAN ; DURÃO, LUIZ ; COSTA-MARTINS, ANDRÉ GUILHERME ; SCHUCH, VIVIANE ; MORAES-VIEIRA, PEDRO M. ; NAKAYA, HELDER I. . Gene Signatures of Autopsy Lungs from Obese Patients with COVID-19. CLINICAL NUTRITION ESPEN JCR, v. 43, p. 986, 2021. **Citações:** WEB OF SCIENCE ℠ 8 | SCOPUS 8

1.

TANOSHI, C. A. ; **NOMA, I. H. Y.** ; GANAZA, K. L. T. ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. ; ARISTIDES, S. M. A. . DESPESAS EM MORBIDADE HOSPITALAR DEVIDO A LEISHMANIOSE VISCERAL EM UMA REGIÃO DO SUL DO BRASIL. In: V Congresso Internacional do CCS, III Congresso Internacional de Tratamento da Obesidade e VII Encontro do PCS. Universidade Estadual de Maringá, 2018, Maringá. Anais V Congresso Internacional do CCS, III Congresso Internacional de Tratamento da Obesidade e VII Encontro do PCS, 2018. p. 5.

2.

GANAZA, K. L. T. ; TANOSHI, C. A. ; **NOMA, I. H. Y.** ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; CONSOLARO, M. E. L. ; ARISTIDES, S. M. A. ; DEMARCHI, I. G. ; LONARDONI, M. V. C. . ANÁLISE DA MORBIDADE HOSPITALAR POR LEISHMANIOSE CUTÂNEA NO ESTADO DO PARANÁ, BRASIL, 2008-2018. In: V Congresso Internacional do CCS, III Congresso Internacional de Tratamento da Obesidade e VII Encontro do PCS. Universidade Estadual de Maringá, 2018, Maringá. Anais V Congresso Internacional do CCS, III Congresso Internacional de Tratamento da Obesidade e VII Encontro do PCS, 2018.

3.

NOMA, I. H. Y.; GANAZA, K. L. T. ; TANOSHI, C. A. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; ARISTIDES, S. M. A. ; DEMARCHI, I. G. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. . LEISHMANIOSE MUCOCUTÂNEA NO PARANÁ, AVALIAÇÃO DA MORBIDADE HOSPITALAR DE 2008 A 2018.. In: V Congresso Internacional do CCS, III Congresso Internacional de Tratamento da Obesidade e VII Encontro do PCS. Universidade Estadual de Maringá, 2018, Maringá. Anais V Congresso Internacional do CCS, III Congresso Internacional de Tratamento da Obesidade e VII Encontro do PCS, 2018. p. 6.

4.

TANOSHI, C. A. ; GANAZA, K. L. T. ; **NOMA, I. H. Y.** ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; CONSOLARO, M. E. L. ; LONARDONI, M. V. C. ; ARISTIDES, S. M. A. . A prevalência de uma doença negligenciada: epidemiologia e morbidade das leishmanioses no Parana. In: V International Meeting of Biosciences and Physiopathology e IX Simpósio de Biociências Aplicadas à Farmácia., 2018, Maringá. Anais IX Semana Acadêmica de Biomedicina, 2018.

5.

NOMA, I. H. Y.; ECKER, A. B. S. ; MOREIRA, D. C. ; SILVA, L. C. ; PEREIRA, P. M. ; SZCZEREPA, M. M. A. ; TOGNIM, M. C. B. . VARIABILIDADE DE β-LACTAMASES PRESENTES EM BACILOS GRAM NEGATIVOS ISOLADOS DURANTE A PERMANÊNCIA DE PACIENTES EM HOSPITAL DE ENSINO. In: 26.o Encontro Anual de Iniciação Científica e 6.o Encontro Anual de Iniciação Científica Júnior, 2017, Maringá. Anais XXVI EAIC VI EAIC Jr., 2017. p. 67.

6.

ALBUQUERQUE, E. P. A. ; MONTANHA, F. F. ; **NOMA, I. H. Y.** ; GREMASCHI, L. B. ; BLANCO, L. F. V. ; BRAZ, N. M. ; ROMAO, P. A. S.

S. ; VALVERDE, T. L. ; VICENTINI, V. E. P. . Dr. Genética: informação a serviço da comunidade. In: Fórum de Extensão e Cultura da UEM Extensão Universitária: compromisso e transformação social, 2016, Maringá. Anais do 14o Fórum de Extensão e Cultura da UEM : Extensão Universitária : compromisso e transformação social, 2016. v. 23. p. 13-16.

7.

NOMA, I. H. Y.; PEREIRA, V. M. ; SANUKI, M. S. ; BORGHI, S. M. ; VERRI JUNIOR, W. A. ; SILVA, P. K. S. ; ARISTIDES, S. M. A. . RELAÇÃO DA NOCICEPÇÃO COM A PRODUÇÃO DE ÓXIDO NÍTRICO EM ANIMAIS INFECTADOS COM LEISHMANIA (LEISHMANIA) AMAZONENSIS. In: 25.o Encontro Anual de Iniciação Científica e 5.o Encontro Anual de Iniciação Científica Júnior, 2016, Maringá. Anais XXV EAIC V EAIC Jr., 2016. p. 62.

8.

NOMA, I. H. Y.; WATANABE, A. S. M. ; SILVA, M. R. T. ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . Avaliação da patogenicidade e fatores de virulência de Candida albicans no modelo experimental de Candidemia. In: III Semana Acadêmica de Biomedicina ? UEM, 2015, Maringá. ANAIS DA TERCEIRA SEMANA ACADÊMICA DE BIOMEDICINA, 2015.

9.

WATANABE, A. S. M. ; SILVA, M. R. T. ; **NOMA, I. H. Y.** ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . AVALIAÇÃO DOS FATORES DE VIRULÊNCIA DE CANDIDA TROPICALIS APÓS CANDIDEMIA EXPERIMENTAL. In: III Semana Acadêmica de Biomedicina ? UEM, 2015, Maringá. ANAIS DA TERCEIRA SEMANA ACADÊMICA DE BIOMEDICINA, 2015.

10.

SILVA, M. R. T. ; WATANABE, A. S. M. ; **NOMA, I. H. Y.** ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . AVALIAÇÃO DO PERFIL DE PATOGENICIDADE DE CANDIDA ALBICANS APÓS CONTATOS SUBSEQUENTES COM O HOSPEDEIRO. In: III Semana Acadêmica de Biomedicina ? UEM, 2015, Maringá. ANAIS DA TERCEIRA SEMANA ACADÊMICA DE BIOMEDICINA, 2015.

Resumos publicados em anais de congressos

1.

RODRIGUES, R. F. ; SILVA, L. L. ; TANOSHI, C. A. ; **NOMA, I. H. Y.** ; SILVA, G. E. C. . ANÁLISE DO PERFIL EPIDEMIOLÓGICO DE ACIDENTES POR ANIMAIS PEÇONHENTOS NO ESTADO DO PARANÁ: NO PERÍODO DE 2015 A 2019. In: VII International Meeting of Biosciences and Physiopathology e XI Simpósio de Biociências Aplicadas à Farmácia, 2020, Maringá. Anais do VII International Meeting of Biosciences and Physiopathology e XI Simpósio de Biociências Aplicadas à Farmácia. Maringá: Isabella Harumi Yonehara Noma, 2020. v. 1.

2.

NOMA, I. H. Y.; TANOSHI, C. A. ; SANTOS, D. S. ; RODRIGUES, R. F. ; SILVA, L. L. ; CONSOLARO, M. E. L. ; SOUZA, E. M. ; PELLOSO, S. M.

; PEDROSO, R. B. . THE PREVALENCE OF SYPHILIS IN PRENATAL CARE WOMEN IN PARANA STATE, BRAZIL. In: III INTERNATIONAL SYMPOSIUM OF EXPERIMENTAL PATHOLOGY, 2019, Londrina. Annais III International Symposium os Experimental Pathology. Londrina : UEL, 2019: Eduardo José de Almeida Araújo ...[et al.], 2019. p. 48.

3.

TANOSHI, C. A. ; **NOMA, I. H. Y.** ; RODRIGUES, R. F. ; SILVA, L. L. ; ROSSONI, D. F. ; PEDROSO, R. B. ; ARISTIDES, S. M. A. . CHALLENGES TO HUMAN LEISHMANIASIS: DISCUSSION OF THE DISEASE BASED ON ITS BEHAVIOR. In: III INTERNATIONAL SYMPOSIUM OF EXPERIMENTAL PATHOLOGY, 2019, Londrina. Annais III International Symposium os Experimental Pathology, 2019. p. 40.

4.

NOMA, I. H. Y.; SUEHIRO, T. T. ; MORELLI, F. ; SOUZA, M. V. F. ; MESQUITA, C. S. S. ; SILVA, V. R. S. ; CONSOLARO, M. E. L. . Co-infecções entre Papillomavirus Humano (HPV) e micoplasmas genitais em mulheres assintomáticas. In: VI International Meeting of Biosciences and Physiopathology ? X Simpósio de Biociências Aplicadas à Farmácia, 2019, Ivatuba. Annais VI International Meeting of Biosciences & Physiopathology. Maringá, 2019. p. 227.

5.

NOMA, I. H. Y.; GANAZA, K. L. T. ; TANOSHI, C. A. ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; ARISTIDES, S. M. A. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. . GASTOS PÚBLICOS NA MORBIDADE HOSPITALAR POR LEISHMANIOSE NO PARANÁ, BRASIL. In: V International Meeting of Biosciences and Physiopathology e IX Simpósio de Biociências Aplicadas à Farmácia., 2018, Maringá. Anais de Simpósio do Programa de Biociências e Fisiopatologia- UEM, 2018. v. 1. p. 175.

6.

NOMA, I. H. Y.; ECKER, A. B. S. ; SZCZEREPA, M. M. A. ; TOGNIM, M. C. B. . Disseminação de enterobactérias em pacientes durante a internação em unidade de tratamento intensivo de hospital escola do sul do Brasil. In: IV International Meeting of Biosciences and Physiopathology, 2017, Maringá. VII Simpósio de Biociências Aplicadas à Farmácia, 2017.

7.

MOREIRA, D. C. ; ECKER, A. B. S. ; **NOMA, I. H. Y.** ; FEDRIGO, N. H. ; FABRI, F. V. ; COSTA, B. B. ; ZERBINATI, H. T. ; MENEZES, A. V. ; FERNANDES, A. S. ; TOGNIM, M. C. B. . SURVEILLANCE OF CARBAPENEM-RESISTANT ENTEROBACTERIACEAE IN PATIENTS DURING HOSPITALIZATION IN AN ADULT INTENSIVE CARE UNIT. In: 29º CONGRESSO BRASILEIRO DE MICROBIOLOGIA - CBM, 2017, Foz do Iguaçu. 29º Congresso Brasileiro de Microbiologia 2017, 2017.

8.

SILVA, L. C. ; PEREIRA, P. M. ; DURANTE, P. C. ; MATTOS, M. S. F. ; ZERBINATI, H. T. ; **NOMA, I. H. Y.** ; FERNANDES, A. S. ; CARDOSO, C. L. ; TOGNIM, M. C. B. ; NISHIYAMA, S. A. B. . PRELIMINARY EVALUATION OF THE FREQUENCY OF ACINETOBACTER BAUMANNI ISOLATE AT THE BUCAL AND NASAL CAVITY FROM UNIVERSITY STUDENTS. In: 29° Congresso Brasileiro de Microbiologia, 2017, Foz do Iguaçu. 29º Congresso Brasileiro de Microbiologia 2017, 2017.

9.

FEDRIGO, N. H. ; MATTA, P. E. G. ; PEREIRA, P. M. ; **NOMA, I. H. Y.** ; TOGNIM, M. C. B. . Atividade da fosfomicina contra Acinetobacter baumannii e desempenho de métodos de avaliação de sensibilidade. In: VII Simpósio de Biociências Aplicadas à Farmácia e III International Meeting of Biosciences and Physiopathology, 2016, Maringá. Anais Simpósio VII Simpósio de Biociências Aplicadas à Farmácia, 2016.

10.

NOMA, I. H. Y.; PEREIRA, V. M. ; SILVA, P. K. S. ; SANUKI, M. S. ; SILVEIRA, T. G. V. ; ARISTIDES, S. M. A. . RELAÇÃO DA NOCICEPÇÃO COM A PRODUÇÃO DE ÓXIDO NÍTRICO EM ANIMAIS INFECTADOS COM Leishmania (Leishmania) amazonensis. In: IV Semana Acadêmica de Biomedicina - UEM, 2016, Maringá. Anais da IV Semana de Biomedicina, 2016.

11.

MATTA, P. E. G. ; ECKER, A. B. S. ; ZARPELLON, M. N. ; MOREIRA, D. C. ; **NOMA, I. H. Y.** ; TAMURA, N. K. ; GARCIA, L. B. ; TOGNIM, M. C. B. . PREVALÊNCIA DE RESISTÊNCIA AOS CARBAPENÊMICOS NOS PRINCIPAIS BACILOS GRAM-NEGATIVOS ISOLADO SEM UM HOSPITAL DE ENSINO. In: XIV Congresso Brasileiro de Controle de Infecção e Epidemiologia Hospitalar, 2014, Curitiba. Journal of Infection Control, 2014. v. 3. p. 139.

12.

VIANA, G. F. ; ZAGO, M. C. B. ; SAALFELD, S. M. S. ; **NOMA, I. H. Y.** ; MATTA, P. E. G. ; MITSUGUI, C. S. ; ABREU FILHO, B. A. ; TOGNIM, M. C. B. . ALTERAÇÃO NO MODO DE DISSEMINAÇÃO DE ISOLADOS DE Acinetobacter baumannii EM UM HOSPITAL UNIVERSITÁRIO. In: XIV Congresso Brasileiro de Controle de Infecção e Epidemiologia Hospitalar, 2014, Curitiba. Journal of Infection Control, 2014. v. 3. p. 148.

13.

ZAGO, M. C. B. ; VIANA, G. F. ; SAALFELD, S. M. S. ; **NOMA, I. H. Y.** ; FARIAS, A. P. M. ; HELBEL, C. ; TOGNIM, M. C. B. . DISSEMINAÇÃO DE Acinetobacter baumannii EM UM HOSPITAL TERCIÁRIO NA REGIÃO NORTE DO PARANA. In: XIV CONGRESSO BRASILEIRO DE CONTROLE DE INFECÇÃO E EPIDEMIOLOGIA HOSPITALAR, 2014, Curitiba. DISSEMINAÇÃO DE Acinetobacter baumannii EM UM HOSPITAL TERCIÁRIO NA REGIÃO NORTE DO PARANÁ, 2014. v. 3. p. 135.

Apresentações de Trabalho

1.

NOMA, I. H. Y.; CRUZ, E. ; DUTRA, A. C. ; NEGRI, M. F. N. . Geospatial Analysis of Diagnostic Imaging Equipment in Brazil. 2020. (Apresentação de Trabalho/Conferência ou palestra).

2.

CRUZ, E. ; **NOMA, I. H. Y.** ; DUTRA, A. C. ; NEGRI, M. F. N. . Life-Sustaining Equipment: A Demographic Geospace Analysis in National Territory. 2020. (Apresentação de Trabalho/Conferência ou palestra).

3.

RODRIGUES, R. F. ; SILVA, L. L. ; TANOSHI, C. A. ; **NOMA, I. H. Y.** ; SILVA, G. E. C. . ANÁLISE DO PERFIL EPIDEMIOLÓGICO DE ESCORPIONISMO NOS ESTADOS DO SUL DO BRASIL: UM ESTUDO REFERENTE AO PERÍODODE 2015 A 2019. 2020. (Apresentação de Trabalho/Outra).

4.

NOMA, I. H. Y.; SUEHIRO, T. T. ; MORELLI, F. ; SOUZA, M. V. F. ; MESQUITA, C. S. S. ; SILVA, V. R. S. ; CONSOLARO, M. E. L. . Co-infecções entre Papillomavirus Humano (HPV) e micoplasmas genitais em mulheres assintomáticas. 2019. (Apresentação de Trabalho/Simpósio).

5.

TANOSHI, C. A. ; **NOMA, I. H. Y.** ; RODRIGUES, R. F. ; SILVA, L. L. ; PEDROSO, R. B. ; ARISTIDES, S. M. A. . Challenges to human leishmaniasis: discussion of the disease based on its behavior. 2019. (Apresentação de Trabalho/Simpósio).

6.

NOMA, I. H. Y.; TANOSHI, C. A. ; RODRIGUES, R. F. ; SILVA, L. L. ; CONSOLARO, M. E. L. ; PEDROSO, R. B. . The prevalence of syphilis in prenatal care women in Paraná State, Brazil. 2019. (Apresentação de Trabalho/Simpósio).

7.

NOMA, I. H. Y.; GANAZA, K. L. T. ; TANOSHI, C. A. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; ARISTIDES, S. M. A. ; DEMARCHI, I. G. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. . Leishmaniose mucocutânea no Paraná, avaliação da morbidade hospitalar de 2008 a 2018.. 2018. (Apresentação de Trabalho/Congresso).

8.

GANAZA, K. L. T. ; TANOSHI, C. A. ; **NOMA, I. H. Y.** ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; CONSOLARO, M. E. L. ; ARISTIDES, S. M. A. ; DEMARCHI, I. G. ; LONARDONI, M. V. C. . Análise da morbidade hospitalar por leishmaniose cutânea no estado do Paraná, Brasil, 2008-2018. 2018. (Apresentação de Trabalho/Congresso).

9.

TANOSHI, C. A. ; **NOMA, I. H. Y.** ; GANAZA, K. L. T. ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. ; ARISTIDES, S. M. A. . Despesas em morbidade hospitalar devido a leishmaniose visceral em uma região do sul do Brasil.. 2018. (Apresentação de Trabalho/Congresso).

10.

TANOSHI, C. A. ; GANAZA, K. L. T. ; **NOMA, I. H. Y.** ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; CONSOLARO, M. E. L. ; LONARDONI, M. V. C. ; ARISTIDES, S. M. A. . A prevalência de uma doença infecciosa negligenciada: epidemiologia e morbidade das leishmanioses no Paraná. 2018. 2018. (Apresentação de Trabalho/Simpósio).

11.

NOMA, I. H. Y.; GANAZA, K. L. T. ; TANOSHI, C. A. ; DEMARCHI, I. G. ; PEDROSO, R. B. ; CARVALHO, M. D. B. ; PELLOSO, S. M. ; ARISTIDES, S. M. A. ; LONARDONI, M. V. C. ; CONSOLARO, M. E. L. . GASTOS PÚBLICOS NA MORBIDADE HOSPITALAR POR LEISHMANIOSE NO PARANA, BRASIL. 2018. (Apresentação de Trabalho/Simpósio).

12.

ECKER, A. B. S. ; MOREIRA, D. C. ; **NOMA, I. H. Y.** ; VIANA, G. F. ; MITSUGUI, C. S. ; PEREIRA, P. M. ; TOGNIM, M. C. B. . VARIABILIDADE CLONAL E PESQUISA DE GENES DE BETA LACTAMASES DE ACINETOBACTER BAUMANNII ISOLADOS DURANTE A PERMANÊNCIA DE PACIENTES EM UNIDADE DE TERAPIA INTENSIVA. 2017. (Apresentação de Trabalho/Congresso).

13.

PEREIRA, P. M. ; MATTA, P. E. G. ; **NOMA, I. H. Y.** ; SILVA, L. C. ; FARIAS, A. P. M. ; ZARPELLON, M. N. ; TOGNIM, M. C. B. . ATIVIDADE DE FOSFOMICINA CONTRA ISOLADOS DE ACINETOBACTER BAUMANNII: AVALIAÇÃO DE DIFERENTES METODOLOGIAS. 2017. (Apresentação de Trabalho/Congresso).

14.

NOMA, I. H. Y.; ECKER, A. B. S. ; MOREIRA, D. C. ; SILVA, L. C. ; PEREIRA, P. M. ; SZCZEREPA, M. M. A. ; TOGNIM, M. C. B. . VARIABILIDADE DE β-LACTAMASES PRESENTES EM BACILOS GRAM NEGATIVOS ISOLADOS DURANTE A PERMANÊNCIA DE PACIENTES EM HOSPITAL DE ENSINO. 2017. (Apresentação de Trabalho/Outra).

15.

NOMA, I. H. Y.; ECKER, A. B. S. ; SZCZEREPA, M. M. A. ; TOGNIM, M. C. B. . Disseminação de enterobactérias em pacientes durante a internação em unidade de tratamento intensivo de hospital escola do sul do Brasil.. 2017. (Apresentação de Trabalho/Simpósio).

16.

SILVA, L. C. ; PEREIRA, P. M. ; DURANTE, P. C. ; MATTOS, M. S. F. ; ZERBINATI, H. T. ; **NOMA, I. H. Y.** ; FERNANDES, A. S. ; CARDOSO, C. L. ; TOGNIM, M. C. B. ; NISHIYAMA, S. A. B. . PRELIMINARY EVALUATION OF THE FREQUENCY OF ACINETOBACTER BAUMANNI ISOLATE AT THE BUCAL AND NASAL CAVITY FROM UNIVERSITY STUDENTS. 2017. (Apresentação de Trabalho/Congresso).

17.

MOREIRA, D. C. ; ECKER, A. B. S. ; **NOMA, I. H. Y.** ; FEDRIGO, N. H. ; FABRI, F. V. ; COSTA, B. B. ; ZERBINATI, H. T. ; MENEZES, A. V. ; FERNANDES, A. S. ; TOGNIM, M. C. B. . SURVEILLANCE OF CARBAPENEM-RESISTANT ENTEROBACTERIACEAE IN PATIENTS DURING HOSPITALIZATION IN AN ADULT INTENSIVE CARE UNIT.. 2017. (Apresentação de Trabalho/Congresso).

18.

ROMAO, P. A. S. S. ; ALBUQUERQUE, E. P. A. ; MONTANHA, F. F. ; **NOMA, I. H. Y.** ; GREMASCHI, L. B. ; BLANCO, L. F. M. ; BRAZ, N. M. ; VALVERDE, T. L. ; VICENTINI, V. E. P. . Dr. Genética: Conteúdo informacional online de apoio a exames genéticos. 2016. (Apresentação de Trabalho/Outra).

19.

NOMA, I. H. Y.; PEREIRA, V. M. ; SANUKI, M. S. ; BORGHI, S. M. ; VERRI JUNIOR, W. A. ; SILVA, P. K. S. ; ARISTIDES, S. M. A. . RELAÇÃO DA NOCICEPÇÃO COM A PRODUÇÃO DE ÓXIDO NÍTRICO EM ANIMAIS INFECTADOS COM LEISHMANIA (LEISHMANIA) AMAZONENSIS. 2016. (Apresentação de Trabalho/Outra).

20.

FEDRIGO, N. H. ; MATTA, P. E. G. ; PEREIRA, V. M. ; **NOMA, I. H. Y.** ; TOGNIM, M. C. B. . Atividade da fosfomicina contra Acinetobacter baumannii e desempenho de métodos de avaliação de sensibilidade. 2016. (Apresentação de Trabalho/Simpósio).

21.

NOMA, I. H. Y.; WATANABE, A. S. M. ; SILVA, M. R. T. ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . Avaliação da patogenicidade e fatores de virulência de Candida albicans no modelo experimental de Candidemia. 2015. (Apresentação de Trabalho/Outra).

22.

LODI, F. G. ; ZAGO, M. C. B. ; MATTA, P. E. G. ; **NOMA, I. H. Y.** ; TAMURA, N. K. ; AOKI, E. E. ; TOGNIM, M. C. B. . Evolução temporal da concentração inibitória minima para daptomicina em cocos gram-positivos. 2015. (Apresentação de Trabalho/Congresso).

23.

FEDRIGO, N. H. ; TREVIZOLI, T. M. B. ; **NOMA, I. H. Y.** ; MITSUGUI, C. S. ; AOKI, E. E. ; TAMANINI, J. B. ; TOGNIM, M. C. B. . Análise temporal da resistência aos carbapenêmicos em bacilos gram-negativos não fermentadores. 2015. (Apresentação de Trabalho/Congresso).

24.

WATANABE, A. S. M. ; SILVA, M. R. T. ; **NOMA, I. H. Y.** ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . AVALIAÇÃO DOS FATORES DE VIRULÊNCIA DE CANDIDA TROPICALIS APÓS CANDIDEMIA EXPERIMENTAL. 2015. (Apresentação de Trabalho/Outra).

25.

SILVA, M. R. T. ; WATANABE, A. S. M. ; **NOMA, I. H. Y.** ; NEGRI, M. F. N. ; BONFIM-MENDONCA, P. S. ; COTICA, E. S. K. . AVALIAÇÃO DO PERFIL DE PATOGENICIDADE DE CANDIDA ALBICANS APÓS CONTATOS SUBSEQUENTES COM O HOSPEDEIRO. 2015. (Apresentação de Trabalho/Outra).

26.

MATTA, P. E. G. ; ECKER, A. B. S. ; ZARPELLON, M. N. ; MOREIRA, D. C. ; **NOMA, I. H. Y.** ; TAMURA, N. K. ; GARCIA, L. B. ; TOGNIM, M. C. B. . PREVALÊNCIA DE RESISTÊNCIA AOS CARBAPENÊMICOS NOS PRINCIPAIS BACILOS GRAM-NEGATIVOS ISOLADO SEM UM HOSPITAL DE ENSINO.. 2014. (Apresentação de Trabalho/Congresso).

27.

VIANA, G. F. ; ZAGO, M. C. B. ; SAALFELD, S. M. S. ; **NOMA, I. H. Y.** ; MATTA, P. E. G. ; MITSUGUI, C. S. ; ABREU FILHO, B. A. ; TOGNIM, M. C. B. . ALTERAÇÃO NO MODO DE DISSEMINAÇÃO DE ISOLADOS DE Acinetobacter baumannii EM UM HOSPITAL UNIVERSITÁRIO. 2014. (Apresentação de Trabalho/Congresso).

28.

ZAGO, M. C. B. ; VIANA, G. F. ; SAALFELD, S. M. S. ; **NOMA, I. H. Y.** ; FARIAS, A. P. M. ; HELBEL, C. ; TOGNIM, M. C. B. . DISSEMINAÇÃO DE Acinetobacter baumannii EM UM HOSPITAL TERCIÁRIO NA REGIÃO NORTE DO PARANÁ. 2014. (Apresentação de Trabalho/Congresso).

Produção técnica

Redes sociais, websites e blogs

1.

NOMA, I. H. Y.. É POSSÍVEL RECRIAR PELE ENVELHECIDA IN VITRO?. 2020; Tema: Divulgação científica do Laboratório de Biologia da Pele e Melanoma. (Rede social).

Participação em eventos, congressos, exposições e feiras

1.

19th International Congress of the Society for Melanoma Research. PRDX2 as a possible phenotype switcher in NRAS-mutated melanomas. 2022. (Congresso).

2.

Simpósio em comemoração dos 50 anos do Programa de Pós Graduação em Farmácia Fisiopatologia e Toxicologia.Targeting Peroxiredoxin 2 as a potential treatment for NRAS mutated metastatic melanoma. 2022. (Simpósio).

3.

XXII Brazilian Congress of Toxicology. Gliotoxin and its potential cytotoxicity against NRAS mutated-melanoma. 2022. (Congresso).

4.

A importância do estudo de Irritação/Corrosão Dérmica in vitro na avaliação toxicológica. 2021. (Outra).

5.

Host-Pathogen Interaction Meeting. 2021. (Encontro).

6.

I Simpósio Desafios em Oncologia Molecular. 2021. (Simpósio).

7.

Brazilian Congress in Biomedical Engineering (CBEB2020). GEOSPATIAL ANALYSIS OF DIAGNOSTIC IMAGING EQUIPMENT IN BRAZIL. 2020. (Congresso).

8.

Ciclo de Palestras FisioTox. 2020. (Seminário).

9.

Congresso Acadêmico Unifesp Virtual 2020 - Ciência e Universidade: Transformações para a Sociedade. 2020. (Congresso).

10.

II Encontro De Genética, Hemoterapia e Patologias Hematológicas. 2020. (Encontro).

11.

Increasing CRISPR editing efficiency with novel guide RNA methods. 2020. (Seminário).

12.

Infecção por HPV- Infecto em Série. 2020. (Seminário).

13.

Perspectivas Diagnósticas e Terapêuticas utilizando a Tecnologia CRISPR. 2020. (Outra).

14.

PÍLULAS DE ONCOLOGIA: Princípios de hormonioterapia no tratamento oncológico. 2020. (Seminário).

15.

PÍLULAS DE ONCOLOGIA: Princípios do Tratamento Oncológico e Mecanismos da Quimioterapia. 2020. (Seminário).

16.

PÍLULAS DE ONCOLOGIA: Terapia-alvo e Imunoterapia na Oncologia Moderna. 2020. (Seminário).

17.

PÍLULAS DE ONCOLOGIA - Casos clínicos em oncologia: aplicação de conceitos básicos. 2020. (Seminário).

18.

VII International Meeting of Biosciences and Physiopathology e XI Simpósio de Biociências Aplicadas à Farmácia. 2020. (Simpósio).

19.

Festival Varilux-UEM de Cinema Francês 2019. 2019. (Exposição).

20.

III INTERNATIONAL SYMPOSIUM OF EXPERIMENTAL PATHOLOGY.THE PREVALENCE OF SYPHILIS IN PRENATAL CARE WOMEN IN PARANA STATE, BRAZIL. 2019. (Simpósio).

21.

VI International Meeting of Biosciences and Physiopathology ? X Simpósio de Biociências Aplicadas à Farmácia.Co-infecções entre Papillomavirus Humano (HPV) e micoplasmas genitais em mulheres assintomáticas. 2019. (Encontro).

22.

8.o Encontro Anual de Iniciação Tecnológica e Inovação.Coordenador(a) de sessão. 2018. (Encontro).

23.

II Workshop Brasil-Estados Unidos em câncer cervical. 2018. (Outra).

24.

O SUS e a Saúde Pública brasileira: cenário atual e perspectivas. 2018. (Outra).

25.

Vamos falar sobre Doenças Raras?. 2018. (Outra).

26.

V Congresso Internacional do CCS, III Congresso Internacional de Tratamento da Obesidade e VII Encontro do PCS. Universidade Estadual de Maringá. Leishmaniose mucocutânea no Paraná, avaliação da morbidade hospitalar de 2008 a 2018. 2018. (Congresso).

27.

V International Meeting of Biosciences and Physiopathology e IX Simpósio de Biociências Aplicadas à Farmácia..GASTOS PUBLICOS NA MORBIDADE HOSPITALAR POR LEISHMANIOSE NO PARANA, BRASIL. 2018. (Simpósio).

28.

26.o Encontro Anual de Iniciação Científica e 6.o Encontro Anual de Iniciação Científica Júnior.VARIABILIDADE DE ?-LACTAMASES PRESENTES EM BACILOS GRAM NEGATIVOS ISOLADOS DURANTE A PERMANÊNCIA DE PACIENTES EM HOSPITAL DE ENSINO. 2017. (Encontro).

29.

I Workshop de genética médica. 2017. (Outra).

30.

25.o Encontro Anual de Iniciação Científica e 5.o Encontro Anual de Iniciação Científica Júnior.RELAÇÃO DA NOCICEPÇÃO COM A PRODUÇÃO DE ÓXIDO NÍTRICO EM ANIMAIS INFECTADOS COM LEISHMANIA (LEISHMANIA) AMAZONENSIS. 2016. (Encontro).

31.

I Simpósio Regional de Fisiologia Integrativa e do Exercício. 2016. (Simpósio).

32.

IV Semana Acadêmica de Biomedicina - UEM.Relação da nocicepção com a produção de óxido nítrico em animais infectados com Leishmania (Leishmania) amazonenses. 2016. (Outra).

33.

PROSED.Projeto Socioeducacional da III Semana Acadêmica de Biomedicina - UEM. 2016. (Oficina).

34.

Conexão Saúde. 2015. (Outra).

35.

III Semana Acadêmica de Biomedicina ? UEM.Avaliação da patogenicidade e fatores de virulência de Candida albicans no modelo experimental de Candidemia. 2015. (Outra).

36.

4º Congresso Paranaense de Ciências Biomédicas e 1ª Oficina Pró-saúde. 2014. (Congresso).

37.

III Mostra de Cinema e Divulgação Cientifica em Biociências. 2014. (Outra).

38.

II Semana Acadêmica de Biomedicina - UEM. 2014. (Outra).

39.

I Fórum de Discussão Estudantil.Fontes alternativas de energia (biodiesel e biogás). 2011. (Outra).

Organização de eventos, congressos, exposições e feiras

1.

NOMA, ISABELLA HARUMI YONEHARA. II Ciclo de Palestra da FisioTox, do Programa de Pós-Graduação em Farmácia (Fisiopatologia e Toxicologia). 2021. (Outro).

2.

NOMA, I. H. Y.. VII International Meeting of Biosciences and Physiopathology e XI Simpósio de Biociências Aplicadas à Farmácia. 2020. (Outro).

3.

NOMA, I. H. Y.. VI International Meeting of Biosciences and Physiopathology ? X Simpósio de Biociências Aplicadas à Farmácia. 2019. (Outro).

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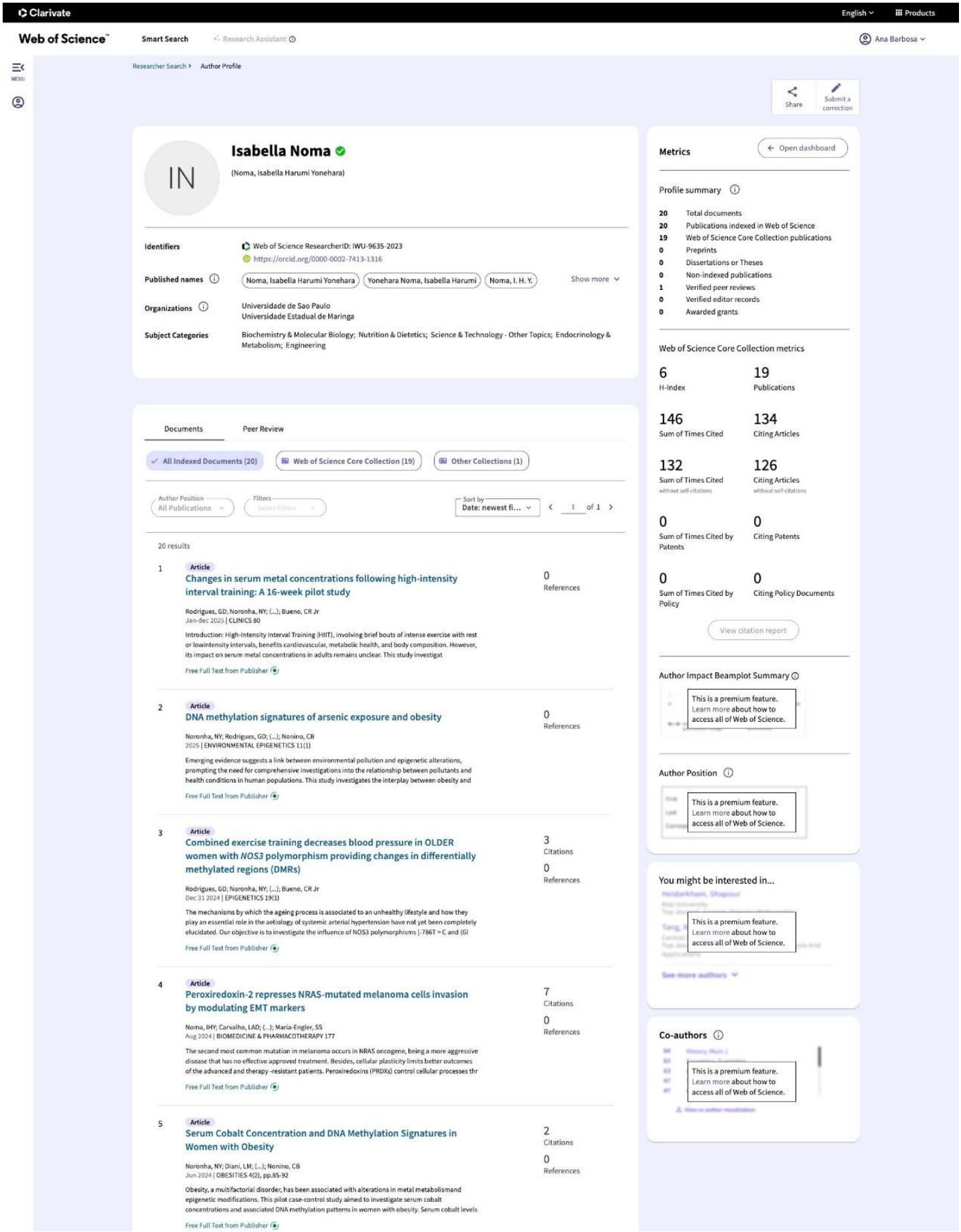
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Noronha, NY; Rodrigues, GD; (...); Nonino, CB
2025 | ENVIRONMENTAL EPIGENETICS 11(1)
Emerging evidence suggests a link between environmental pollution and epigenetic alterations, prompting the need for comprehensive investigations into the relationship between pollutants and health conditions in human populations. This study investigates the interplay between obesity and
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References
3
Article
Combined exercise training decreases blood pressure in OLDER women with NOS3 polymorphism providing changes in differentially methylated regions (DMRs)
Rodrigues, GD; Noronha, NY; (...); Bueno, CR Jr
Dec 31 2024 | EPIGENETICS 19(1)
The mechanisms by which the ageing process is associated to an unhealthy lifestyle and how they play an essential role in the aetiology of systemic arterial hypertension have not yet been completely elucidated. Our objective is to investigate the influence of NOS3 polymorphisms [-786T > C and (GI
Free Full Text from Publisher
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References
4
Article
Peroxiredoxin-2 represses NRAS-mutated melanoma cells invasion by modulating EMT markers
Noma, IHY; Carvalho, LAD; (...); Maria-Engler, SS
Aug 2024 | BIOMEDICINE & PHARMACOTHERAPY 177
The second most common mutation in melanoma occurs in NRAS oncogene, being a more aggressive disease that has no effective approved treatment. Besides, cellular plasticity limits better outcomes of the advanced and therapy -resistant patients. Peroxiredoxins (PRDXs) control cellular processes thr
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Citations
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References
5
Article
Serum Cobalt Concentration and DNA Methylation Signatures in Women with Obesity
Noronha, NY; Diani, LM; (...); Nonino, CB
Jun 2024 | OBESITIES 4(2), pp.85-92
Obesity, a multifactorial disorder, has been associated with alterations in metal metabolismand epigenetic modifications. This pilot case-control study aimed to investigate serum cobalt concentrations and associated DNA methylation patterns in women with obesity. Serum cobalt levels
Free Full Text from Publisher
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References
Metrics
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Profile summary
20 Total documents
20 Publications indexed in Web of Science
19 Web of Science Core Collection publications
0 Preprints
0 Dissertations or Theses
0 Non-indexed publications
1 Verified peer reviews
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6 H-Index
19 Publications
146 Sum of Times Cited
134 Citing Articles
132 Sum of Times Cited without self-citations
126 Citing Articles without self-citations
0 Sum of Times Cited by Patents
0 Citing Patents
0 Sum of Times Cited by Policy
0 Citing Policy Documents
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6 Article
Modeling Melanoma Heterogeneity In Vitro: Redox, Resistance and Pigmentation Profiles
Carvalho, LAD; Noma, IHY; (...); Maria-Engler, SS
May 2024 | ANTIOXIDANTS 13(5)
Microenvironment and transcriptional plasticity generate subpopulations within the tumor, and the use of BRAF inhibitors (BRAFis) contributes to the rise and selection of resistant clones. We stochastically isolated subpopulations (C1, C2, and C3) from na & iuml;ve melanoma and found that
Free Full Text from Publisher
3 Citations
0 References

7 Article
Association between the relative abundance of phyla actinobacteria, vitamin C consumption, and DNA methylation of genes linked to immune response pathways
Noronha, NY; Noma, IHY; (...); Nonino, CB
Apr 4 2024 | FRONTIERS IN NUTRITION 11
Introduction: There is an emerging body of evidence that vitamin C consumption can modulate microbiota abundance and can also impact DNA methylation in the host, and this could be a link between diet, microbiota, and immune response. The objective of this study was to evaluate
Free Full Text From Publisher
3 Citations
0 References

8 Meeting Abstract
Unveiling PAI-1 as a new player in chemoresistant melanoma in aged human skin reconstructed in vitro
da Silva, RO; Carvalho, LA; (...); Engler, SSM
Annual Meeting of the American-Association-for-Cancer-Research (AACR)
Mar 15 2024 | CANCER RESEARCH 84(6)
Full text at publisher
0 References

9 Article
14-Week exercise training modifies the DNA methylation levels at gene sites in non-Alzheimer's disease women aged 50 to 70 years
Rodrigues, GD; Noronha, NY; (...); Bueno, CR Jr
Feb 2024 | EXPERIMENTAL GERONTOLOGY 186
Exercise training emerges as a key strategy in lifestyle modification, capable of reducing the risk of developing Alzheimer's disease (AD) due to risk factors such as age, family history, genetics and low level of education associated with AD. We aim to analyze the effect of a 14-week combined ex
Free Full Text from Publisher
4 Citations
0 References

10 Article
The influence of serum selenium in differential epigenetic and transcriptional regulation of CPT1B gene in women with obesity
Watanabe, LM; Pereira, VAB; (...); Nonino, CB
May 2024 | JOURNAL OF TRACE ELEMENTS IN MEDICINE AND BIOLOGY 83
Introduction: The increasing prevalence of obesity has become a major health problem worldwide. The causes of obesity are multifactorial and could be influenced by dietary patterns and genetic factors. Obesity has been associated with a decrease in micronutrient intake and consequently
Full Text at Publisher
7 Citations
0 References

11 Article
Eight Weeks of Physical Training Decreases 2 Years of DNA Methylation Age of Sedentary Women
Rodrigues, GD; Noma, IHY; (...); Bueno, CR Jr
Apr 2 2024 | RESEARCH QUARTERLY FOR EXERCISE AND SPORT 95(2), pp.405-415
Purpose: The acceleration of epigenetic age is a predictor of mortality and contributes to the increase in chronic diseases. Adherence to a healthy lifestyle is a strategy to reduce epigenetic age. The present study aimed to determine whether eight weeks of combined (aerobic and strength) trainin
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9 Citations
0 References

12 Article
2-Methoxyestradiol-3,17-*O*,*O*-bis-sulfamate (STX140) Inhibits Proliferation and Invasion via Senescence Pathway Induction in Human BRAFi-Resistant Melanoma Cells
Franco, YA; de Moraes, MO Jr; (...); Maria-Engler, SS
Jul 2023 | INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 24(14)
The endogenous estradiol derivative 2-Methoxyestradiol (2-ME) has shown good and wide anticancer activity but suffers from poor oral bioavailability and extensive metabolic conjugation. However, its sulfamoylated derivative, 2-methoxyestradiol-3,17-O,O-bis-sulfamate (STX140), has superior potenti
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1 Citation
0 References

13 Article
14-weeks combined exercise epigenetically modulated 118 genes of menopausal women with prediabetes
Noronha, NY; Rodrigues, GD; (...); Nonino, CB
Aug 15 2022 | FRONTIERS IN ENDOCRINOLOGY 13
BackgroundPre-diabetes precedes Diabetes Mellitus (DM) disease and is a critical period for hyperglycemia treatment, especially for menopausal women, considering all metabolic alterations due to hormonal changes. Recently, the literature has demonstrated the role of physical exercise in
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21 Citations
0 References

14 Article
Sample Preparation to Bioinformatics Analysis of DNA Methylation: Association Strategy for Obesity and Related Trait Studies
Noronha, NY; Rodrigues, GD; (...); Nonino, CB
May 2022 | JOVE-JOURNAL OF VISUALIZED EXPERIMENTS (183)
Obesity is directly connected to lifestyle and has been associated with DNA methylation changes that may cause alterations in the adipogenesis and lipid storage processes contributing to the development of the disease. We demonstrate a complete protocol from selection to epigenetic data
9 Citations
0 References

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15 **Article**

Trend change estimation for interrupted time series with heteroscedastic and autocorrelated errors: application in syphilis occurrences in Brazil

de Souza, EM; Sodré, D; (...); Pedroso, RB
Jan-dec 2022 | ACTA SCIENTIARUM-TECHNOLOGY 44

The impact evaluation of exogenous policies over time is of great importance in several areas. Unfortunately, an adequate time-series analysis has not always been taken into account in the literature, mainly in health problems. When regression models are used in the known interrupted

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1 Citation
0 References

16 **Proceedings Paper**

Life-Sustaining Equipment: A Demographic Geospace Analysis in National Territory

Cruz, E; Noma, IHY; (...); Negri, M
27th Brazilian Congress on Biomedical Engineering (CBEB)
2022 | XXVII BRAZILIAN CONGRESS ON BIOMEDICAL ENGINEERING, CBEB 2020, pp.2131-2136

Equitable supply of life-sustaining equipment (LSE) is an important guide for assessing public health service provision. However, the inadequate geospatial distribution of this equipment can pose a difficulty in accessing it. In this attempt, the objective of this study was to analyze the geospat

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17 **Proceedings Paper**

Geospatial Analysis of Diagnostic Imaging Equipment in Brazil

Noma, IHY; Cruz, E; (...); Negri, M
27th Brazilian Congress on Biomedical Engineering (CBEB)
2022 | XXVII BRAZILIAN CONGRESS ON BIOMEDICAL ENGINEERING, CBEB 2020, pp.2137-2142

Diagnostic imaging equipment is important for assistance in the Unified Health System, however, the lack of adequate geographical distribution of these equipment can hamper access to services. The objective was to analyze the geospatial distribution by data from the SUS Information Technology

Full Text at Publisher

0 References

18 **Article**

Gene signatures of autopsy lungs from obese patients with COVID-19

Silva, JCSE; Vasconcelos, AP; (...); Nakaya, HI
Aug 2021 | CLINICAL NUTRITION ESPEN 44, pp.475-478

Background & aims: Obesity is associated with low grade systemic inflammation and insulin resistance. Although metabolic and immunological changes may contribute to the increased risk for COVID-19 mortality in obese, little is known about the impact of obesity in the lungs of patients with

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11 Citations
0 References

19 **Journal Article**

Association of Righ-Risk Human Papillomavirus and Ureaplasma parvum Co-Infections with Increased Risk of Low-Grade Squamous Intraepithelial Cervical Lesions.

Noma, Isabella Harumi Yonehara; Shinobu-Mesquita, Cristiane Suemi; (...); Consolaro, Marcia Edilaine Lopes
2021-04-01 | Asian Pacific journal of cancer prevention : APJCP 22(4), pp.1239-1246

OBJECTIVE: The present report investigated the rates of coinfections between high-rik human papillomavirus (hrHPV) and the most important human mycoplasmas including Mycoplasma hominis, M. genitalium, Ureaplasma urealyticum and U. parvum in cervical samples of asymptomatic

14 Citations
0 References

20 **Article**

BROAD RESISTANCE TO PLANT-VIRUSES IN TRANSGENIC PLANTS CONFERRED BY ANTISENSE INHIBITION OF A HOST GENE ESSENTIAL IN S-ADENOSYLMETHIONINE-DEPENDENT TRANSMETHYLATION REACTIONS

MASUTA, C; TANAKA, H; (...); NOMA, I
Jun 20 1995
| PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA 92(13), pp.6117-6121

S-Adenosylhomocysteine hydrolase (SAHH) is a key enzyme in transmethylation reactions that use S-adenosylmethionine as the methyl donor. Because of the importance of SAHH in a number of S-adenosylmethionine-dependent transmethylation reactions, particularly the 5' capping of mRNA

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87 Citations
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Page size 50 ‹ 1 of 1 ›

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Our innovation data comes from PATSTAT, which contains over 90 million legal patent and bibliographic records from more than 100 industrialized and developing countries. It provides a unique foundation for conducting sophisticated bibliographic and legal status data analyses.

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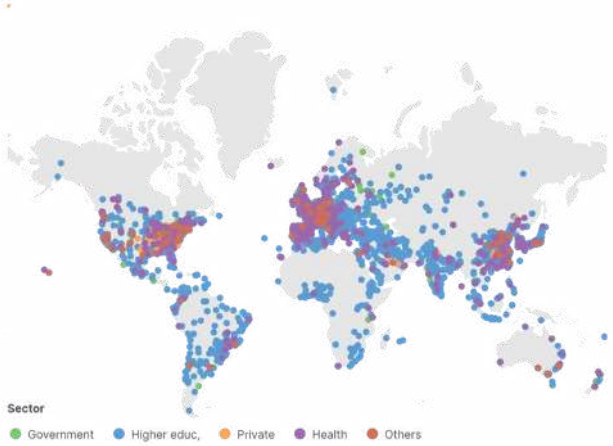
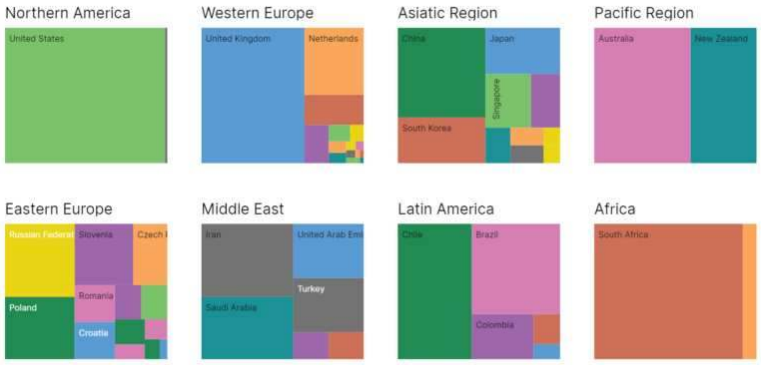


Institutions that conduct research worldwide. Source: SCImago Institutions Rankings using SCImago Graphica



Distribution of Q1 journals by geographic region. Source: SCImago Journal & Country Rank using SCImago Graphica

Scientific validity

All the indicators we use for generating rankings have been previously validated by the scientific community and supported by scientific publications in high-impact journals.

Aguillo, IF, Bar-Ilan, J., Levene, M. et al. **Comparing university rankings.** Scientometrics 85, 243–256 (2010). https://doi.org/10.1007/s11192-010-0190-z

Arencibia-Jorge, R., Vega-Almeida, RL, Chinchilla-Rodríguez, Z., Corera-Álvarez, E., Moya-Anegón, F. **Patterns of specialization in national health research** . Cuban Journal of Public Health 38, 5 (2012). https://doi.org/10.1590/s0864-34662012000500007

Bornmann, L., De Moya Anegón, F., Leydesdorff, L. (2012) **The new Excellence Indicator in the World Report of the SCImago Institutions Rankings 2011** . Journal of Informetrics, 6 (2), pp. 333-335. https://doi.org/10.1016/j.joi.2011.11.006

Bornmann, L., & Moya Anegón, F. (2014a). **What proportion of excellent papers makes an institution one of the best worldwide? Specifying thresholds for the interpretation of the results of the SCImago Institutions Ranking and the Leiden Ranking** . Journal of the Association for Information Science and Technology, 65 (4), 732-736. https://doi.org/10.1002/asi.23047

Bornmann, L., Stefaner, M., de Moya Anegón, F. and Mutz, R. (2014), " **Ranking and mapping of universities and research-focused institutions worldwide based on highly-cited papers: A visualization of results from multi-level models** ", Online Information Review, Vol. 38 No. 1, pp. 43-58. https://doi.org/10.1108/OIR-12-2012-0214

Chinchilla-Rodríguez, Z., Vargas-Quesada, B., Hassan-Montero, Y., González-Molina, A., & Moya-Anegóna, F. (2010). **New Approach to the Visualization of International Scientific Collaboration.** Information Visualization, 9(4), 277–287. https://doi.org/10.1057/ivs.2009.31

Chinchilla-Rodríguez, Z., Benavent-Pérez, M., de Moya-Anegón, F. and Miguel, S. (2012), **International Collaboration in Medical Research in Latin America and the Caribbean (2003–2007).** J Am Soc Inf Sci Tec, 63: 2223-2238. https://doi.org/10.1002/asi.22669

Chinchilla-Rodríguez, Z., Zacca-González, G., Vargas-Quesada, B. et al. **Benchmarking scientific performance by decomposing leadership of Cuban and Latin American institutions in Public Health.** Scientometrics 106, 1239–1264 (2016). https://doi.org/10.1007/s11192-015-1831-z

González-Pereira, B., Guerrero-Bote,V., Moya-Anegón, F. (2010). **A new approach to the metric of journal's scientific prestige: The SJR indicator.** Journal of Informetrics, 4(3), pp. 379–391. https://doi.org/10.1016/j.joi.2010.03.002

Guerrero-Bote, VP, Moya-Anegón, F. (2012) **A further step forward in measuring journals' scientific prestige: The SJR2 indicator.** Journal of Informetrics, 6 (4), pp. 674-688. https://doi.org/10.1016/j.joi.2012.07.001

Guerrero Bote, VP, Olmeda-Gómez, C. and de Moya-Anegón, F. (2013), **Quantifying the benefits of international scientific collaboration.** J Am Soc Inf Sci Tec, 64: 392-404. https://doi.org/10.1002/asi.22754

Lancho-Barrantes, BS, Guerrero-Bote, VP & de Moya-Anegón, F. **Citation increments between collaborating countries.** Scientometrics 94, 817–831 (2013). https://doi.org/10.1007/s11192-012-0797-3

Lancho Barrantes, BS, Guerrero Bote, VP, Rodríguez, ZC and de Moya Anegón, F. (2012), **Citation flows in the zones of influence of scientific collaborations** . J. Am. Soc. Inf. Sci., 63: 481-489. https://doi.org/10.1002/asi.21682

López-Illescas, C., de Moya-Anegón, F. & Moed, HF **A ranking of universities should account for differences in their disciplinary specialization.** Scientometrics 88, 563–574 (2011). https://doi.org/10.1007/s11192-011-0398-6

Miguel, S., Chinchilla-Rodriguez, Z. and de Moya-Anegón, F. (2011), **Open access and Scopus: A new approach to scientific visibility from the standpoint of access.** J. Am. Soc. Inf. Sci., 62: 1130-1145. https://doi.org/10.1002/asi.21532

Moya-Anegón, F., Chinchilla-Rodríguez, Z., Vargas-Quesada, B. et al. **Coverage analysis of Scopus: A journal metric approach.** Scientometrics 73, 53–78 (2007). https://doi.org/10.1007/s11192-007-1681-4

Moed, HF, Moya-Anegón, F., López-Illescas, C., Visser, M. (2011). **Is concentration of university research associated with better research performance?** Journal of Informetrics. 5 (4) 649-658. https://doi.org/10.1016/j.joi.2011.06.003

Moya-Anegón, F. (2012). **Leadership and excellence in Spanish science.** Profesional De La Información, 21(2), 125–128. https://doi.org/10.3145/epi.2012.mar.01

Moya-Anegón, F., Guerrero-Bote, VP, Bornmann, L. et al. **The research guarantors of scientific papers and the output counting: a promising new approach.** Scientometrics 97, 421–434 (2013). https://doi.org/10.1007/s11192-013-1046-0

Moya-Anegón, F. (dir.), Chinchilla-Rodríguez, Z. (coord.), Corera-Álvarez, E., González-Molina, A., Vargas-Quesada, B. (2013) **Main Bibliometric Indicators of Spanish Scientific Activity: 2010.** Madrid: Spanish Foundation for Science and Technology. https://www.fecyt.es/es/system/files/publications/attachments/2014/11/indicadoresbibliometricos_2010.pdf

Moya-Anegón, F. (dir.), Chinchilla-Rodríguez, Z. (coord.), Corera-Álvarez, E., González-Molina, A., Vargas-Quesada, B. (2013) **Excellence and leadership of Spanish scientific production 2003-2010.** Madrid: Spanish Foundation for Science and Technology.

Moya-Anegón, F., Chinchilla-Rodríguez, Z. **Technological impact of Ibero-American university production.** In: The transfer of R&D, innovation and entrepreneurship in universities. Higher Education in Ibero-America. 2015 Report. Santiago, Chile: Inter-University Center for Development, 2015, pp. 83-94. http://hdl.handle.net/10261/115266

OECD and SCImago Research Group (CSIC) (2015), **Compendium of Bibliometric Science Indicators 2014** . https://www.oecd.org/sti/inno/scientometrics.htm

Romo-Fernández, LM, Lopez-Pujalte, C., Guerrero Bote, VP, Moya-Anegon, F. (2011). **Analysis of Europe's scientific production on renewable energies.** Renewable Energy, 36 (9), pp. 2529-2537. https://doi.org/10.1016/j.renene.2011.02.001

Tibaná-Herrera, G., Fernández-Bajón, M.-T., & De-Moya-Anegón, F. (2018). **Output, collaboration and impact of e-learning research: Bibliometric analysis and visualizations at the country and institutional level (Scopus 2003-2016).** Profesional De La Información, 27(5), 1082–1096. https://doi.org/10.3145/epi.2018.sep.12

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E.4. Evidence of publication in Biomedicine & Pharmacotherapy (2024)

Biomedicine & Pharmacotherapy 177 (2024) 116953

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journal homepage: www.elsevier.com/locate/biopha

Peroxiredoxin-2 represses NRAS-mutated melanoma cells invasion by modulating EMT markers

Isabella Harumi Yonehara Noma [a,1], Larissa Anastacio da Costa Carvalho [a,1], Denisse Esther Mallaupoma Camarena [a], Renaira Oliveira Silva [a], Manoel Oliveira de Moraes Junior [a], Sophia Tavares de Souza [a], Julia Newton-Bishop [b], Jérémie Nsengimana [c], Silvya Stuchi Maria-Engler [a,*]

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ARTICLE INFO

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Gliotoxin (PubChem CID: 6223)

ABSTRACT

The second most common mutation in melanoma occurs in NRAS oncogene, being a more aggressive disease that has no effective approved treatment. Besides, cellular plasticity limits better outcomes of the advanced and therapy-resistant patients. Peroxiredoxins (PRDXs) control cellular processes through direct hydrogen peroxide oxidation or by redox-relaying processes. Here, we demonstrated that PRDX2 could act as a modulator of multiple EMT markers in NRAS-mutated melanomas. PRDX2 knockdown lead to phenotypic changes towards invasion in human reconstructed skin and the treatment with a PRDX mimetic (gliotoxin), decreased migration in PRDX2-deficient cells. We also confirmed the favorable clinical outcome of patients expressing PRDX2 in a large primary melanoma cohort. This study contributes to our knowledge about genes involved in phenotype switching and opens a new perspective for PRDX2 as a biomarker and target in NRAS-mutated melanomas.

1. Introduction

Melanoma of the skin is the fifth most common type of cancer [1] and the deadliest form of skin cancer due to its high metastatic potential, development of resistance and tumor recurrence [2]. The second most frequent mutation in melanoma occurs in NRAS oncogene, found in approximately 25 % of cutaneous melanoma cases, after BRAF, which affects 40–45 % of all patients [3]. NRAS-mutated melanomas are more aggressive, resulting in lower patient overall survival, and it has no specific therapy approved [4]. Immunotherapy is the first-line treatment for surgically incurable NRAS-mutated melanoma at high stages, such as anti-PD-1(nivolumab or pembrolizumab), and the combination of anti-PD-1 and CTLA-4 blockade (ipilimumab). Second-line treatment includes MEK inhibitors and combination of targeted-therapies as BRAF and MEK inhibitors. Altogether, the therapeutic success is limited for these patients by drug resistance and clinical relapse, emphasizing the need for new therapeutic approaches and new targeted combinations [3, 5].

One of the major challenges in treating metastatic melanoma is the residual disease associated with intratumoral heterogeneity and cellular plasticity. These well-characterized phenotypic switches between differentiated (proliferative) and undifferentiated (invasive) occur as cells undergo phenotypic transitions as an adaptative mechanism under stressful conditions. These phenotypes have different gene signatures, therapeutic sensitivity, and metastatic potential [6].

A common stressful condition in cancer, is when the physiological balance in the redox state is disrupted. The transient production of ROS is necessary for cell signaling, but its persistent production and exposure, can trigger tumor initiation and/or progression and also be cytotoxic. Once ROS have a dual role in cancer, agents that modulates its levels are interesting targets [7]. In this sense, cells regulate the expression of cellular peroxidase enzymes to maintain the redox homeostasis [8].

Peroxiredoxins (PRDXs) are one of the major hydrogen peroxide

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Table 1
Identification of NRAS-mutated melanoma cell lines.

Cell lines	Mutation	Short-tandem-repeat
SK-MEL-147	NRAS p.Gln61Arg (c.182 A>G); Q61R (PubMed=24576830; PubMed=25728708).	Amelogenin (X); CSF1PO (10, 12); D13S317 (9, 11); D16S539 (12, 13); D5S818 (10, 13); D7S820 (9, 11); THO1 (6, 9.3); TPOX (8, 12); vWA (17, 18).
SK-MEL-173	NRAS p.Gln61Lys (c.181 C>A): Q61K (PubMed=21725359; PubMed=24576830).	Amelogenin (X, Y); CSF1PO (10, 11); D13S317 (9, 11); D16S539 (11, 12); D5S818 (9, 11); D7S820 (9, 11); THO1 (7, 9.3); TPOX (8); vWA (18).

Available: https://www.cellosaurus.org/

consumers, controlling cellular processes through direct hydrogen peroxide oxidation or by redox-relaying processes. The PRDX family is classified according to the number of active-site cysteine residues into the 1-Cys subfamily and 2-Cys (PRDX1, 2, 3, and 4), with PRDX1 and 2 being more abundant in the cytosol and nucleus [8]. Our group have previously demonstrated that PRDXs could act as biomarkers and are involved in melanoma heterogeneity and resistance [9,10]. The importance of redox regulation and the use of PRDXs as biomarkers, have also been highlighted before [11]. It has been demonstrated that PRDX2 suppresses BRAF-mutated melanoma cell proliferation and migration by increasing the E-cadherin/β-catenin complexes in adherens junctions, a critical step for metastatic ability [12]. Studies in invasive melanoma cells and metastatic tumors have already demonstrated a downregulation or silencing of PRDX2 [8,9,13,14].

Once NRAS-mutated melanomas have no effective treatment, we investigated the role of PRDX2 in this context and demonstrated that it could modulate the invasion profile, impacting the overall patient survival and also can act as a biomarker or possible target.

2. Materials and methods

2.1. Cell culture

The cell lines SK-MEL-147, SK-MEL-173 (kindly donated by Dr. María Soengas, Centro Nacional de Investigaciones Oncológicas, Madrid, Spain) were cultured in DMEM (Gibco, USA) with 10 % FBS, 25 μg/mL ampicillin, and 100 μg/mL streptomycin). Cells were cultured at 37 °C with 5 % CO_2 in a humidified chamber and passage numbers were less than 100. Cells were assayed for mycoplasma contamination every two weeks with conventional PCR. The short-tandem-repeat profiling (DNA fingerprinting) was performed using PowerPlex® 16 System (Promega) on following short-tandem-repeat loci. The results in table showed the perfect match with known profile (Table 1).

2.2. Cell viability assays

Cell viability was evaluated using the death curve assay after exposure to Gliotoxin - GT (Sigma-Aldrich /G9893–5MG) treatment. GT was solubilized in DMSO (10 mM). In a 24-well plate, 2×10^4 cells were seeded and, after adherence, they were treated with different concentrations of GT (0, 50, 100, 200 and 400 nM) for 24 hours of incubation. After staining using Trypan Blue dye (T6146, Sigma-Aldrich, St. Louis, MO, USA), viable cells were counted in a Neubauer chamber.

2.3. Growth curve

The growth curve was evaluated by counting cells in a Neubauer chamber, using the Trypan Blue dye exclusion method. Cell lines were plated at a concentration of 10^4 cells/well in triplicate. After a period of 24, 72, 120, and 168 hours the cells were collected. Then, they were incubated with Trypan Blue solution (0.4 % in PBS) for approximately 5 minutes. In this assay, non-viable cells can be identified by blue staining and counted under an optical microscope, while viable cells are not permeable to the dye [15].

2.4. Western blotting

Cell lysates for Western Blotting were prepared in RIPA buffer with a cocktail of protease inhibitors (Roche, Penzberg, Upper Bavaria, Germany) and with a cocktail of phosphatase inhibitors I and II (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Total protein (40 μg) run to SDS gel electrophoresis with a 4–20 % gradient under reducing conditions and subsequent transfer to a PVDF membrane (Hybond-P, Amersham Pharmacia Biotech, Piscataway, NJ, USA). The membrane was blocked with 5 % BSA diluted in TBS-Tween 20 (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1 % Tween-20) for 1 hour and the antibodies were

Table 2
shRNA sequences for PRDX2 inhibition.

Bacterial stock code	ID	Sequences	PRDX2- 201	PRDX2- 202
TRCN0000064905	PRDX2–0	CAGACGCTTGTCTGAGGATTA	Éxon 4	Éxon 4
TRCN0000064906	PRDX2–1	GTGAAGCTGTCGGACTACAAA	Éxon 2	Éxon 2
TRCN0000064907	PRDX2–2	GCCTGGCAGTGACACGATTAA	Éxon 6	Éxon 5

tested at concentrations recommended by the manufacturer (Table S1). Protein bands were detected by enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech, Piscataway, NJ, USA) and semi-quantified using a Image J software.

2.5. Gene expression by real-time PCR

Total RNA from cell lines was extracted using the RNeasy Plus Mini Kit (Qiagen), according to the manufacturer's instructions. The RNA was heated to 65°C for 5 minutes, and quantified using a spectrophotometer at 260 nm to evaluate the sample's quality. RT-PCR reaction (real-time polymerase chain reaction) was performed using the Taqman® method (Life Technologies, USA). The cDNAs obtained from the samples were diluted (2 μg/μL). Reactions had a final volume of 10 μL containing 0.5 μL of primer mix (forward and reverse) for each gene of interest (Table S2), 1 μL of cDNA, 5 μL of 2x Taqman® Gene Expression Master Mix (Life Technologies, USA), and 3.5 μL of RNAse-free water. The relative level of gene expression was calculated using the delta delta Ct method (cycle threshold method) with GAPDH as endogenous controls [16].

2.6. Clonogenic assay

The cells were seeded at 10^3 cells/well in 6-well dishes and cultured in DMEM for 2 and 3 weeks. After cell adhesion (24 hours), they were treated at different concentrations, based on the results obtained in the death curve. The culture medium was changed every 2–3 days and gliotoxin was added for a period of 15 days, varying according to the cell line used. After the colony formation period, the wells were washed with PBSA. The colonies were then fixed and stained with a solution of 49.75 % methanol, 49.75 % deionized water, and 0.5 % crystal violet. The plates were photographed to compare the treatment effects. The analysis of the number of colonies and the area they occupied was carried out according to Guzmán et al.[17].

2.7. Transwell assay

The invasion capacity of melanoma cells was evaluated after treatment of the cell lines on Transwell membranes (8 μm pore size, Becton Dickinson). These were coated with 30 μL of Matrigel (BD Biosciences, San Jose, CA, diluted 1:6 in FBS-free DMEM). Cells were resuspended (5×10^4 cells/well) in FBS-free DMEM and added to the upper compartment of the chamber. Then, 750 μL of DMEM supplemented with 10 % FBS was added to the lower chamber. Treatments were used in both upper and lower compartments. After 24 h, cells that migrated through Matrigel were fixed in 4 % formaldehyde (in 1% PBS), stained with 1 % crystal violet (in methanol) for 20 minutes before counting in an inverted microscope (Axiovert S100, Zeiss, Germany). In each well, 5 representative and independent random fields were counted (20x magnification).

2.8. Wound-healing assay

Evaluation of the ability of melanoma cells to migrate after treatment: 5×10^4cells/well were seeded in 24-well plates. After reaching approximately 90 % confluence, slits ("wounds") were made in the center of each well using a 200 μL pipette tip. The cells were then washed with 1x PBS, and treatments with DMEM culture medium + 1 % FBS were added. The experiment was carried out until the slits were closed in the controls, with pictures taken through a microscope. Analysis of cell-free areas was performed using ImageJ software.

2.9. PRDX2 gene silencing through lentiviral vectors

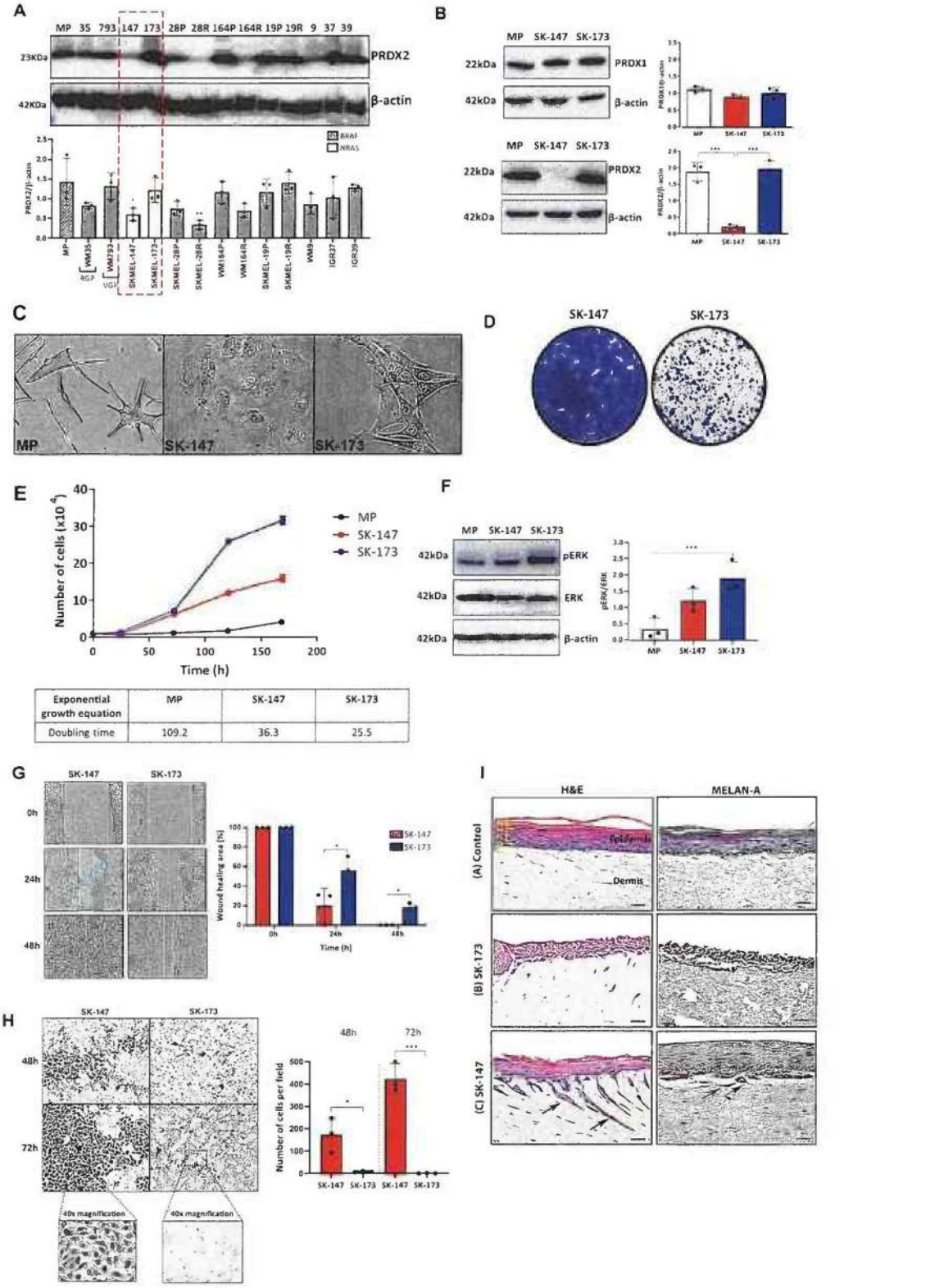
The silencing of the PRDX2 gene in cell lines was performed using short-hairpin RNA (shRNA) sequences to inhibit the messenger RNA (mRNA) of this gene. This process involved using bacterial cultures transfected with the Mission shRNA plasmid sequence (Sigma, USA). Three different shRNA sequences for PRDX2 were used to inhibit distinct regions of the mRNA. Bacterial stocks were cultivated for subsequent extraction and purification of plasmid DNA, following the Sigma Mission cell cloning protocol. Lentiviral vectors with a random sequence of nucleotides (scramble) were used to generate controls, which underwent the same manipulation conditions as the shRNA sequences. The shRNA vectors were co-transfected with lentiviral packaging plasmids into HEK293T cells, according to the manufacturer's instructions. These cells were selected using puromycin antibiotic (Sigma-Aldrich, USA). Afterward, the manipulated PRDX2 gene was validated by performing Western blotting and RT-PCR experiments. The knockdown cells (inhibited) were identified as scramble for the control (CN), PRDX2#0 for the cell line infected with shRNA sequence 0 PRDX2, PRDX2#1 for the cell line infected with shRNA PRDX2 sequence 1, and PRDX2#2 for the cell line infected with shRNA PRDX2 sequence 2 (Table 2).

2.10. Reconstruction of human skin (RHS) with melanoma

Primary human cells were isolated from skin samples extracted obtained from breast plastic surgery and/or postectomy in University Hospital of USP (CEP/HU-USP 943/09, SISNEP CAAE 0062.0.198.000–9). Approved was granted by Ethics Committee (CEP/FCF-USP 534). After extraction, the cells were tested for the presence of microorganisms (Human *Papillomavirus*, *Herpes Simplex* Virus, Cytomegalovirus, Hepatitis B, Hepatitis C, HIV-1, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Treponema pallidum* and *Trichomonas vaginalis*) by PCR. The reconstructed human skin model (RHS) followed the protocol described by Brohem *et al.* (2011) [18], with the following changes regarding the number of cells 50×10^4 melanoma cells/skin. The RHS were cultured and incubated submerged in DMEM:HAM F-12 1x (Gibco#21700–026) medium (1:3) + supplements (cholera toxin (1 μM), insulin (5 mg/mL), apo-transferrin (5 mg/mL), hydrocortisone-21 (0,1 mg/mL) and EGF (1 mg/mL)) for 24 hours, followed by 11 days of exposure to air-liquid interface to promote epithelial differentiation. After this period, the skins were collected, and fixed with 4 % paraformaldehyde and processed for histological analysis.

2.11. Histology and immunohistochemistry

Samples were processed according to Camarena et al., 2020 [19]. The immunostaining assay was performed using an anti-melan A antibody (ab51061) at a 1:50 dilution. A commercial kit with a goat secondary antibody against rabbit and mouse immunoglobulins (EnVision Flex/HRP, Dako Omnis, Santa Clara, CA, USA) was used in combination with 3,3′-diaminobenzidine (DAB; EnVision Flex DAB + Chromogen, Dako Omnis, Santa Clara, CA, USA) according to the manufacturer's instructions. All images were observed and photographed with an



Exponential growth equation	MP	SK-147	SK-173
Doubling time	109.2	36.3	25.5

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Fig. 1. PRDX2 expression in NRAS-mutated melanoma with different phenotypic profile. A Screening of the PRDX2 protein expression in different mutations and stages of melanoma cell lines (melanoma cell lines, including radial growth phase (RGP), vertical growth phase (VGP), NRAS-mutant melanoma, and BRAF-mutant melanoma). B Western blot was used to detect the protein expression of PRDX1 and PRDX2. C Cell morphology in 40x magnification. D Colony formation assay after ~15 days. E Growth curve at times 24, 72, 120 and 168 hours by trypan blue exclusion. F Western blot was used to detect the protein expression of ERK-phosphorylated. G Wound healing assay after 24 and 48 hours with wound area measurement. H Invasion assay after 48 and 72 hours and quantification of number of cells per field below of the chamber Matrigel. I Control skin without melanoma, skin with NRAS-mutant melanoma stained with Hematoxylin & Eosin and immunohistochemical staining of Melan-A, 20x magnification. Arrows indicate the sites of melanoma invasion in the dermis. Epidermis included SB: *stratum basale*, SS: *stratum spinosum*, SG: *stratum granulosum*, SC: *stratum corneum*. **A-H** All values are indicated as mean ± standard deviation (SD) of three independent experiments. P values are based on a one-way analysis of variance (ANOVA) followed by Tukey's test, *p<0.05 **p<0.01 and ***p<0.001. Images are representative of three independent biological experiments. Abbreviations: Primary melanocyte (MP), SK-MEL-147 (SK-147), and SK-MEL-173 (SK-173).

Eclipse i80 Nikon camera using the NIS-Elements Viewer (Nikon) program for image acquisition [19].

2.12. Statistical analysis

GraphPad Prism 7.0 was used for all in vitro data analysis. An independent Student's t-test was applied to two-group comparisons, while ANOVA with Tukey correction was applied to multiple groups/multiple predictors. Doubling times were obtained by non-linear regression analysis. Pairwise gene expression correlations were performed after normalizing the data, and the Pearson correlation was represented graphically. Analyses were considered significant when p-values were ≤ 0.05. Values were indicated as mean ± standard error of triplicate results from three independent experiments.

2.13. Patient data analysis

The Leeds melanoma cohort is a prospective cohort of patients diagnosed with primary melanoma in the North of England. These patients consented to be followed up starting from 3 to 6 months after diagnosis to monitor the risk of recurrence following the initial surgery. Death records were retrieved from the UK Office of National Statistics and confirmed by checking death certificates. Transcriptomic data were generated from primary tumor formalin-fixed paraffin-embedded (FFPE) blocks. Samples were independent and non-matched. Any duplicates included for quality control purposes were removed after data cleaning [20]. Transcriptomic data were generated using the Illumina HT12.4 DASL array. Illumina's software GenomeStudio was used to extract raw data from image files before they were exported into R Bioconductor for further processing. This processing included background correction and quantile normalization using Lumi [21], followed by batch correction by Singular Value Decomposition (SVD) in Swamp [22].

To explore the utility of in vitro results in patients, we performed the following analyses on this cohort using STATA 18 (StataCorp. 2023). Transcripts and clinical data from the Leeds Melanoma cohort are accessible in the European Genome-phenome Archive under accession number EGAS00001002922 [20]. This is one of the largest transcriptomic datasets on primary melanoma worldwide, with a sample size of 703 patients and long follow-up (>10 years).

Associations between transcripts of PRDX2 and candidate targets related to tumor progression were performed by linear regression in the entire cohort and stratified by oncogenic mutations BRAF and NRAS. Regression coefficients of these analyses were represented in a heatmap. We tested the association between PRDX2 expression and the presence/absence of BRAF and NRAS mutations using analysis of variance (ANOVA). Pearson's correlation was calculated between the expression of PRDX2 and the log-transformed Breslow thickness (the transformation allowed for an approximate normality distribution). A similar analysis was performed to test for association with tumor staging (AJCC, American Joint Committee on Cancer), and the results were plotted as violin plots or box plots.

We tested the effect of PRDX2 on melanoma-specific survival (using only deaths confirmed to be caused by melanoma) using Cox proportional hazards regression. Survival profiles were plotted with Kaplan-Meier curves. Survival analysis was performed after dividing PRDX2 expression into 5 groups (lower 20 % = 1st quintile, upper 20 % = 5th quintile). The number of 5 was arbitrarily chosen, but it is possible to merge similar groups. Finally, the top 4 quintiles (80 % of tumors with the highest PRDX2 expression) were grouped together because their effect on prognosis appeared identical.

3. Results

3.1. Investigating the role of PRDX2 in proliferation, colony formation, migration, and invasion in NRAS-mutated melanoma

Initially, we tested several cell lines with different mutations (BRAF and NRAS) and tumor stages (vertical or horizontal growth, and metastatic melanoma) to investigate the protein expression of PRDX2. Our data highlighted that, in NRAS-mutated cells, PRDX2 exhibited a distinct expression pattern (Fig. 1A). This observation led us to further investigate PRDX2 in N-RAS mutated cell as SK-MEL-147, derived from metastatic melanoma, and SK-MEL- 173, from primary melanoma.

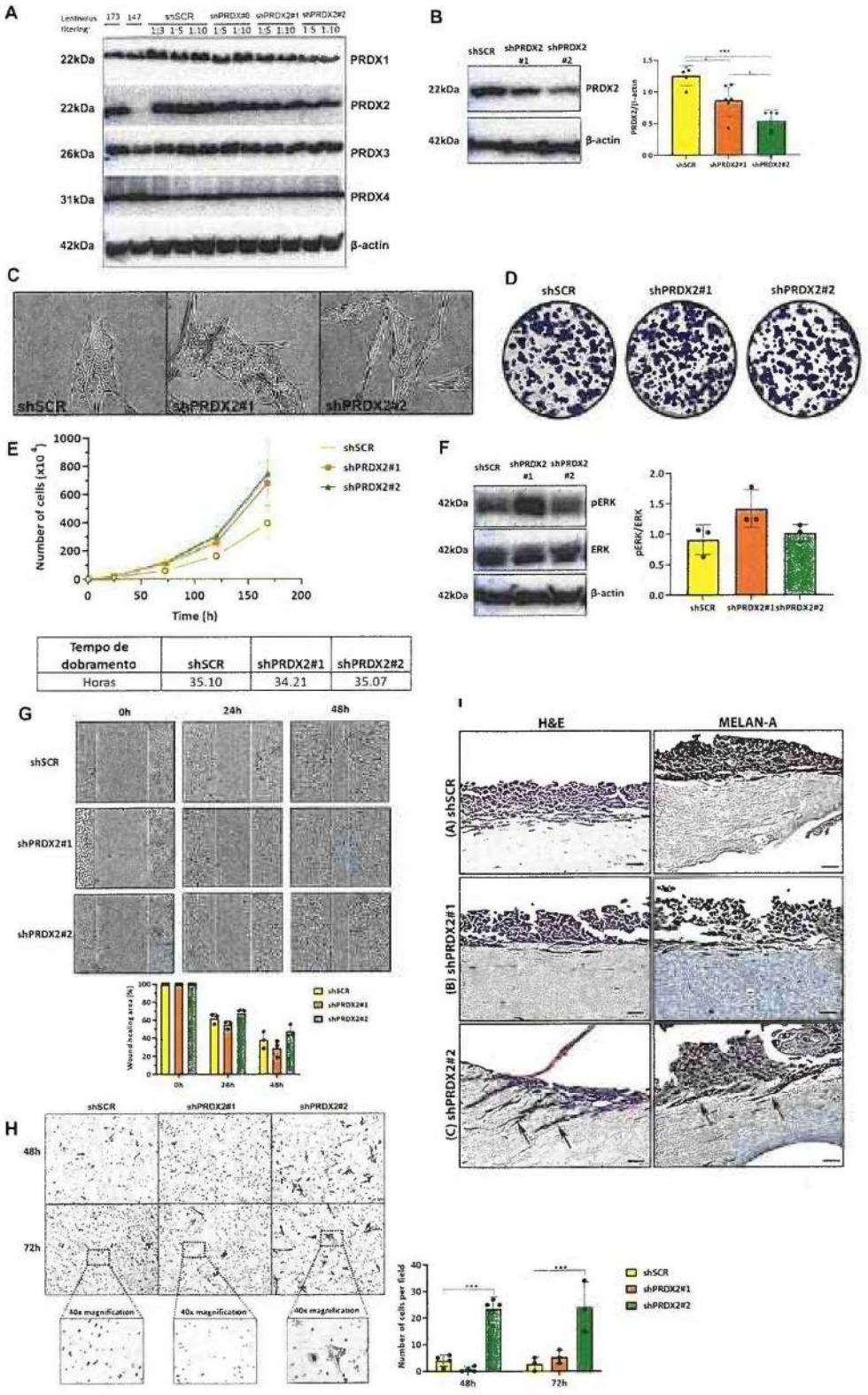
We assessed PRDX2 protein expression and observed high levels in our control melanocyte (MP) and in SK-MEL-173 compared to SK-MEL-147. Interestingly, PRDX1 expression did not vary among the cells tested (Fig. 1B). These cells with different mutations in NRAS, exhibited different morphologies and colony formations (Fig. 1C - D). SK-MEL-173 displayed a shorter doubling time and higher phosphorylated ERK levels (Fig. 1E – F), indicating a more proliferative profile than SK-MEL-147. Conversely, SK-MEL-147 showed greater potential for migration and invasion, observed by wound healing and invasion assay (Fig. 1G - H).

Using reconstructed human skin (RHS) models, we compared the profiles of these cell lines. We observed the presence of skin layers (corneal layer of the epidermis, dermis) and the melanoma cells which were stained with Melan-A. RHS-SK-MEL-173 melanoma cells demonstrated higher proliferation, growing over the stratum corneum, whereas RHS-SK-MEL-147 melanoma cells showed more pronounced invasion, infiltrating into the dermis (Fig. 1I).

3.2. Altering the phenotypic profile of SK-MEL-173 by knocking down PRDX2

We employed shRNAs to modulate PRDX2 expression and conducted knockdown (KD) experiments. The specificity of the shRNA was validated through Western Blot analysis, which showed a reduction specifically in the PRDX2 isoform. With shRNA PRDX2 sequence 1 (shPRDX2#1), we observed partial inhibition, and with shRNA PRDX2 sequence 2 (shPRDX2#2), there was more than a 60 % inhibition compared to the control – scramble (shSCR). We discarded shPRDX2#0 due to its lack of inhibitory effect (Fig. 2A - B).

Throughout our study, no differences were noted in the cell lines' morphology or colony formation characteristics (Fig. 2C - D). Post-KD experiments, the cells maintained their doubling time and phosphorylated ERK levels without statistically significant changes (Fig. 2E – F). The cells with shPRDX2#2 demonstrated more invasiveness capacity in transwell assay and in the reconstructed human skin model. However, we did not observe significative changes in the migration assay (Fig. 2G – H).



Tempo de dobramento	shSCR	shPRDX2#1	shPRDX2#2
Horas	35.10	34.21	35.07

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Fig. 2. Knockdown (KD) of PRDX2 by shRNA changed phenotypic profile in melanoma SK-MEL-173. A Western blotting analysis of 2-cys PRDX group proteins (PRDX1, PRDX2, PRDX3 and PRDX4). B Western blot was used to detect the protein expression of PRDX2 in cells KD PRDX2. C Cell morphology in 40x magnification. D Colony formation assay after ~15 days. E Growth curve at times 24, 72, 120 and 168 hours by trypan blue exclusion. F Western blot was used to detect the protein expression of ERK-phosphorylated. G Wound healing assay after 24 and 48 hours with wound area measurement. H Invasion assay after 48 and 72 hours and quantification of number of cells per field below of the chamber Matrigel. I Control skin without melanoma, skin with melanoma KD PRDX2 (shPRDX2#1 and shPRDX2#2 compared to shSCR- scramble) stained with Hematoxylin & Eosin and immunohistochemical staining of Melan-A, 20x magnification. Arrows indicate the sites of melanoma invasion in the dermis. A-H All values are indicated as mean ± standard deviation (SD) of three independent experiments. P values are based on a one-way analysis of variance (ANOVA) followed by Tukey's test, *p<0.05 **p<0.01 and ***p<0.001. Images are representative of three independent biological experiments.

In the RHS model, the control (shSCR) exhibited the same pattern as naive SK-MEL-173 (Fig. 1I). KD with shPRDX2#1 showed no difference, but KD with shPRDX2#2 revealed a notable increase in skin invasion (Fig. 2I). The skins were also subjected to immunohistochemistry (IHC) staining for Melan-A to confirm the invasion of melanoma cells. These results corroborate the observed differences in invasion in the 2D model following PRDX2 knockdown.

3.3. Expression of PRDX2 alters several genes and proteins related to invasion markers: a comparison between melanoma cell lines SK-MEL-147 and SK-MEL-173

Following the observation of phenotypic changes associated with PRDX2 downregulation, we investigated the potential proteins and genes that might be influenced by PRDX2. Our data revealed a higher expression of MITF in SK-MEL-173 (where PRDX2 levels are higher), contrasting with the expression of AXL (Fig. 3A-B). However, there was no significant difference in the expression of SOX10 (Fig. 3C). Only SK-MEL-147 (where PRDX2 levels are lower) showed expression of N-cadherin (Fig. 3D). β-catenin exhibited a pattern similar to MITF, being more highly expressed in SK-MEL-173 (Fig. 3E). E-cadherin was exclusively expressed in melanocytes (MP) and not in the melanoma cells (Fig. 3F). Additionally, real-time PCR analysis of gene expression confirmed higher expression of PRDX2 and MITF, and lower expression of AXL, FN1, SNAI1, and SMAD3 in SK-MEL-173 compared to SK-MEL-147 (Fig. 3G).

*3.4. **Comparing PRDX2 knockdown in melanoma SK-MEL-173: control (shSCR) vs. shPRDX2#1 and shPRDX2#2***

We observed that PRDX2 KD decreased the expression of MITF compared to control (scramble). The PRDX2 KD did not affect SOX10 expression, but it increased Snail expression at the protein level (Fig. 3H-K). In SK-MEL-173, AXL, N-cadherin, and E-cadherin were not expressed; therefor, the knockdown of PRDX2 in Sk-MEL-173 did not impact its expression (data not shown). Real-time PCR analysis for gene expression revealed that PRDX2 KD led to decreased levels of PRDX2, MITF, and SMAD2, and an increase in AXL and SNAI1 (Fig. 3L).

3.5. Gliotoxin (GT) treatment in NRAS-mutated melanoma

We evaluated gliotoxin (GT) as PRDX mimetic with antioxidant properties. Our viability tests revealed that melanoma cells were more sensitive to GT compared to fibroblast (FB) cells. However, there was no significant difference in sensitivity between melanocytes and the tested melanoma cell lines (Fig. 4A). The ability to form colonies was significantly reduced across all cell lines (Fig. 4B). Additionally, SK-MEL-173 cells showed no change in migration after GT treatment, whereas SK-MEL-147 cells exhibited a decrease in migration following GT treatment (Fig. 4C-D). Although GT did not affect cell proliferation, it altered the migration capability in SK-MEL-147 cells, which have lower PRDX2 levels.

3.6. Patient analysis in the Leeds Melanoma Cohort (LMC) of primary melanoma tumors

3.6.1. Gene correlation with PRDX2 in the total cohort and specifically in tumors exhibiting NRAS mutations

We validated our *in vitro* findings through bioinformatic analysis of patient data from the Leeds Melanoma Cohort (LMC, ethical approval MREC 1/3/57, PIAG 3–09(d)/2003). This analysis showed a positive correlation of PRDX2 with STAT3, CTNNB1, and CDH1, consistent with our *in vitro* assays, and a negative correlation with ZEB2, MAPK1, HIF1A, and PDGFB, which are genes implicated in tumor progression (Fig. 5A, all correlation coefficients are highly significant with $P<0.0001$). The cohort was subdivided into subgroups based on mutation type (BRAF, NRAS, and double wild type WT). It was observed that melanomas with NRAS mutations exhibited lower PRDX2 expression compared to those with BRAF mutations (Fig. 5B, $p=10^{-5}$). Despite that, PRDX2 did not have a difference in patient survival from the NRAS melanoma cohort (Sup. Fig. 1), suggesting PRDX2 is melanoma mutation-independent. Within the NRAS-mutated melanomas, PRDX2 showed varying correlations with different genes. It was negatively correlated with CSNK1A1, NRAS, AXL, and FN1, and positively correlated with ROS1, KRT18, AXIN1, and others, corroborating some of our *in vitro* results (Fig. 5C).

*3.6.2. **PRDX2 as a positive prognostic factor in the Leeds Melanoma Cohort (LMC)***

Primary melanomas from LMC patients were analyzed to corroborate our *in vitro* data. The results indicated that patients in the top 4 quintiles for PRDX2 expression (80 % high expressors) had a better survival profile compared to those with lower tumor PRDX2 expression (i.e. low expression has a double of hazard of high expression) (Fig. 5D-E). Consistent with this, lower expression of PRDX2 was associated with a more advanced stage of melanoma as indicated by AJCC stage (Fig. 5F) (mean values are 7.84 for AJCC stage I, 7.57 for AJCC stage 2 and 3) or Breslow thickness (Fig. 5G). Put together, our findings in *in vitro* and clinical datasets (especially in a large unbiased population-based cohort) suggest that PRDX2 expression in tumors is a favorable prognostic factor in primary melanomas, regardless of mutation status.

4. Discussion

PRDX2 is an antioxidant enzyme that plays an important role in redox homeostasis during carcinogenesis and in several cellular processes. While some studies have investigated the role of PRDX2 in melanomas, they were limited to BRAF-mutated melanomas, and the mechanism by which this protein contributes to melanoma progression has not been completely explored. Therefore, our aim was to understand the role of PRDX2 in NRAS-mutated melanoma and how it may influence cellular phenotypic pathways.

Our results showed that cell lines with higher PRDX2 expression have a more proliferative profile, whereas those with lower PRDX2 expression exhibit a more invasive profile. This aligns with other studies that linked PRDX2 silencing to increased migration and invasion in BRAF-mutated melanomas and gastric tumors [12,23]. Several studies have noted that MITF expression promotes proliferation and suppresses invasiveness [6,24]. Additionally, β-catenin, involved in adherens

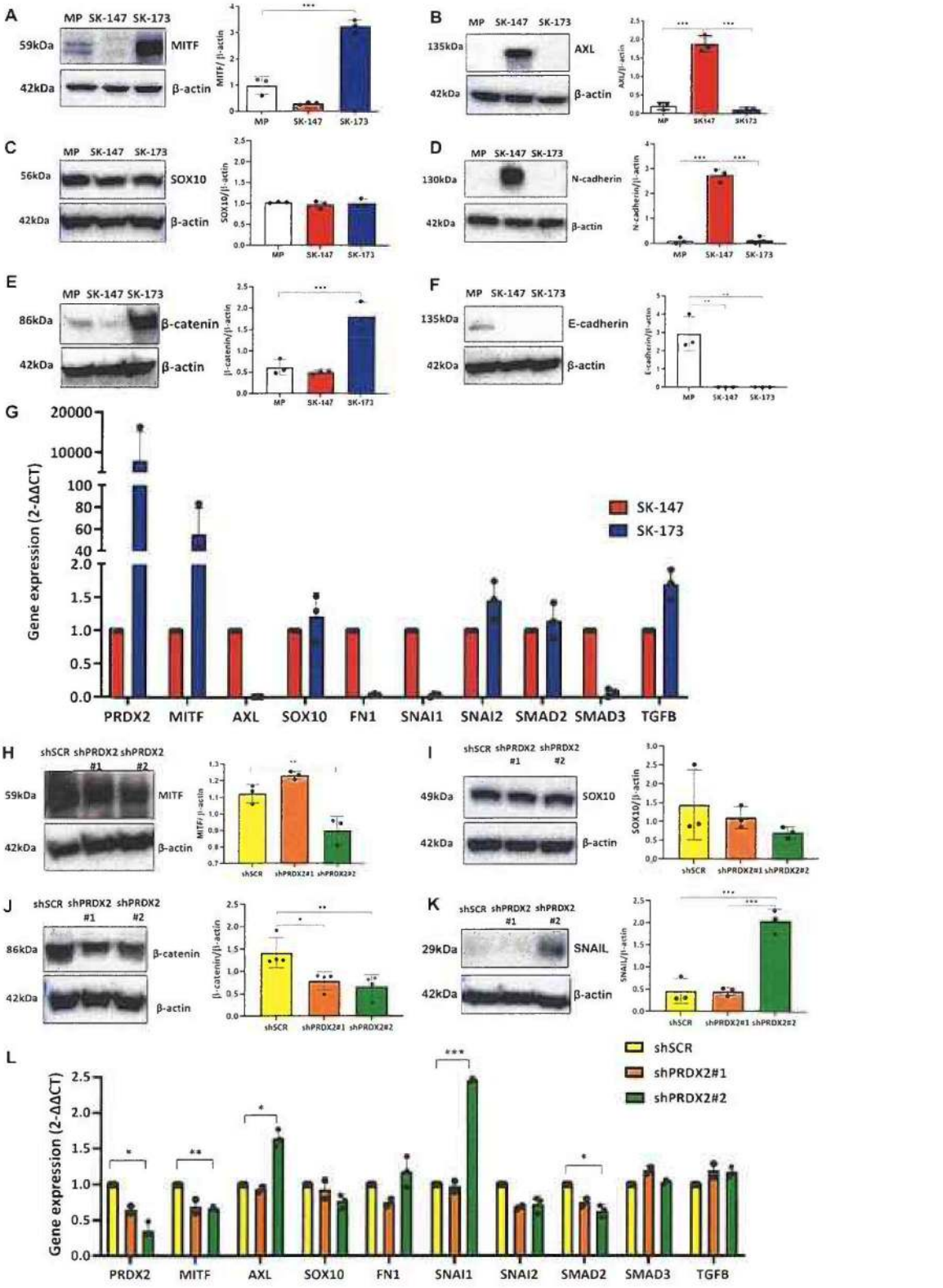

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Fig. 3. Lack of PRDX2 expression promotes invasive properties by regulation of genes and proteins related MITF and EMT markers in NRAS-mutated melanoma. A Western blot was used to detect the protein expression of MITF, (B) AXL, (C) SOX10, (D) N-cadherin, (E) β-catenin and (F) E-cadherin in NRAS-mutant melanoma cell lines (SK-MEL-147 and SK-MEL-173). G Genes expressions in NRAS-mutant melanoma cell lines based in delta delta CT. H Western blot was used to detect the protein expression of MITF, (I) SOX10, (J) β-catenin and (K) Snail in melanoma KD PRDX2. L Genes expressions in melanoma KD PRDX2 based in delta delta CT. **A-L** All values are indicated as mean ± standard deviation (SD) of three independent experiments. P values are based on a one-way analysis of variance (ANOVA) followed by Tukey's test, *p<0.05 **p<0.01 and ***p<0.001. Images are representative of three independent biological experiments. Abbreviations: Primary melanocyte (MP), SK-MEL-147 (SK-147), SK-MEL-173 (SK-173), PRDX2 knockdown using shRNA: scramble sequence (shSCR), sequence 1 (shPRDX2#1), and sequence 2 (shPRDX2#2).

junctions complexes [12], cooperates with the canonical Wnt pathway [25]. This pathway is important for MAP kinase signaling, regulating MITF expression and activity, and promoting melanoma proliferation [25]. Our data demonstrated that silencing PRDX2 correlates with decreased β-catenin and MITF levels, leading to increased cell migration and invasion.

Alterations in MITF expression levels are linked to phenotypic plasticity, and the acquisition of migration and invasion capabilities. Extremely low MITF expression is characteristic of invasive melanoma cells, while high MITF expression characterizes non-invasive melanoma cells. Phenotypic plasticity refers to the ability of malignant melanoma cells to undergo transient and reversible morphological and functional changes, similar to the embryonic neural crest invasion program. Although this phenotype switching resembles epithelial-mesenchymal transition (EMT), this term is not entirely appropriate for melanoma, as melanocytes are not epithelial cells. Instead, EMT-like processes, which play a key role during the formation and migration of neural crest cells, are considered [26,27]. Epithelial-mesenchymal transition-regulating transcription factors can induce EMT-like phenotype switching in melanoma, involving proteins like SNAIL, TWIST, and ZEB [28]. Melanocyte proliferation and differentiation from neural crest precursors heavily depend on the canonical WNT signaling pathway, mediated through β-catenin, which regulates MITF [26].

In the non-canonical pathway, blocking β-catenin expression and increasing Snail expression leads to metastasis. Snail (encoded by the SNAI1 gene) and Slug (encoded by the SNAI2 gene) are transcriptional repressors. Snail is expressed in mesenchymal cells and is upregulated during EMT, while Slug is expressed in epithelial cells. This is in line with our findings, where PRDX2 knockdown increased Snail expression and invasion. This coincides with an increase in N-cadherin, a major component of the cytoskeleton in mesenchymal cells and a marker of EMT [29,30]. FN1, the gene encoding fibronectin, shows a negative correlation with PRDX2 expression. Fibronectin is an extracellular matrix component facilitating tumor progression, expressed in the metastatic niches of lung melanoma cells and identified as a regulator of the Wnt/β-catenin pathway [31]. SMAD4 mediates canonical TGF-β signaling, essential for melanoma cell proliferation *in vivo*. A TGF-β family member, BMP7, stimulates melanoma cell proliferation [26].

We validated our 2D results in three-dimensional reconstructed human skin (RHS) models. This model replicates human skin histology and is used for functional analysis of melanoma development. The radial growth phase of melanoma, occurs proliferation of cancer cells in the dermal reconstruct, and the vertical growth phase, the cells invaded deep into the dermis, similar to *in vivo* scenarios [18,32]. The RHS using SK-MEL-173, the cell line containing higher levels of PRDX2, exhibited higher rates of cell proliferation, leading to an increased proportion of melanoma in the skin, surpassing the epidermis. Conversely, the SK-MEL-147 cell line, that showed lower levels of PRDX2, displayed invasion sites in the dermis due to its high invasive capacity observed in transwell assays (2D model). In the RHS using the melanoma cell line SK-MEL-173 KD for PRDX2 (shPRDX2#2), the reduction of PRDX2 in the more proliferative cell line, resulted in an increased invasion sites, evidencing the phenotypic modulatory potential of PRDX2. Our data suggest a metastatic potential in NRAS-mutated melanoma with lower PRDX2 expression, which is consistent with several studies that demonstrated the negative regulation of PRDX2 gene in metastatic melanomas [9,12,33].

Gliotoxin, an antioxidant compound described as a 2-Cys-PRDX mimetic, can reduces H_2O_2 and be restored by accepting electrons from NADPH via the TR-Trx redox system [34]. Viability assays conducted post-GT treatment revealed no differences between lineages. However, continuous GT exposure for over three weeks decreased colony formation in all tested cell lines. Notably, only the SK-MEL-147 cell line, lacking PRDX2, exhibited a difference in migration in response to GT treatment. This suggests that GT may have a compensatory effect in the absence of PRDX in SK-MEL-147 cells, changing their profile after mimetic use. This observation aligns with another study which observed a reduction in migration using GT in BRAF-melanoma [12].

In the analysis of patient samples, several genes exhibited distinct correlations with PRDX2, with notable differences observed in ZEB2 and STAT3. ZEB2 (Zinc finger E-box binding homeobox 2) negatively correlated with PRDX2 and is known to be associated with the E-cadherin promoter, thus activating the EMT pathway and contributing to metastatic invasions [35,36]. PRDX2 also regulates STAT3-mediated transcriptional activity through redox mechanisms, transferring oxidative equivalents to STAT3, hence their positive correlation [37].

Interestingly, melanomas with NRAS mutations had lower PRDX2 expression than those with BRAF mutations. Which may explain in part the more aggressive NRAS-mutated melanoma profile. When analyzed by mutation type, the NRAS group showed different gene correlations. Within the NRAS-mutant melanoma cohort, there were increases in ROS1 and KRT18 expression, and a decrease in CSNK1A1, all correlated with PRDX2. ROS1, a receptor tyrosine kinase involved in various cancers, regulates cell proliferation, differentiation, and growth [38]. KRT18 contributes to cytoskeletal filaments that influence cell architecture [39]. Additionally, CSNK1A1 plays a role in cytoskeletal dynamics and cell motility, with its increased expression associated with metastasis [40]. An inverse relationship between PRDX2 and EMT gene signatures was observed.

We analyzed primary tumors from the Leeds' cohort *in silico*. PRDX2 had a significant impact on survival across the entire cohort of primary tumor patients (n=703, 204 deaths); very low expression of PRDX2 was detrimental (only deaths from melanoma were analyzed). Additionally, these data revealed that tumors with lower PRDX2 expression exhibited higher Breslow thickness and staging, indicating higher malignancy levels [41,42]. These clinical findings suggest the potential of PRDX2 as a biomarker in metastatic melanoma. In an osteosarcoma study, PRDX2 has already been used as a biomarker to predict treatment response [43].

Although we have not conducted *in vivo* analyses, our data aligns with findings already observed in studies using mouse models with BRAF melanoma. Furthermore, we established an *in silico* relationship using patient data, which is highly relevant for prospecting this target in a clinical context. Nevertheless, our PRDX2 baseline results with high and low PRDX2 expression strongly indicate these relationships. Despite these limitations, this study has contributed to understanding the role of PRDX2 as a target and biomarker for metastatic melanoma, particularly in NRAS-mutated.

In conclusion, our data demonstrated that PRDX2 modulates EMT-like proteins such as MITF, β-catenin, N-cadherin, and Snail, which participate in the migration/invasion process. Also, PRDX2 is associated with favorable clinical features, and Gliotoxin could be used as a potential treatment. Furthermore, we established an *in silico* relationship using patient data, which is highly relevant for prospecting this target in a clinical context. This study contributes to our knowledge about genes

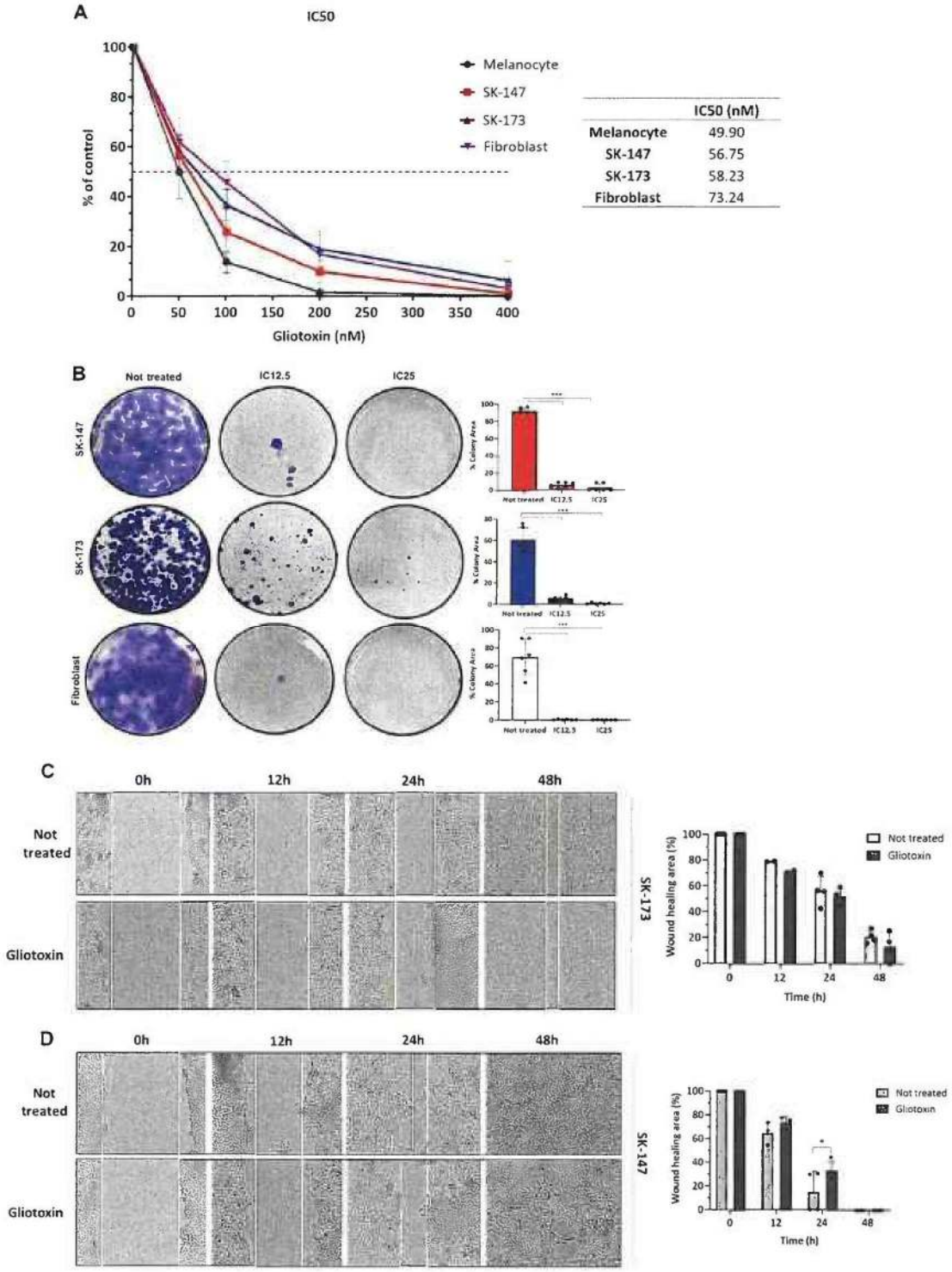

Fig. 4. Mimetic of PRDX2 inhibits cell proliferation, colony formation and migration ability in NRAS-melanoma cells. A Viability curve and table with calculated IC50 values. B Glyotoxin sensitivity test on NRAS-mutated melanoma and fibroblast (FP) cell lines for colony formation compared to untreated control using IC25 and IC12.5 concentration. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's test, ***p<0.001, **p<0.01 and *p<0.05. C-D Wound healing test for SK-MEL-173 and SK-MEL-147 with IC75 (99.7 nM for SK-MEL-147 and 114.1 nM for SK-MEL-173). Statistical analysis was performed by two-way analysis of variance (ANOVA) followed by Tukey's test, ***p<0.001, **p<0.01 and *p<0.05. A-D Values are indicated as mean ± SD of triplicate results from three independent experiments. Abbreviations: Primary melanocyte (MP), SK-MEL-147 (SK-147), and SK-MEL-173 (SK-173).

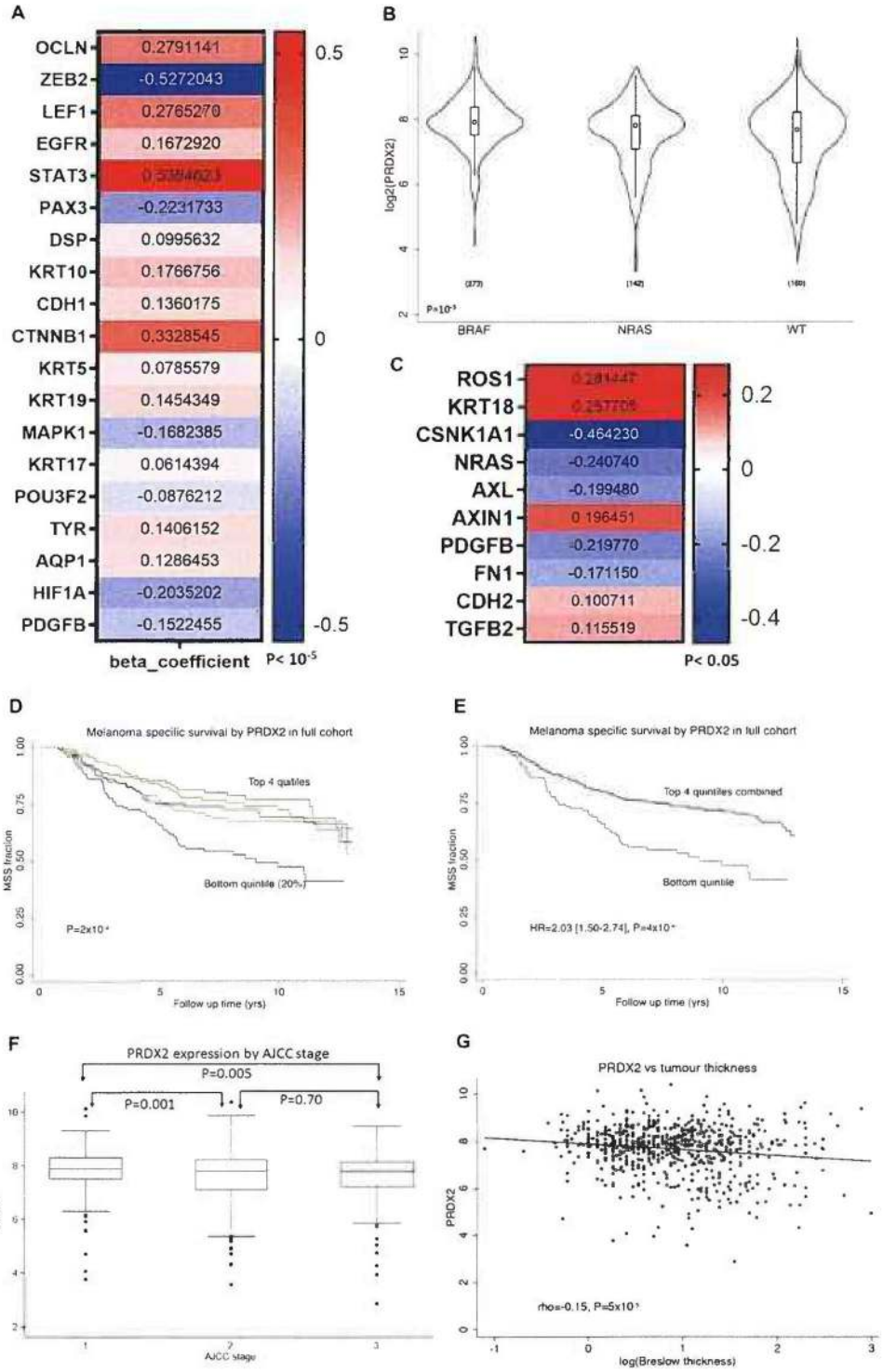

Fig. 5. Bioinformatic analysis in LMC from melanoma primary tumors: PRDX2 correlation with other genes and clinic features. A and C Regression coefficients between the expression of PRDX2 and other genes in primary melanomas in the whole LMC (n=703). Negative coefficients indicate a negative correlation while positive coefficients indicate a positive correlation. B PRDX2 tumor expression by mutation status (WT=double wild type, Pvalue=10^{-5}). C Regression coefficients between expressions of PRDX2 and other genes in NRAS-mutated primary melanoma subset of LMC (all Pvalues<0.001). D-E Melanoma specific survival (MSS) by PRDX2 in full cohort: the first 4 quintiles (80 % with high PRDX2 expression) were grouped after they appeared to have identical prognosis profiles. F Correlation of PRDX2 expression with primary tumor staging (AJCC). G Correlation of PRDX2 expression with the Breslow thickness of primary melanomas.

involved in phenotype switching and opens a new perspective for PRDX2 as a biomarker and target in NRAS-mutated melanomas.

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CRediT authorship contribution statement

Jérémie Nsengimana: Formal analysis, Data curation. **Julia-Newton Bishop:** Formal analysis, Data curation. **Sophia Tavares de Souza:** Methodology. **Manoel Oliveira de Moraes Junior:** Methodology, Formal analysis. **Renaira Oliveira Silva:** Methodology, Formal analysis. **Denisse Esther Mallaupoma Camarena:** Methodology, Formal analysis. **Larissa Anastacio da Costa Carvalho:** Writing – review & editing, Methodology, Data curation, Conceptualization, Formal analysis. **Isabella Harumi Yonehara Noma:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Silvya Stuchi Maria-Engler:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2024.116953.

References

[1] R.L. Siegel Mph, K.D. Miller, N. Sandeep, W. Mbbs, | Ahmedin, J. Dvm, R.L. Siegel, Cancer statistics, 2023, CA, Cancer J. Clin. 73 (2023) 17–48, https://doi.org/10.3322/CAAC.21763.

[2] F. Huang, F. Santinon, R.E. Flores González, S.V. del Rincón, Melanoma Plasticity: Promoter of Metastasis and Resistance to Therapy, Front. Oncol. 11 (2021), https://doi.org/10.3389/FONC.2021.756001.

[3] T. Randic, I. Kozar, C. Margue, J. Utikal, S. Kreis, NRAS mutant melanoma: Towards better therapies, Cancer Treat. Rev. 99 (2021), https://doi.org/10.1016/J.CTRV.2021.102238.

[4] D.B. Johnson, I. Puzanov, Treatment of NRAS-mutant melanoma, Curr. Treat. Options Oncol. 16 (2015) 15, https://doi.org/10.1007/s11864-015-0330-z.

[5] M. Berger, G. Richtig, K. Kashofer, A. Aigelsreiter, E. Richtig, The window of opportunities for targeted therapy in BRAFwt/NRASwt/KITwt melanoma: biology and clinical implications of fusion proteins and other mutations, G. Ital. Di Dermatol. e Venereol. Organo Uff. Soc. Ital. Di Dermatol. e Sifilogr. 153 (2018) 349–360, https://doi.org/10.23736/S0392-0488.18.05970-9.

[6] F. Rambow, J.C. Marine, C.R. Goding, Melanoma plasticity and phenotypic diversity: therapeutic barriers and opportunities, Genes Dev. 33 (2019) 1295–1318, https://doi.org/10.1101/GAD.329771.119.

[7] J. Wang, D. Sun, L. Huang, S. Wang, Y. Jin, Targeting Reactive Oxygen Species Capacity of Tumor Cells with Repurposed Drug as an Anticancer Therapy, Oxid. Med. Cell. Longev. 2021 (2021), https://doi.org/10.1155/2021/8532940.

[8] S.W. Kang, S. Lee, J.H.S. Lee, Cancer-Associated Function of 2-Cys Peroxiredoxin Subtypes as a Survival Gatekeeper, Antioxid. (Basel, Switz.) 7 (2018), https://doi.org/10.3390/ANTIOX7110161.

[9] L.A.C. Carvalho, R.G. Queijo, A.L.B. Baccaro, Á.D.D. Siena, W.A. Silva, T. Rodrigues, S.S. Maria-Engler, Redox-Related Proteins in Melanoma Progression, Antioxid. (Basel, Switz.) 11 (2022), https://doi.org/10.3390/ANTIOX11030438.

[10] L.A.C. Carvalho, I.H.Y. Noma, A.H. Uehara, Á.D.D. Siena, L.H. Osaki, M.P. Mori, N. C. Souza-Pinto, V.M. Freitas, W.A.S. Junior, K.S.M. Smalley, S.S. Maria-Engler, Modeling Melanoma Heterogeneity In Vitro: Redox, Resistance and Pigmentation Profiles, Antioxid. (Basel, Switz.) 13 (2024), https://doi.org/10.3390/antiox13050555.

[11] H.R. Hintsala, Y. Soini, K.M. Haapasaari, P. Karihtala, Dysregulation of redox-state-regulating enzymes in melanocytic skin tumours and the surrounding microenvironment, Histopathology 67 (3) (2015) 348–357, https://doi.org/10.1111/his.12659. PMID: 25627040.

[12] D.J. Lee, D.H. Kang, M. Choi, Y.J. Choi, J.Y. Lee, J.H. Park, Y.J. Park, K.W. Lee, S. W. Kang, Peroxiredoxin-2 represses melanoma metastasis by increasing E-cadherin/β-catenin complexes in adherens junctions, Cancer Res 73 (2013) 4744–4757, https://doi.org/10.1158/0008-5472.CAN-12-4226/650915/AM/PEROXIREDOXIN-2-REPRESSES-MELANOMA-METASTASIS-BY.

[13] S.W. Kang, S.G. Rhee, T.S. Chang, W. Jeong, M.H. Choi, 2-Cys peroxiredoxin function in intracellular signal transduction: therapeutic implications, Trends Mol. Med. 11 (2005) 571, https://doi.org/10.1016/J.MOLMED.2005.10.006.

[14] N. Chandimali, D.K. Jeong, T. Kwon, Peroxiredoxin II Regulates Cancer Stem Cells and Stemness-Associated Properties of Cancers, Cancers (Basel) 10 (2018), https://doi.org/10.3390/CANCERS10090305.

[15] F. Liu, Y. Fu, F.L. Meyskens, MiTF regulates cellular response to reactive oxygen species through transcriptional regulation of APE-1/Ref-1, J. Invest. Dermatol. 129 (2009) 422–431, https://doi.org/10.1038/JID.2008.255.

[16] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods 25 (2001) 402–408, https://doi.org/10.1006/METH.2001.1262.

[17] C. Guzmán, M. Bagga, A. Kaur, J. Westermarck, D. Abankwa, ColonyArea: an ImageJ plugin to automatically quantify colony formation in clonogenic assays, PLoS One 9 (2014) e92444, https://doi.org/10.1371/journal.pone.0092444.

[18] C.A. Brohem, L.B. Da Silva Cardeal, M. Tiago, M.S. Soengas, S.B. De Moraes Barros, S.S. Maria-Engler, Artificial skin in perspective: concepts and applications, Pigment Cell Melanoma Res 24 (2011) 35–50, https://doi.org/10.1111/J.1755-148X.2010.00786.X.

[19] D.E.M. Camarena, L.S.A.S. Matsuyama, S.S. Maria-Engler, L.H. Catalani, Development of Epidermal Equivalent from Electrospun Synthetic Polymers for In Vitro Irritation/Corrosion Testing, Nanomater. 2020, Vol. 10, Page 2528 10 (2020) 2528, https://doi.org/10.3390/NANO10122528.

[20] J. Nsengimana, J. Laye, A. Filia, S. O'Shea, S. Muralidhar, J. Pożniak, A. Droop, M. Chan, C. Walker, L. Parkinson, J. Gascoyne, T. Mell, M. Polso, R. Jewell, J. Randerson-Moor, G.P. Cook, D.Timothy Bishop, J. Newton-Bishop, β-Catenin-mediated immune evasion pathway frequently operates in primary cutaneous melanomas, J. Clin. Invest. 128 (2018) 2048–2063, https://doi.org/10.1172/JCI95351.

[21] P. Du, W.A. Kibbe, S.M. Lin, lumi: a pipeline for processing Illumina microarray, Bioinformatics 24 (2008) 1547–1548, https://doi.org/10.1093/BIOINFORMATICS/BTN224.

[22] M. Lauss, I. Visne, A. Kriegner, M. Ringnér, G. Jönsson, M. Höglund, Monitoring of technical variation in quantitative high-throughput datasets, Cancer Inf. 12 (2013) 193–201, https://doi.org/10.4137/CIN.S12862.

[23] S.H. Hong, C. Min, Y. Jun, D.J. Lee, S.H. Kim, J.H. Park, J.H. Cheong, Y.J. Park, S. Y. Kim, S. Lee, S.W. Kang, Silencing of peroxiredoxin II by promoter methylation is necessary for the survival and migration of gastric cancer cells, Exp. Mol. Med. 50 (2018), https://doi.org/10.1038/EMM.2017.267.

[24] J. Müller, O. Krijgsman, J. Tsoi, L. Robert, W. Hugo, C. Song, X. Kong, P.A. Possik, P.D.M. Cornelissen-Steijger, M.H.G. Foppen, K. Kemper, C.R. Goding, U. McDermott, C. Blank, J. Haanen, T.G. Graeber, A. Ribas, R.S. Lo, D.S. Peeper, Low MITF/AXL ratio predicts early resistance to multiple targeted drugs in melanoma, Nat. Commun. 5 (2014), https://doi.org/10.1038/NCOMMS6712.

[25] M.R. Webster, C.H. Kugel, A.T. Weeraratna, The Wnts of change: How Wnts regulate phenotype switching in melanoma, Biochim. Biophys. Acta 1856 (2015) 244–251, https://doi.org/10.1016/J.BBCAN.2015.10.002.

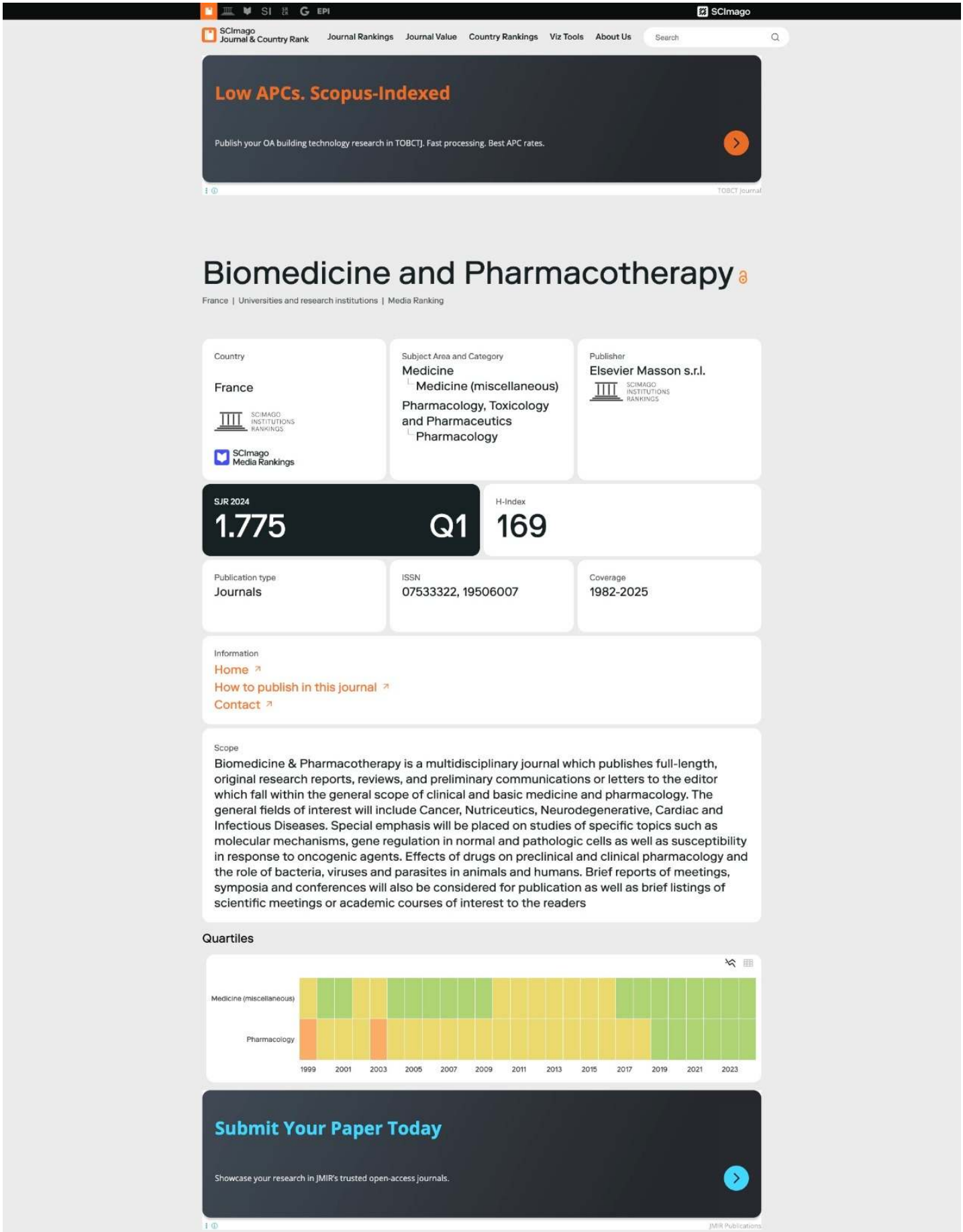
[26] S.M. Hossain, M.R. Eccles, Phenotype Switching and the Melanoma Microenvironment; Impact on Immunotherapy and Drug Resistance, Int. J. Mol. Sci. 24 (2023), https://doi.org/10.3390/ijms24021601.

[27] E. Tuncer, R.R. Calçada, D. Zingg, S. Varum, P. Cheng, S.N. Freiberger, C.-X. Deng, I. Kleiter, M.P. Levesque, R. Dummer, L. Sommer, SMAD signaling promotes melanoma metastasis independently of phenotype switching, J. Clin. Invest. 129 (2019) 2702–2716, https://doi.org/10.1172/JCI94295.

[28] B.De Craene, G. Berx, Regulatory networks defining EMT during cancer initiation and progression, Nat. Rev. Cancer 13 (2013) 97–110, https://doi.org/10.1038/nrc3447.

[29] L. Hao, J.R. Ha, P. Kuzel, E. Garcia, S. Persad, Cadherin switch from E- to N-cadherin in melanoma progression is regulated by the PI3K/PTEN pathway

through Twist and Snail, Br. J. Dermatol. 166 (2012) 1184–1197, https://doi.org/10.1111/J.1365-2133.2012.10824.X.
[30] D. Ribatti, R. Tamma, T. Annese, Epithelial-Mesenchymal Transition in Cancer: A Historical Overview, Transl. Oncol. 13 (2020), https://doi.org/10.1016/J.TRANON.2020.100773.
[31] S. Spada, A. Tocci, F. Di Modugno, P. Nisticò, Fibronectin as a multiregulatory molecule crucial in tumor matrisome: from structural and functional features to clinical practice in oncology, J. Exp. Clin. Cancer Res. 40 (2021), https://doi.org/10.1186/S13046-021-01908-8.
[32] A. Leikeim, M. Wußmann, F.F. Schmidt, N.G.B. Neto, F. Benz, K. Tiltmann, C. Junger, M.G. Monaghan, B. Schilling, F.K. Groeber-Becker, A preclinical model of cutaneous melanoma based on reconstructed human epidermis, Sci. Rep. 12 (2022), https://doi.org/10.1038/S41598-022-19307-0.
[33] F. Carta, P.R. Demuro, C. Zanini, A. Santona, D. Castiglia, S. D'Atri, P.A. Ascierto, M. Napolitano, A. Cossu, B. Tadolini, F. Turrini, A. Manca, M.C. Sini, G. Palmieri, C. Rozzo, Analysis of candidate genes through a proteomics-based approach in primary cell lines from malignant melanomas and their metastases, Melanoma Res 15 (2005) 235–244, https://doi.org/10.1097/00008390-200508000-00002.
[34] H.S. Choi, J.S. Shim, J.A. Kim, S.W. Kang, H.J. Kwon, Discovery of gliotoxin as a new small molecule targeting thioredoxin redox system, Biochem. Biophys. Res. Commun. 359 (2007) 523–528, https://doi.org/10.1016/J.BBRC.2007.05.139.
[35] M. Fardi, M. Alivand, B. Baradaran, M. Farshdousti Hagh, S. Solali, The crucial role of ZEB2: From development to epithelial-to-mesenchymal transition and cancer complexity, J. Cell. Physiol. 234 (2019) 14783–14799, https://doi.org/10.1002/JCP.28277.
[36] A. Sathyanarayanan, K.S. Chandrasekaran, D. Karunagaran, microRNA-145 modulates epithelial-mesenchymal transition and suppresses proliferation, migration and invasion by targeting SIP1 in human cervical cancer cells, Cell. Oncol. (Dordr.). 40 (2017) 119–131, https://doi.org/10.1007/S13402-016-0307-3.
[37] M.C. Sobotta, W. Liou, S. Stöcker, D. Talwar, M. Oehler, T. Ruppert, A.N.D. Scharf, T.P. Dick, Peroxiredoxin-2 and STAT3 form a redox relay for H2O2 signaling, Nat. Chem. Biol. 11 (2015) 64–70, https://doi.org/10.1038/NCHEMBIO.1695.
[38] A.T. Shaw, S.-H.I. Ou, Y.-J. Bang, D.R. Camidge, B.J. Solomon, R. Salgia, G.J. Riely, M. Varella-Garcia, G.I. Shapiro, D.B. Costa, R.C. Doebele, L.P. Le, Z. Zheng, W. Tan, P. Stephenson, S.M. Shreeve, L.M. Tye, J.G. Christensen, K.D. Wilner, J.W. Clark, A. J. Iafrate, Crizotinib in ROS1-rearranged non-small-cell lung cancer, N. Engl. J. Med. 371 (2014) 1963–1971, https://doi.org/10.1056/NEJMOA1406766.
[39] A.M. Fortier, E. Asselin, M. Cadrin, Keratin 8 and 18 loss in epithelial cancer cells increases collective cell migration and cisplatin sensitivity through Claudin1 up-regulation, J. Biol. Chem. 288 (2013) 11555, https://doi.org/10.1074/JBC.M112.428920.
[40] D. Sun, M. Zhou, C.M. Kowolik, V. Trisal, Q. Huang, K.H. Kernstine, F. Lian, B. Shen, Differential expression patterns of capping protein, protein phosphatase 1 and casein kinase 1 may serve as diagnostic markers for malignant melanoma, Melanoma Res 21 (2011) 335, https://doi.org/10.1097/CMR.0B013E328346B715.
[41] M.A. El Sharouni, R.V. Rawson, V. Sigurdsson, A.J. Witkamp, C.H. van Gils, R. A. Scolyer, J.F. Thompson, P.J. van Diest, S.N. Lo, The progressive relationship between increasing Breslow thickness and decreasing survival is lost in patients with ultrathick melanomas (≥15 mm in thickness), J. Am. Acad. Dermatol. 87 (2022) 298–305, https://doi.org/10.1016/J.JAAD.2022.01.040.
[42] R.A. Scolyer, R.V. Rawson, J.E. Gershenwald, P.M. Ferguson, V.G. Prieto, Melanoma pathology reporting and staging, Mod. Pathol. 33 (2020) 15–24, https://doi.org/10.1038/S41379-019-0402-X.
[43] D. Kubota, K. Mukaihara, A. Yoshida, H. Tsuda, A. Kawai, T. Kondo, Proteomics study of open biopsy samples identifies peroxiredoxin 2 as a predictive biomarker of response to induction chemotherapy in osteosarcoma, J. Proteom. 91 (2013) 393–404, https://doi.org/10.1016/j.jprot.2013.07.022.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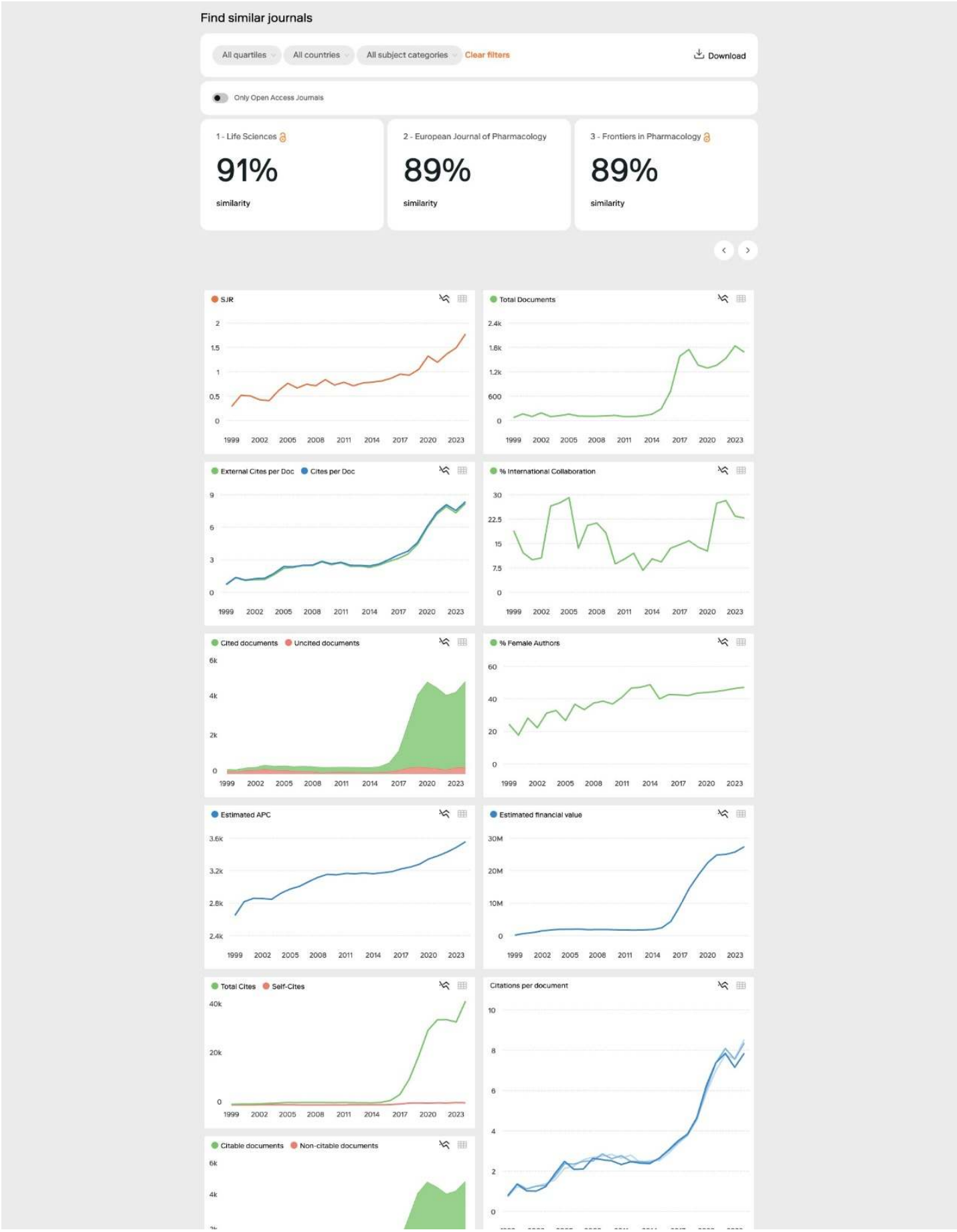
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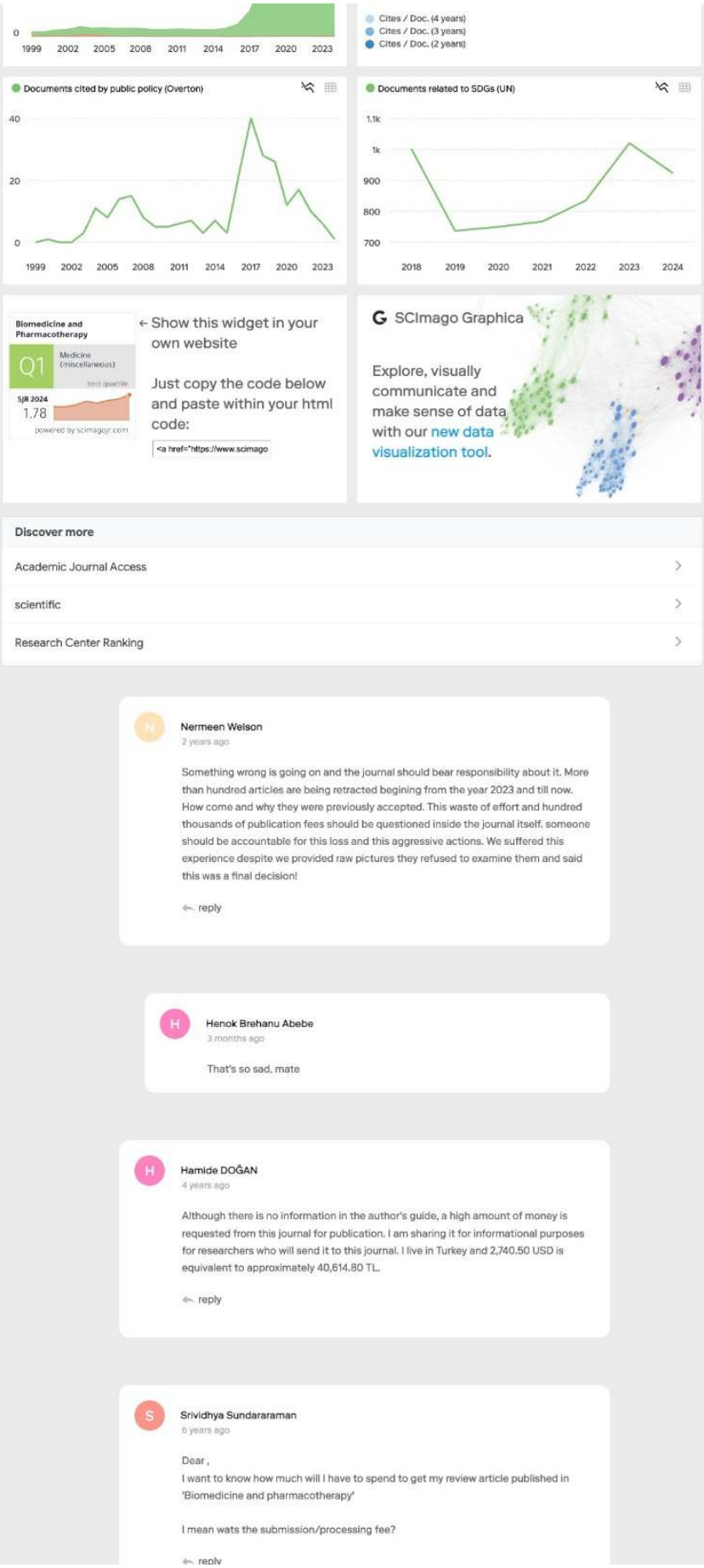
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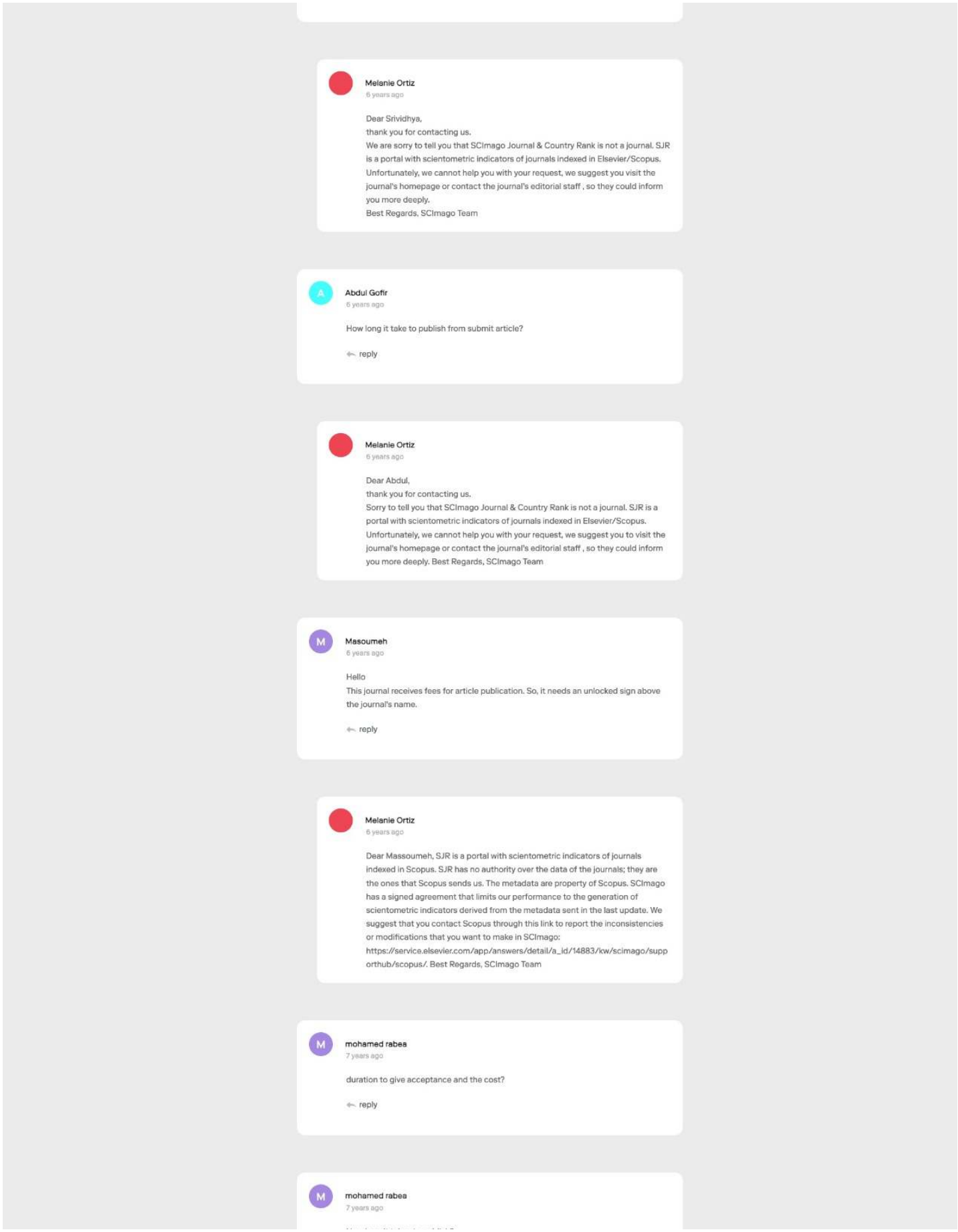

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Modeling Melanoma Heterogeneity In Vitro: Redox, Resistance and Pigmentation Profiles

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Abstract: Microenvironment and transcriptional plasticity generate subpopulations within the tumor, and the use of BRAF inhibitors (BRAFis) contributes to the rise and selection of resistant clones. We stochastically isolated subpopulations (C1, C2, and C3) from naïve melanoma and found that the clones demonstrated distinct morphology, phenotypic, and functional profiles: C1 was less proliferative, more migratory and invasive, less sensitive to BRAFis, less dependent on OXPHOS, more sensitive to oxidative stress, and less pigmented; C2 was more proliferative, less migratory and invasive, more sensitive to BRAFis, less sensitive to oxidative stress, and more pigmented; and C3 was less proliferative, more migratory and invasive, less sensitive to BRAFis, more dependent on OXPHOS, more sensitive to oxidative stress, and more pigmented. Hydrogen peroxide plays a central role in oxidative stress and cell signaling, and PRDXs are one of its main consumers. The intrinsically resistant C1 and C3 clones had lower MITF, PGC-1α, and PRDX1 expression, while C1 had higher AXL and decreased pigmentation markers, linking PRDX1 to clonal heterogeneity and resistance. PRDX2 is depleted in acquired BRAFi-resistant cells and acts as a redox sensor. Our results illustrate that decreased pigmentation markers are related to therapy resistance and decreased antioxidant defense.

Keywords: melanoma; heterogeneity; pigmentation; peroxiredoxin 1; peroxiredoxin 2; BRAFi resistance

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1. Introduction

Melanoma is the most aggressive type of skin cancer [1,2]. Although BRAF inhibitors (BRAFi) have revolutionized therapy, residual disease persists and relapse is very common [3–6]. Melanoma is a heterogeneous tumor and BRAFi treatment contributes to the selection of intrinsic and acquired resistant subpopulations [7–9]. The key biomarker for this phenotype switching is the melanocyte lineage-specific transcription factor MITF (microphthalmia-associated transcription factor) [10,11]. It has been demonstrated that the $MITF^{high}/AXL^{low}$ phenotype is linked to BRAFi sensitivity while $MITF^{low}/AXL^{high}$ predicts resistance [12]. MITF is also linked to oxidative metabolism by controlling the transcriptional cofactor PGC-1α (peroxisome proliferator-activated receptor γ coactivator 1), which activates mitochondrial biogenesis and oxidative phosphorylation (OXPHOS) [13,14]. Increased expression of PGC-1α can restore OXPHOS in melanoma and, consequently, lead to higher ROS production by the mitochondria [14,15]. Acquired mutations in BRAF

suppresses OXPHOS, MITF, and PGC-1α expression, while BRAFi increases OXPHOS, MITF, PGC-1α, and ROS production. Increased ROS levels makes the tumor more sensitive to oxidative stress [16,17], rendering cells highly dependent on antioxidant defense. BRAFi-resistant cells frequently have adaptive antioxidant mechanisms to tolerate ROS [18]. PGC-1 coactivators are regulators of melanin production via regulation of MITF and melanin production itself is an oxidative process that generates ROS [19,20]. It is known that PGC-1s regulates broad protective programs against ROS [21] and MITF has also been implicated in controlling ROS [22]. Coregulation of melanogenesis and an anti-ROS program by PGC-1s may thus be critical for protecting activated melanocytes from ROS damage [13]. However, the role of melanin on metastasis and resistance remains poorly explored at the heterogeneity level [23].

Transient production of ROS is necessary for cell signaling, but persistent production could be cytotoxic. In this sense, cancer cells require increased levels of ROS for continuous proliferation, but overexpress cellular peroxidases such as peroxiredoxins (PRDXs) to maintain homeostasis [24]. PRDXs are antioxidant enzymes that react with ROS and RNS, and they play a central role in redox signaling acting as redox sensors [25–27]; also, the oxidation state of PRDXs, provides information about redox homeostasis [28,29]. Moreover, PRDXs are differentially expressed in patients during melanoma progression [30] and decreased expression of PRDX1 and PRDX2 is a biomarker in melanoma compared to nevus [31]. Additionally, the role of PRDX2 in preventing invasion in BRAF-mutant melanoma has been described in a heterogenous population [32], but its roles in heterogeneity, resistance, and pigmentation are not known [31]. In this study, we investigated clonal heterogeneity and demonstrated a link among pigmentation, resistance, and oxidative stress that could help better understand therapy failure.

2. Materials and Methods

2.1. Human Melanoma Cell Culture

The human melanoma cell line (SK-MEL-28) was kindly donated by Dr. Maria S. Soengas (Melanoma Group, CNIO, Spain). The SK-MEL-28 BRAFi-resistant cells (SK-MEL-28R) were generated using vemurafenib (PLX4032) as previously described [33]. STR profiling was performed in naïve cells. Cells were cultured in DMEM supplemented with 10% FBS and antibiotics at 37 °C, 5% CO_2 and humidified atmosphere. All cells were tested for mycoplasma.

2.2. Clonal Subpopulations Isolation

Stochastic isolation was performed by seeding naïve SK-MEL-28 one cell per well. Cells were cultivated until confluence was reached and three distinct clones were isolated, called C1, C2, and C3.

2.3. Cell Proliferation

Cells (1×10^4 cells/well) were harvested at times 24, 72, 120, and 168 h, and the viable cells were counted using trypan blue in a Neubauer chamber. Doubling times were obtained by non-linear regression analysis with GraphPad Prism 5 (La Jolla, CA, USA).

2.4. Wound Healing Assay

The cell (5×10^4 cells/well) monolayer was scratched using a pipette tip after reaching more than 90% confluence and maintained in DMEM containing 1% FBS. Images were taken at 0, 12, and 24 h until the control wound was fully closed. Images were processed and quantified in ImageJ 8 (Bethesda, MD, USA). Results represent the measurements of each wounded area in three independent experiments.

2.5. Evaluation of Intrinsic BRAFi Resistance

Cells (2×10^4 cells/well) were treated with 0, 1, or 6 μM of BRAFi. At times 24, 48, and 72 h, viable cells were counted using trypan blue in a Neubauer chamber.

2.6. Colony Formation Assay

Cells (1000 cells/well) were treated with 0, 1, and 6 μM BRAFi every 2–3 days for 4 weeks. They were then washed with PBS, fixed, and stained with violet crystal solution for 10 min at RT.

2.7. Protein Levels by Western Blot

Cells were lysed with RIPA buffer (Tris 10 mM pH 7.5, NaCl 150 mM, NP 40 1%, SDS 0.1%, Sodium Deoxycolate 1%, EDTA 5 mM, EGTA 5 mM, NaF 25 mM and Na_2VO_4 1 mM) and a mixture of protease and phosphatase inhibitors. For non-reducing Western blot, N-ethylmaleimide 30 mM was added. Proteins were quantified by Bradford, separated by SDS-PAGE under reducing or non-reducing buffer, transferred to a PVDF membrane, and incubated for 1 h at RT with blocking solution (BSA 5%). Then, the membrane was incubated overnight at 4 °C with the primary antibody (1:1000). Next day, the membrane was incubated for 1 h with the secondary antibody (1:2500) and protein bands detected by an ECL system (Luminata™ Forte Western HRP Substrate) (Millipore Corporation, FL, USA) in an Amersham Imager 680 device. Relative densitometry was semi-quantified by ImageJ.

2.8. mRNA Levels by RT-qPCR

RNA was extracted with the RNeasy Plus Mini Kit (Qiagen, Hilden, Germany). Purity was evaluated by absorbance at 260/280 and 260/230 nm. The cDNA was obtained by ThermoScript™ RT-PCR System kit from Invitrogen and amplified with the Taqman Mix® Master and Real Time PCR Assays (Thermo Fischer, Waltham, MA, USA). Transcriptions were normalized with β-actin levels in the samples using the Real-Time PCR System from Applied Biosystems™ StepOne™. Relative expression was calculated using the ΔΔCt method [34].

2.9. Oxygen Consumption Rate (OCR)—Seahorse XF24 Flux Analyzer

OCR was measured in intact cells using the XF24 Extracellular Flow Analyzer (Seahorse Bioscience, North Billerica, MA, USA). Cells (3×10^4 cells/well) were plated in XF24 V7 cell culture microplates. After 12–16 h, XF test medium supplemented with D-glucose 10 mM, sodium pyruvate 1 mM, and GlutaMAX™ (Gibco™) (Thermo Fischer, Waltham MA, USA)2 mM was added and incubated in a free-of-CO_2 atmosphere at 37 °C for 1 h. Respiratory parameters were analyzed based on the protocol for the Seahorse XF cell stress test kit with minor modifications. Three background measurements were performed before the addition of mitochondrial inhibitors. Sequential additions of 1.5 μM oligomycin, 30 μM dinitrophenol (DNP), and 1 μM rotenone plus antimycin A were made. The results are shown relative to the background.

2.10. Redox State Assessment by Dihydrorhodamine 123 (DHR) Probe

Cells (5×10^4 cells/well) were incubated in PBS containing 1 g/L of glucose and the DHR fluorescent probe (ThermoFischer, D23806, 10 μM) was added. After 3 h, fluorescence was read (Synergy, Biotek, VT, USA) with excitation at 485 nm and emission at 528 nm.

2.11. Evaluation of Hydrogen Peroxide Cytotoxicity

Cells (2×10^4 cells/well) were treated with hydrogen peroxide (0, 50, 100, 200, 300, and 400 μM) for 3 h. Hydrogen peroxide was quantified at 240 nm ($\varepsilon_{240nm} = 43.6\ M^{-1}cm^{-1}$). MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 1 mg/mL) was added and cells incubated for 3 h. Reduced formazan was read at 570 nm (BioTek Instruments, Winooski, VT, USA). IC50 values were obtained by non-linear regression analysis with GraphPad Prism 5.

2.12. Evaluation of Gliotoxin (GT) Cytotoxicity

Cells (2×10^4 cells/well) were treated with 0, 100, 200, 350, and 500 nM GT (Sigma-Aldrich G9893) (Sigma-Aldrich, Sao Paulo, Brail). Viable cells were counted using trypan

blue in a Neubauer chamber. IC50 values were obtained by non-linear regression analysis with GraphPad Prism 5.

2.13. Reconstructed Human Skin

Normal human skin cells were obtained from donated foreskin samples from the University of São Paulo Hospital (HU/USP) (CAAE:76737917.5.1001.0067). Cells were isolated as described before [33]. Fibroblasts were cultured in DMEM with antibiotics and 10% FBS; melanocytes were cultured in medium 254; and keratinocytes were cultured in KBM medium. The reconstructed human skin containing melanoma cells was prepared as described previously [33]. Briefly, fibroblasts (1.5×10^4 cells/skin) were mixed in type I collagen and incubated at 37 °C for polymerization of the dermal matrix. Then, keratinocytes (2.5×10^4 cells/skin), melanocytes (0.8×10^4 cells/skin), and melanoma cells (50×10^4 cells/skin) were added. In the next day, skins were transferred to an air–liquid interface for epithelial differentiation for 10 days.

2.14. Hematoxylin and Eosin

Reconstructed skins were paraffin included, sectioned (5 μm), and stained with H&E for morphological analysis. All images were obtained by optical microscopy and analyzed by the NIS Elements software 5.02 (Melville, NY, USA).

2.15. Immunofluorescence

Slides were deparaffinized, submitted to antigen retrieval with 10 mM sodium citrate, and washed with PBS. A quantity of 10% goat serum was used to prevent unspecific binding of antibodies, followed by incubation with primary antibodies. Sections were incubated with secondary antibodies for 1 h. Slides were mounted in SlowFade antifade mounting with DAPI (Thermo Scientific) for nuclear counterstaining. All images were acquired with a fluorescence microscope (Axio observer, Zeiss, Oberkochen, Germany) using ZEN software 3.4.

2.16. In Silico Analysis

Public data were used from 472 samples of primary and metastatic melanoma of the TCGA (*The Cancer Genome Atlas*), which were downloaded from the repository of the University of Carolina Santa Cruz—UCSC (https://xena.ucsc.edu/) (accessed on 1 February 2020). Samples were separated in reference to their expression values normalized by the first and last quartile as "low" and "high", respectively. The expression values of the gene of interest were verified for these groups and then *boxplots* were generated with the results. The statistical analysis corresponds to the *two-tail* T test among the samples presented.

2.17. cBioPortal Database Analysis

To analyze the integrative relationships of the genes and their clinical characteristics in skin cutaneous melanoma, the cBioPortal for Cancer Genomics was used. The cBioPortal for Cancer genomics is an open access resource (http://www.cbioportal.org/) (accessed on 5 February 2020) [35,36]. The terms "MITF", "PPARGC1A", "SOX10", "PRDX1", "PRDX2", and "AXL" were searched for in the cBioPortal database for skin cutaneous melanoma (TCGA, Firehose Legacy), n = 479 samples. The selected genomic profiles were mRNA expression and protein expression with a z-score threshold of 1.5.

2.18. scRNAseq Analysis

The analysis of single-cell datasets was performed using Interactive Single-Cell Visual Analytics (ISCVA), a computational tool consisting of two major components. The first is a collection of Bash and R scripts that uses many of the widely used algorithms in single cells, such as Seurat for general processing [37], SingleR for cell-type recognition [38], and single-cell signature explorer for gene set signature scoring [39], which processed the scRNA-Seq data offline. The second is a web-based component including react.js from Facebook,

tensorflow.js from Google, and Plotly.js. These components allow real-time interactive exploration and ad hoc analysis. As a part of the analytical modules, the heterogeneity analyses implemented in SinCHet [40] were also performed. A node.js backend was created to serve the on-demand queries of the web application, allowing for real-time interactive investigation of genes expressed in selected samples or subsets of cells. Data analyzed are from previous published work (GSE174401 and GSE77940) [41,42].

2.19. Statistical Analysis

Results are expressed as mean ± standard error of three independent experiments. One-way analysis of variance (ANOVA) was used followed by the Newman–Keuls post-test to test for multiple comparisons with a given significance level of $p < 0.05$. Alternatively, the *t*-test was used when indicated. Significant differences between the control and treated groups are indicated by * $p > 0.05$, ** $p > 0.01$, and *** $p > 0.001$. All experiments were performed in independent triplicates. All data analyzed and images generated were obtained using the R platform and Prism GraphPad 5.

3. Results

3.1. Clones Exhibited Distinct Morphology, Proliferation Rate, Intrinsic Resistance, Migration, and Invasion Capacity

Clone C1 is more spherical than C2 and C3, while clone C3 showed a more elongated shape. A mixture of these morphologies can be seen in the parental cell line (Figure 1A). Clone C2 (24.3 h) and SK-MEL-28P (24.5 h) are more proliferative than C1 (45.5 h) and C3 (48.6 h, Figure 1B). Confirming our results, the phosphorylation of ERK is decreased in C1 and C3 (Figure 1C). Clone C2 seems to be the less migratory than the others (Figure 1D). After a long-term treatment (3 weeks) with BRAFi (acquired resistance), C1 and C3 were capable of surviving and forming viable colonies, unlike C2 (Figure 1E). We tested the intrinsic resistance by short-term treatment with 24, 48, and 72 h of BRAFi exposure and demonstrated that the SK-MEL-28P and C2 were sensitive at 24 h, while C1 and C3 needed 48h to respond (Figure 1F).

In a 3D skin model, SK-MEL-28P invaded the dermis and proliferated on the corneal layer (Figure 2A). The SK-MEL-28R also invaded the dermis even in the presence of BRAFi. Among the clones, C1 showed the most invasive profile, followed by C3 and C2. We confirmed the melanoma invasion within the dermis with S100 and HMB45 staining in Figure 2B. Further studies demonstrated that clone C1 and the SK-MEL-28R cells showed higher levels of AXL and beta-catenin (Figure 2C,D).

3.2. Clones Exhibited Distinct Oxidative Metabolism

PGC-1α levels were decreased in all clones, with C1 and C3 showing the lowest levels (Figure 3A). C1 was less dependent on OXPHOS, C3 was more dependent, and C2 resembled the profile of SK-MEL-28P (Figure 3B). Clones C1 and C3 showed higher ROS production compared to C2. The SK-MEL-28P exhibited higher levels of ROS, probably because of the presence of other clones (Figure 3C). The antioxidant system in general was decreased in C1 (Figure 3D). C1 and C3 expressed less PRDX1 when compared to SK-MEL-28P. In addition, SK-MEL-28P and the clones C1 and C3 exhibited decreased expression of PRDX1 when compared to primary human melanocytes, while clone C2 and SK-MEL-28R showed comparable levels to melanocytes (Figure 3E). No differences in PRDX2 levels were seen among the clones (Figure 3E), but PRDX2 was depleted in the SK-MEL-28R while PRDX1 was increased in SK-MEL-28R compared to SK-MEL28P (Figure 3E). Once PRDX dimers were formed after oxidation, non-reducing Western blot was performed to probe oxidative stress. As expected, cells that showed increased levels of PRDX1 also had increased expression of monomers and dimers. We calculated the dimer/monomer ratio to evaluate the redox status [43] (Figure 3F, upper figure) and found no differences among the cells. In contrast, levels of PRDX2 monomers were lower in C1 and C3 while the dimers were higher, providing evidence that these had a more oxidative environment (Figure 3F, lower figure), in agreement

with the data obtained with DHR (Figure 3C). Once PRDX1 levels are variable among the clones and PRDX2 levels are maintained, this result suggests that PRDX2, but not PRDX1, could be used as an oxidative stress probe. We then tested the sensitivity of the clones to hydrogen peroxide (Figure 3G) and gliotoxin (GT), an antioxidant and PRDX mimetic (Figure 3H). C1 was the most sensitive to hydrogen peroxide (IC50 = 93.95 μM), followed by C3 (IC50 = 103.1 μM), SK-MEL28R (106.5 μM), SK-MEL-28P (113.6 μM), and C2 (134.6 μM). By contrast, clone C2 was the most sensitive to GT (IC50 = 85.01 nM), followed by SK-MEL-28P (IC50 = 131.2 nM), SK-MEL-28R (IC50 = 151.2 nM), clone C3 (IC50 = 151.8 nM), and clone C1 (IC50 = 160.3 nM). In summary, C1 and C3, which were the most sensitive to oxidative stress, were also the most resistant to GT. So, it seems that C1 and C3 produce more ROS and/or exhibit a weaker antioxidant system compared to clone C2.

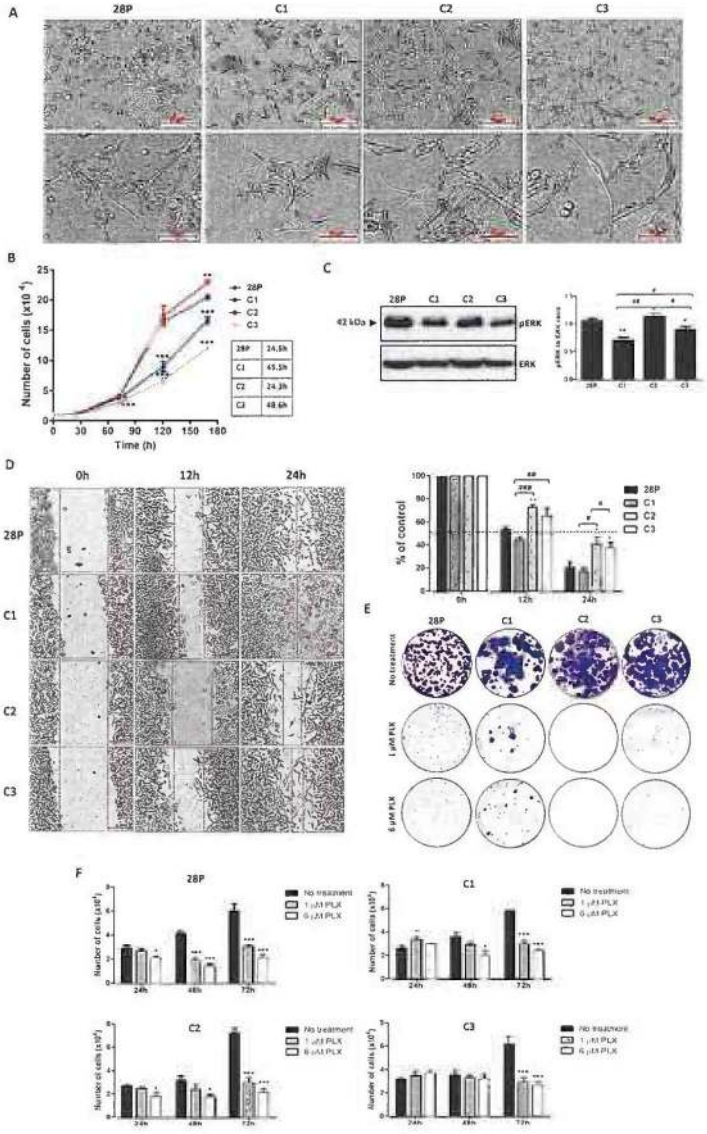

Figure 1. The isolated clones exhibited distinct morphology, proliferation rate, migration capacity, and intrinsic resistance. (**A**) Cell morphology. Scale 100 μm, magnification 10× (above) and 40× (below).

(**B**) Proliferation rate. The cells (1×10^4 cells/well) were plated and the viable ones were counted with trypan blue exclusion after 24, 72, 120, and 168 h. (**C**) Evaluation of proliferation by expression of pERK and ERK. (**D**) Wound healing assay. The cells (2×10^5 cells/well) were plated, and after the scratch, photos were taken at 0, 12, and 24h. Scale 50 μm, magnification 10×. (**E**) Colony formation assay. The cells (1000 cells/well) were treated with PLX (0, 1, and 6 μM) for 3 weeks and stained with crystal violet. (**F**) Intrinsic resistance to BRAFi. The cells (2×10^4 cells/well) were plated and treated with PLX (0, 1 and 6 μM), and the viable ones were counted with trypan blue exclusion after 24, 48, and 72 h. All the images are representative of three independent experiments. The values are indicated by mean ± standard error of three independent experiments. The statistical analysis was performed by one-way analysis of variance (ANOVA) followed by a Newmann–Keuls test; *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ when compared to control and # when compared among the groups. 28P: SK-MEL-28 parental cell line. PLX: vemurafenib (BRAFi) (### $p < 0.001$, ## $p < 0.01$, and # $p < 0.05$).

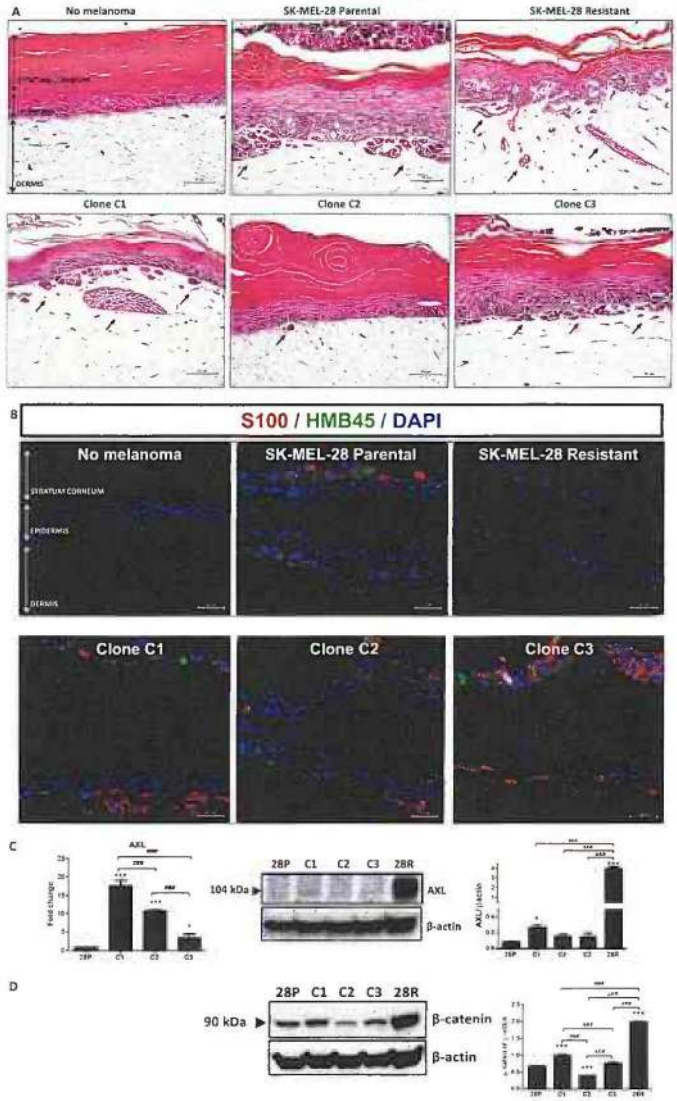

Figure 2. Clones exhibited distinct invasion profiles. (**A**) Human reconstructed skin containing no melanoma, SK-MEL-28 parental, clone C1, clone C2, clone C3, or SK-MEL-28 resistant stained for

H&E. Scale 50 µm, magnification 40×. (**B**) Immunofluorescence for human reconstructed skin containing no melanoma, SK-MEL-28 parental, clone C1, clone C2, clone C3, or SK-MEL-28 resistant stained for S100 and HMB45. Scale 50 µm, magnification 40×. (**C**) mRNA and protein levels of AXL in SK-MEL-28 parental, clones, and SK-MEL-28R. (**D**) Protein levels of beta-catenin in SK-MEL-28 parental, clones and SK-MEL-28R. 28P: SK-MEL-28 parental cell line. 28R: SK-MEL-28-resistant cell line. The statistical analysis was performed by one-way analysis of variance (ANOVA) followed by a Newmann–Keuls test; *** $p < 0.001$ and * $p < 0.05$ when compared to control and # when compared among the groups (### $p < 0.001$).

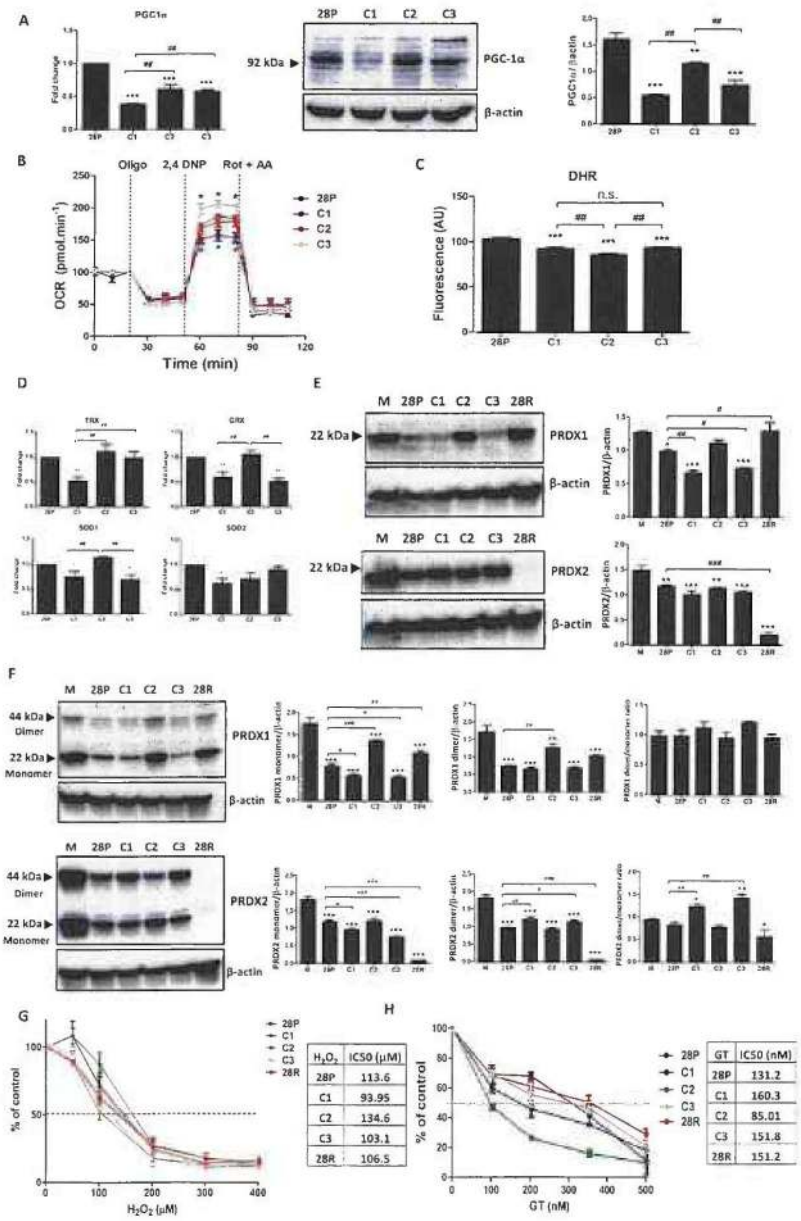

Figure 3. Clones exhibited distinct oxidative metabolism. (**A**) Expression of mRNA and protein of PGC−1α. (**B**) Oxygen consumption rate (OCR). The cells (3×10^4 cells/well) were treated with indicated

drugs (oligomycin 1.5 μM, 2,4-dinitrophenol 30 mM, rotenone 1 μM, and antimycin A 1 μM) at the determined times. The values are indicated by mean ± standard error of three independent experiments. Statistical analysis was performed by *t*-test comparing with the parental SK-MEL-28P control, * $p < 0.05$. (**C**) Detection of total reactive oxygen species using DHR probe. The cells (5×10^4 cells/well) were seeded in a black plate, treated with DHR (10 μM) for 3 h and then fluorescence was read (λexc 485 nm and λem 528 nm). The values are indicated by mean ± standard error of three independent experiments. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Newmann–Keuls test, *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$ compared to parental control (# when compared among groups). (**D**) TRX, GPX, SOD1, and SOD2 mRNA levels in clones. (**E**) Protein levels of PRDX1 and PRDX2 in melanocytes (M), SK-MEL-28 parental, clones, and SKM-MEL-28R. (**F**) Western blot redox for PRDX1 and PRDX2. (**G**) Determination of the sensitivity of the cells to hydrogen peroxide by calculating the IC50. (**H**) Determination of the sensitivity of the cells to gliotoxin by calculating the IC50. The cells (2×10^4 cells/well) were treated with increasing concentrations of hydrogen peroxide (0, 100, 200, 300, and 400 nM). After 24 h and 5 h, viable cells were determined. 28P: SK-MEL-28 parental cell line. 28R: SK-MEL-28-resistant cell line (### $p < 0.001$, ## $p < 0.01$, and # $p < 0.05$).

3.3. Clones Showed Different Profiles of Pigmentation

An analysis of melanocyte lineage/pigmentation markers demonstrated that NRF2 expression was significantly increased in clone C1, while SOX10 and MITF were significantly decreased (Figure 4A–C). It was further noted that Melan-A levels were decreased in the skin containing C1 or SK-MEL-28R (Figure 4D). Skins exhibited different patterns of pigmentation with the SK-MEL-28P and the clones C2 and C3 exhibiting the most pigmented, while C1 and SK-MEL28R showed no pigmentation (Figure 4E). We confirmed these results showing that the pigmentation markers Melan-A, TYR, TRP1, and TRP2 were decreased in C1 and SK-MEL-28R (Figure 4F). We also demonstrated that RAB7 and RAB27 were decreased in C1 and SK-MEL-28R (Figure 4G).

3.4. In Silico Analysis of PRDX1 and PRDX2 Expression in Melanoma Patients

PRDX1 shows significant co-expression with MITF in melanoma patients, with PRDX2 showing a tendency towards mutual exclusivity (Figure 5A). There was also a tendency for co-occurrence between PRDX2 and AXL, whereas PRDX1 and AXL were mutually exclusive (Figure 5A). Patients with low levels of MITF have significantly lower levels of PRDX1 and higher levels of PRDX2, while no differences were found on AXL and PGC1α compared to PRDX1 and PRDX2 (Figure 5B). Additionally, BRAF-mutated patients with decreased expression of PRDX1 demonstrated poor survival rates compared to those with higher expression of PRDX1 (Figure 5C). Using single-cell data from metastatic melanoma patients published by [42], we observed that MITF, SOX10, MLANA, PGC1a, TYR, TYRP1, and TYRP2/DCT are present only or enriched in melanoma cells. Additionally, NRF2 expression was noted in all cell types at a lower expression than that of MITF in melanoma cells. The expression of PRDX1 was higher than that of PRDX2 in melanoma cells and more similar to that of MITF than that of PRDX2. Furthermore, the expressions of TYR, TYRP1, and TYRP2/DCT were similar to those of MITF and MLANA, corroborating our data obtained using the isolated clones, where C1 expresses less MITF, MLANA, TYR, TYRP1, TYPR2/DCT, and PRDX1 (Figure 5D). We also analyzed another dataset published in [41], containing melanoma patients with skin, brain, and leptomeningeal metastasis. As expected, PRDX1 and PRDX2 were distributed across all cell types. However, it was interesting to note that the expressions of PRDX1 and PRDX2 were different among the cells. We found PRDX1 enriched in neurons more than PRDX2, while some populations of melanoma cells express more PRDX2 than PRDX1. Then, PRDX1 expression is similar to MITF in patient melanoma cells, which corroborates our in vitro data using the clones (Figure 5E).

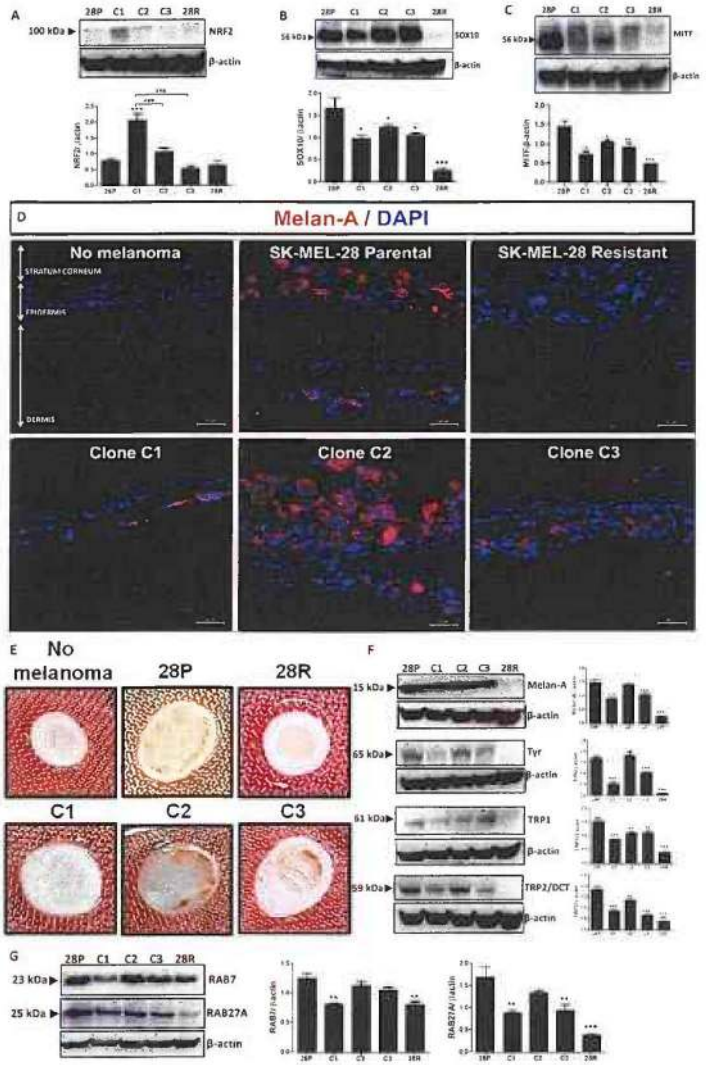

Figure 4. Clones exhibited different patterns of pigmentation. (**A**) Protein levels of NRF2. (**B**) Protein levels of SOX10. (**C**) Protein levels of MITF. (**D**) Immunofluorescence of skin reconstructed containing no melanoma, SK-MEL28 parental, clones, or SK-MEL-28R stained for Melan-A. Scale 50 µm, magnification 40×. (**E**) Images of the reconstructed skins showing the differences in pigmentation. (**F**) Protein levels of Melan-A, TYR, TRP1, and TRP2. (**G**) Protein levels of RAB7 and RAB27. The values are indicated by mean ± standard error of three independent experiments. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Newmann–Keuls test, *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$ when compared to parental control (# when compared among groups). 28P: SK-MEL-28 parental cell line. 28R: SK-MEL-28-resistant cell line (### $p < 0.001$).

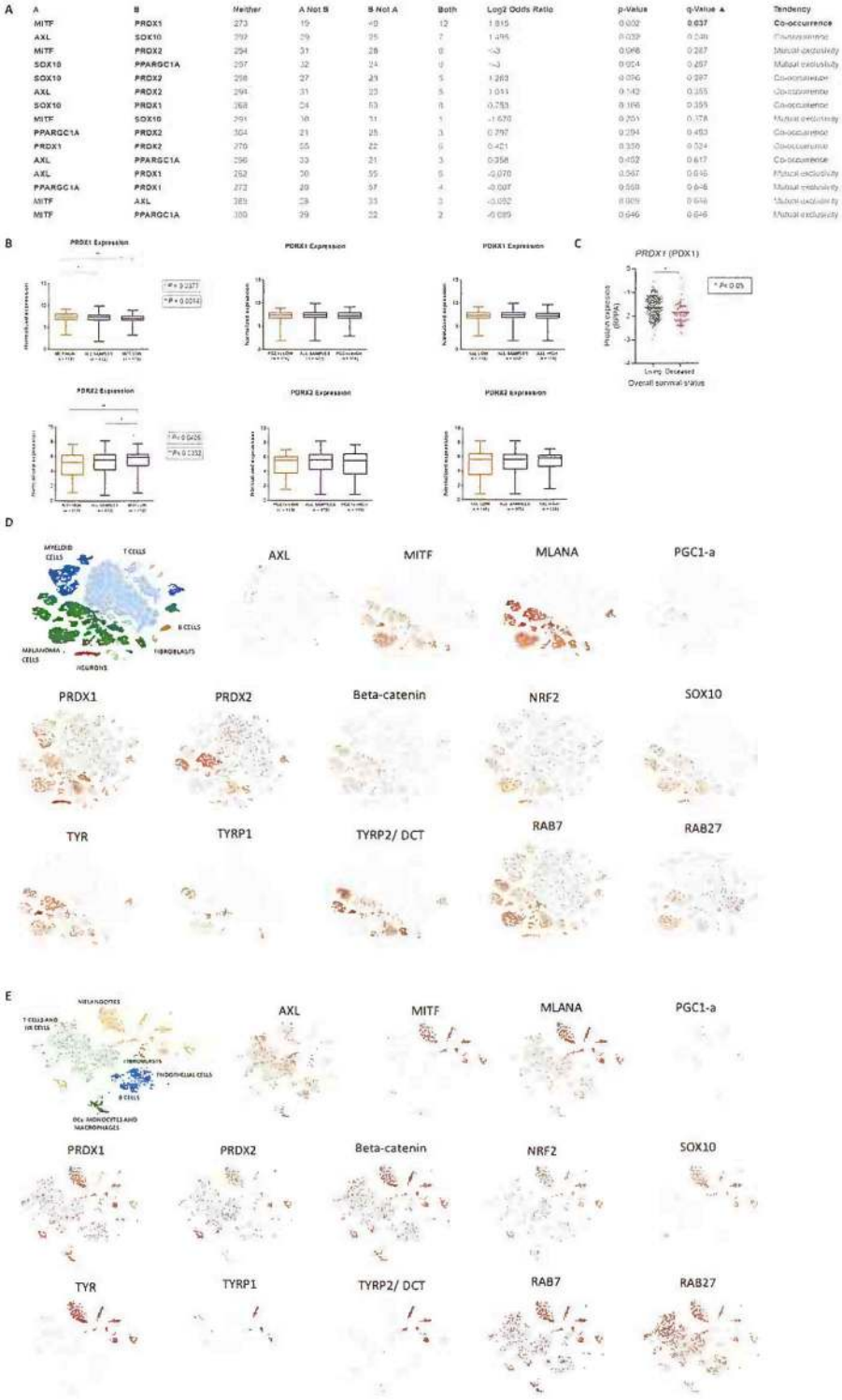

Figure 5. In silico analysis of the expression of PRDX1 and PRDX2 in melanoma patients. (**A**) Correlation between AXL, SOX10, MITF, PGC1−α, PRDX1, and PRDX2 in melanoma patients

according to cBioPortal. (**B**) Expression of PRDX1 and PRDX2 in cells expressing high or lower levels of MITF, PGC1-α and AXL. (**C**) Correlation of overall survival and levels of PRDX1. (**D**,**E**) scRNAseq data of proteins involved in proliferation, invasion, metabolism, redox processes, BRAFi, resistance, and pigmentation.

4. Discussion

Tumor heterogeneity is one of the causes of treatment resistance and failure [44,45]. It arises from complex genetic, epigenetic, and proteomic/transcriptomic modifications that drive phenotypic selection and alterations in response to microenvironment pressures [46]. In fact, a recent finding showed the potential to predict initial BRAFi sensitivity at the single-cell level [47]. The understanding of driver agents, the functional consequences of heterogeneity, and the transcriptional profiles of the clonal subpopulations may provide new insights that can optimize therapy. MITF regulates phenotype switching since $MITF^{high}$ cells exhibit a proliferative profile, while $MITF^{low}$ cells exhibit a more invasive one [10,11]. These data are in agreement with low MITF expression in C1 and C3. Considering that MITF controls the expression of PGC-1α, clones C1 and C3 also exhibited significant decreased expression of PGC-1α. SOX10 contributes to melanoma development by regulating the SOX10-MITF pathway, but also contributes to melanoma cell survival, proliferation, and metastasis formation [48,49]. AXL is linked to the mesenchymal state in the epithelium–mesenchymal transition, while melanoma cells with low AXL expression resemble the epithelial state [50]. The $MITF^{high}/AXL^{low}$ phenotype is linked to BRAFi sensitivity and the $MITF^{low}/AXL^{high}$ phenotype is linked to resistance [12], and we confirmed that C1 had the $MITF^{low}/AXL^{high}$ profile along with intrinsic resistance to BRAFi. Of relevance, cells that are $MITF^{low}/AXL^{high}$ can co-exist with $MITF^{high}/AXL^{low}$ cells and $MITF^{high}$ cells, and can cluster with distinct transcriptional states and be drug-sensitive or resistant [47,51].

BRAFi resistance is characterized by high mitochondrial activity that can occur regardless of PGC-1α expression [18]. Our results corroborate previous data and provide new evidence that naïve and intrinsically BRAFi-resistant cells like C1 could also display low mitochondrial activity. The presence of a BRAF mutation is associated with suppression of OXPHOS, MITF, and PGC-1α expression, while BRAFi can increase OXPHOS, MITF, PGC-1α, and ROS production. Additionally, ROS can be increased through a dependent or independent PGC1α mechanism [13,18]. MITF is redox-sensitive, since oxidative injury induced by hydrogen peroxide drives downregulation of MITF and its downstream targets [52]. Additionally, MITF dysfunction may lead to oxidative damage once MITF deficiency in mice leads to higher levels of ROS. Overexpression of MITF upregulates the antioxidant response and mitochondrial biogenesis by regulating PGC-1α [53]. Therefore, MITF is possibly involved in redox signaling by controlling the PGC1α/mitochondria/OXPHOS/antioxidant axis [53]. MITF also controls the cellular response to ROS by regulating APE-1 [22]. Then, $MITF^{high}$ cells were found to be more resistant to hydrogen peroxide-induced cell death [22], which corroborates our data obtained for C2 treated with hydrogen peroxide. It was demonstrated that APE-1/PRDX1 could act as an anti-inflammatory agent avoiding cancer invasion and metastasis [54]. Additionally, the transcription factor NRF2, which is the major mediator of oxidative stress responses and linked to therapy resistance, suppresses MITF activity and reduces the expression of pigmentation markers in melanoma [55].

MITF targets the pigment genes that encode enzymes necessary for melanin synthesis, TYR, TYRP1, and TYRP2, together with Melan-A [56], and PGC-1s are involved in this regulation [13,19]. The role of melanin in melanoma is contradictory, and a clinical study showed that amelanotic melanoma was associated with poorer patient survival than pigmented melanoma [57]; however, pigmentation is also associated with a worse outcome in metastatic melanoma patients and increased resistance to therapy [58]. Some studies demonstrated that melanin is associated with invasion suppression [59,60] and inhibition of metastasis [24,58,61]. Inhibiting melanoma growth and invasion at early stages, when melanogenesis is deregulated at advanced stages, and its intermediates have an immunosuppressive effect [62], can also induce mutations and DNA damage, and can

switch energy yielding metabolism [63]. RAB7 expression is induced in early-stage tumors and downregulated in invading melanomas [64,65]. RAB27 was identified as a melanoma driver; its levels increase during melanoma progression and its depletion decreases tumor progression and metastasis [66], and it is also linked with decreased survival [67]. In contrast to RAB27, RAB7 expression is not regulated by MITF and high RAB7 expression was linked to a decrease in invasiveness and an increase in patient survival [67]. C1 had decreased expression of pigmentation markers and RAB7 and RAB27, suggesting that the presence of melanin could inhibit invasion and resistance.

Interestingly, depletion of PGC-1α caused a significant decrease in antioxidant protein expression [14]. We confirmed that C1 and C3 had lower PRDX1, so it is likely that the imbalance in antioxidant defense could lead to increased ROS production, supporting our observation that C1 and C3 exhibit high levels of ROS. ROS can play a dual role in cancer: a tumorigenic role by promoting proliferation and genomic instability [68], or a toxic role by inducing cellular damage and death [69]. Redox signaling can be regulated by redox-sensitive proteins such as thiol-containing proteins, which guarantee the high specificity of signal transduction, in addition to avoiding the accumulation of ROS [70]. PRDXs are thiol-containing enzymes that play an important role in redox signaling associated with tumorigenesis [70]. PRDXs can directly react with transcription factors and are involved in regulation of apoptosis, differentiation, and proliferation [71]. We have previously demonstrated that PRDX1 was increased in melanoma patients from the vertical growth phase compared to common nevus, and was decreased in metastatic melanoma compared to dysplastic nevus, whereas PRDX2 is decreased in the vertical growth phase and in melanoma metastasis when compared to dysplastic nevus [30]. We observed that PRDX1 and PRDX2 levels were decreased in SK-MEL-28P compared to normal melanocytes and PRDX1 is increased in resistance, while PRDX2 is depleted in acquired resistant SK-MEL-28R. Using data from melanoma patients, we observed that low levels of MITF expression are associated with decreased PRDX1 expression. Our results suggested that PRDX1 and PRDX2 may play distinct roles in melanoma heterogeneity.

It was demonstrated that PRDXs can contribute to resistance to therapy [72,73] and act as an oncogene or tumor suppressor, depending on the tumor [74] and the melanoma stage [30]. In melanoma it is accepted that they are downregulated in metastasis [30,31,75]. PRDXs are known to be involved in receptor tyrosine kinase-mediated signal transduction [76] and modulation of phosphokinase signaling cascades [25,77,78], and may play an important role as tumor suppressor [77,79]. On the other hand, increased expression of PRDX1 by ROS can prevent cell death and PRDX1 is involved in melanoma resistance [80–82]. Downregulation of PRDX2 in melanoma cells is related to more production of ROS, proliferation, and migration [32]. In fact, it was demonstrated that PRDX2 prevents metastasis by increasing E-cadherin/β-catenin complexes [32], and that PRDX2 is downregulated in melanomas and could be restored by a demethylating agent [83]. GT is a mimetic of PRDX by reducing hydrogen peroxide to water and accelerating NADPH oxidation by the thioredoxin system [84]. Its potent activity has already been demonstrated in different types of cancer [85–89]. In melanoma, its IC50 is 100–200 nM [32,86]. Interestingly, we found an inverse correlation among sensitivity to oxidative stress and antioxidant treatment.

Taken together, our results demonstrated that clones in the same tumor can naturally express less PRDX1, MITF, SOX10, PGC-1α, and pigmentation markers. We linked this genetic profile to a phenotype that is more invasive, less pigmented, and intrinsically resistant. These results provide evidence for the influence of redox-sensitive reactions in melanoma heterogeneity and the role of pigmentation in this context, highlighting the importance of antioxidant response in treatment resistance.

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Á.D.D.S., L.H.O., M.P.M., N.C.d.S.P., V.M.F., W.A.S.J., K.S.M.S. and S.S.M.-E.; supervision, S.S.M.-E.; project administration, S.S.M.-E.; funding acquisition, S.S.M.-E. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are contained within the article.

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References

1. Gray-Schopfer, V.; Wellbrock, C.; Marais, R. Melanoma biology and new targeted therapy. *Nature* **2007**, *445*, 851–857. [CrossRef] [PubMed]
2. Helfrich, I.; Scheffrahn, I.; Bartling, S.; Weis, J.; Von Felbert, V.; Middleton, M.; Kato, M.; Ergün, S.; Augustin, H.G.; Schadendorf, D. Resistance to antiangiogenic therapy is directed by vascular phenotype, vessel stabilization, and maturation in malignant melanoma. *J. Exp. Med.* **2010**, *207*, 491–503. [CrossRef] [PubMed]
3. Chapman, A.; del Ama, L.F.; Ferguson, J.; Kamarashev, J.; Wellbrock, C.; Hurlstone, A. Heterogeneous tumor subpopulations cooperate to drive invasion. *Cell Rep.* **2014**, *8*, 688–695. [CrossRef] [PubMed]
4. Flaherty, K.T.; Robert, C.; Hersey, P.; Nathan, P.; Garbe, C.; Milhem, M.; Demidov, L.V.; Hassel, J.C.; Rutkowski, P.; Mohr, P.; et al. Improved survival with MEK inhibition in BRAF-mutated melanoma. *N. Engl. J. Med.* **2012**, *367*, 107–114. [CrossRef] [PubMed]
5. Smalley, K.S.; Sondak, V.K. Melanoma—An unlikely poster child for personalized cancer therapy. *N. Engl. J. Med.* **2010**, *363*, 876–878. [CrossRef] [PubMed]
6. Sosman, J.A.; Kim, K.B.; Schuchter, L.; Gonzalez, R.; Pavlick, A.C.; Weber, J.S.; McArthur, G.A.; Hutson, T.E.; Moschos, S.J.; Flaherty, K.T.; et al. Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. *N. Engl. J. Med.* **2012**, *366*, 707–714. [CrossRef] [PubMed]
7. Hoek, K.S.; Goding, C.R. Cancer stem cells versus phenotype-switching in melanoma. *Pigment Cell Melanoma Res.* **2010**, *23*, 746–759. [CrossRef] [PubMed]
8. Quail, D.F.; Joyce, J.A. Microenvironmental regulation of tumor progression and metastasis. *Nat. Med.* **2013**, *19*, 1423–1437. [CrossRef] [PubMed]
9. Tasdogan, A.; Faubert, B.; Ramesh, V.; Ubellacker, J.M.; Shen, B.; Solmonson, A.; Murphy, M.M.; Gu, Z.; Gu, W.; Martin, M.; et al. Metabolic heterogeneity confers differences in melanoma metastatic potential. *Nature* **2020**, *577*, 115–120. [CrossRef]
10. Falletta, P.; Sanchez-Del-Campo, L.; Chauhan, J.; Effern, M.; Kenyon, A.; Kershaw, C.J.; Siddaway, R.; Lisle, R.; Freter, R.; Daniels, M.J.; et al. Translation reprogramming is an evolutionarily conserved driver of phenotypic plasticity and therapeutic resistance in melanoma. *Genes Dev.* **2017**, *31*, 18–33. [CrossRef]
11. Rambow, F.; Marine, J.C.; Goding, C.R. Melanoma plasticity and phenotypic diversity: Therapeutic barriers and opportunities. *Genes Dev.* **2019**, *33*, 1295–1318. [CrossRef] [PubMed]
12. Müller, J.; Krijgsman, O.; Tsoi, J.; Robert, L.; Hugo, W.; Song, C.; Kong, X.; Possik, P.A.; Cornelissen-Steijger, P.D.; Geukes Foppen, M.H.; et al. Low MITF/AXL ratio predicts early resistance to multiple targeted drugs in melanoma. *Nat. Commun.* **2014**, *5*, 5712. [CrossRef] [PubMed]
13. Haq, R.; Shoag, J.; Andreu-Perez, P.; Yokoyama, S.; Edelman, H.; Rowe, G.C.; Frederick, D.T.; Hurley, A.D.; Nellore, A.; Kung, A.L.; et al. Oncogenic BRAF regulates oxidative metabolism via PGC1α and MITF. *Cancer Cell* **2013**, *23*, 302–315. [CrossRef] [PubMed]
14. Vazquez, F.; Lim, J.H.; Chim, H.; Bhalla, K.; Girnun, G.; Pierce, K.; Clish, C.B.; Granter, S.R.; Widlund, H.R.; Spiegelman, B.M.; et al. PGC1α expression defines a subset of human melanoma tumors with increased mitochondrial capacity and resistance to oxidative stress. *Cancer Cell* **2013**, *23*, 287–301. [CrossRef] [PubMed]
15. Kluza, J.; Corazao-Rozas, P.; Touil, Y.; Jendoubi, M.; Maire, C.; Guerreschi, P.; Jonneaux, A.; Ballot, C.; Balayssac, S.; Valable, S.; et al. Inactivation of the HIF-1α/PDK3 signaling axis drives melanoma toward mitochondrial oxidative metabolism and potentiates the therapeutic activity of pro-oxidants. *Cancer Res.* **2012**, *72*, 5035–5047. [CrossRef] [PubMed]
16. Kozar, I.; Margue, C.; Rothengatter, S.; Haan, C.; Kreis, S. Many ways to resistance: How melanoma cells evade targeted therapies. *Biochim. Biophys. Acta Rev. Cancer* **2019**, *1871*, 313–322. [CrossRef] [PubMed]

17. Smith, L.K.; Parmenter, T.; Kleinschmidt, M.; Kusnadi, E.P.; Kang, J.; Martin, C.A.; Lau, P.; Patel, R.; Lorent, J.; Papadopoli, D.; et al. Adaptive translational reprogramming of metabolism limits the response to targeted therapy in BRAFV600 melanoma. *Nat. Commun.* **2022**, *13*, 1100. [CrossRef]
18. Corazao-Rozas, P.; Guerreschi, P.; Jendoubi, M.; André, F.; Jonneaux, A.; Scalbert, C.; Garçon, G.; Malet-Martino, M.; Balayssac, S.; Rocchi, S.; et al. Mitochondrial oxidative stress is the Achille's heel of melanoma cells resistant to Braf-mutant inhibitor. *Oncotarget* **2013**, *4*, 1986–1998. [CrossRef] [PubMed]
19. Shoag, J.; Haq, R.; Zhang, M.; Liu, L.; Rowe, G.C.; Jiang, A.; Koulisis, N.; Farrel, C.; Amos, C.I.; Wei, Q.; et al. PGC-1 coactivators regulate MITF and the tanning response. *Mol. Cell* **2013**, *49*, 145–157. [CrossRef]
20. Wittgen, H.G.; van Kempen, L.C. Reactive oxygen species in melanoma and its therapeutic implications. *Melanoma Res.* **2007**, *17*, 400–409. [CrossRef]
21. St-Pierre, J.; Drori, S.; Uldry, M.; Silvaggi, J.M.; Rhee, J.; Jäger, S.; Handschin, C.; Zheng, K.; Lin, J.; Yang, W.; et al. Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell* **2006**, *127*, 397–408. [CrossRef] [PubMed]
22. Liu, F.; Fu, Y.; Meyskens, F.L., Jr. MiTF regulates cellular response to reactive oxygen species through transcriptional regulation of APE-1/Ref-1. *J. Investig. Dermatol.* **2009**, *129*, 422–431. [CrossRef]
23. Slominski, R.M.; Zmijewski, M.A.; Slominski, A.T. The role of melanin pigment in melanoma. *Exp. Dermatol.* **2015**, *24*, 258–259. [CrossRef] [PubMed]
24. Kang, S.W.; Lee, S.; Lee, J.H.S. Cancer-Associated Function of 2-Cys Peroxiredoxin Subtypes as a Survival Gatekeeper. *Antioxidants* **2018**, *7*, 161. [CrossRef] [PubMed]
25. Jarvis, R.M.; Hughes, S.M.; Ledgerwood, E.C. Peroxiredoxin 1 functions as a signal peroxidase to receive, transduce, and transmit peroxide signals in mammalian cells. Free Radic. *Biol. Med.* **2012**, *53*, 522–530. [CrossRef] [PubMed]
26. Sobotta, M.C.; Liou, W.; Stöcker, S.; Talwar, D.; Oehler, M.; Ruppert, T.; Scharf, A.N.; Dick, T.P. Peroxiredoxin-2 and STAT3 form a redox relay for H2O2 signaling. *Nat. Chem. Biol.* **2015**, *11*, 64–70. [CrossRef] [PubMed]
27. Winterbourn, C.C.; Hampton, M.B. Redox biology: Signaling via a peroxiredoxin sensor. *Nat. Chem. Biol.* **2015**, *11*, 5–6. [CrossRef] [PubMed]
28. Poynton, R.A.; Hampton, M.B. Peroxiredoxins as biomarkers of oxidative stress. *Biochim. Biophys. Acta* **2014**, *1840*, 906–912. [CrossRef]
29. Rhee, S.G.; Woo, H.A. Multiple functions of 2-Cys peroxiredoxins, I and II, and their regulations via post-translational modifications. Free Radic. *Biol. Med.* **2020**, *152*, 107–115. [CrossRef]
30. Carvalho, L.A.C.; Queijo, R.G.; Baccaro, A.L.B.; Siena, Á.D.D.; Silva, W.A., Jr.; Rodrigues, T.; Maria-Engler, S.S. Redox-Related Proteins in Melanoma Progression. *Antioxidants* **2022**, *11*, 438. [CrossRef]
31. Hintsala, H.R.; Soini, Y.; Haapasaari, K.M.; Karihtala, P. Dysregulation of redox-state-regulating enzymes in melanocytic skin tumours and the surrounding microenvironment. *Histopathology* **2015**, *67*, 348–357. [CrossRef] [PubMed]
32. Lee, D.J.; Kang, D.H.; Choi, M.; Choi, Y.J.; Lee, J.Y.; Park, J.H.; Park, Y.J.; Lee, K.W.; Kang, S.W. Peroxiredoxin-2 Represses Melanoma Metastasis by Increasing E-Cadherin/β-Catenin Complexes in Adherens Junctions. *Cancer Res.* **2013**, *73*, 4744–4757. [CrossRef] [PubMed]
33. Sandri, S.; Faião-Flores, F.; Tiago, M.; Pennacchi, P.C.; Massaro, R.R.; Alves-Fernandes, D.K.; Berardinelli, G.N.; Evangelista, A.F.; de Lima Vazquez, V.; Reis, R.M.; et al. Vemurafenib resistance increases melanoma invasiveness and modulates the tumor microenvironment by MMP-2 upregulation. *Pharmacol. Res.* **2016**, *111*, 523–533. [CrossRef] [PubMed]
34. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **2001**, *25*, 402–408. [CrossRef]
35. Cerami, E.; Gao, J.; Dogrusoz, U.; Gross, B.E.; Sumer, S.O.; Aksoy, B.A.; Jacobsen, A.; Byrne, C.J.; Heuer, M.L.; Larsson, E.; et al. The cBio cancer genomics portal: An open platform for exploring multidimensional cancer genomics data. *Cancer Discov.* **2012**, *2*, 401–404. [CrossRef] [PubMed]
36. Gao, J.; Aksoy, B.A.; Dogrusoz, U.; Dresdner, G.; Gross, B.; Sumer, S.O.; Sun, Y.; Jacobsen, A.; Sinha, R.; Larsson, E.; et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci. Signal.* **2013**, *6*, pl1. [CrossRef]
37. Butler, A.; Hoffman, P.; Smibert, P.; Papalexi, E.; Satija, R. Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nat. Biotechnol.* **2018**, *36*, 411–420. [CrossRef]
38. Aran, D.; Looney, A.P.; Liu, L.; Wu, E.; Fong, V.; Hsu, A.; Chak, S.; Naikawadi, R.P.; Wolters, P.J.; Abate, A.R.; et al. Reference-based analysis of lung single-cell sequencing reveals a transitional profibrotic macrophage. *Nat. Immunol.* **2019**, *20*, 163–172. [CrossRef]
39. Pont, F.; Tosolini, M.; Fournié, J.J. Single-Cell Signature Explorer for comprehensive visualization of single cell signatures across scRNA-seq datasets. *Nucleic Acids Res.* **2019**, *47*, e133. [CrossRef]
40. Li, J.; Smalley, I.; Schell, M.J.; Smalley, K.S.M.; Chen, Y.A. SinCHet: A MATLAB toolbox for single cell heterogeneity analysis in cancer. *Bioinformatics* **2017**, *33*, 2951–2953. [CrossRef]
41. Smalley, I.; Chen, Z.; Phadke, M.; Li, J.; Yu, X.; Wyatt, C.; Evernden, B.; Messina, J.L.; Sarnaik, A.; Sondak, V.K.; et al. Single-Cell Characterization of the Immune Microenvironment of Melanoma Brain and Leptomeningeal Metastases. *Clin. Cancer Res.* **2021**, *27*, 4109–4125. [CrossRef]
42. Tirosh, I.; Izar, B.; Prakadan, S.M.; Wadsworth, M.H.; Treacy, D.; Trombetta, J.J.; Rotem, A.; Rodman, C.; Lian, C.; Murphy, G.; et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. *Science* **2016**, *352*, 189–196. [CrossRef]

43. McGinnis, A.; Klichko, V.I.; Orr, W.C.; Radyuk, S.N. Hyperoxidation of Peroxiredoxins and Effects on Physiology of Drosophila. *Antioxidants* **2021**, *10*, 606. [CrossRef]
44. Jamal-Hanjani, M.; Quezada, S.A.; Larkin, J.; Swanton, C. Translational implications of tumor heterogeneity. *Clin. Cancer Res.* **2015**, *21*, 1258–1266. [CrossRef] [PubMed]
45. Ramón, Y.; Cajal, S.; Sesé, M.; Capdevila, C.; Aasen, T.; De Mattos-Arruda, L.; Diaz-Cano, S.J.; Hernández-Losa, J.; Castellví, J. Clinical implications of intratumor heterogeneity: Challenges and opportunities. *J. Mol. Med.* **2020**, *98*, 161–177. [CrossRef]
46. Assenov, Y.; Brocks, D.; Gerhäuser, C. Intratumor heterogeneity in epigenetic patterns. *Semin. Cancer Biol.* **2018**, *51*, 12–21. [CrossRef] [PubMed]
47. Smalley, I.; Kim, E.; Li, J.; Spence, P.; Wyatt, C.J.; Eroglu, Z.; Sondak, V.K.; Messina, J.L.; Babacan, N.A.; Maria-Engler, S.S.; et al. Leveraging transcriptional dynamics to improve BRAF inhibitor responses in melanoma. *EBioMedicine* **2019**, *48*, 178–190. [CrossRef]
48. Goding, C.R. Melanocyte development and malignant melanoma. *Forum* **2000**, *10*, 176–187. [PubMed]
49. Tudrej, K.B.; Czepielewska, E.; Kozłowska-Wojciechowska, M. SOX10-MITF pathway activity in melanoma cells. *Arch. Med. Sci.* **2017**, *13*, 1493–1503. [CrossRef]
50. Boshuizen, J.; Koopman, L.A.; Krijgsman, O.; Shahrabi, A.; van den Heuvel, E.G.; Ligtenberg, M.A.; Vredevoogd, D.W.; Kemper, K.; Kuilman, T.; Song, J.Y.; et al. Cooperative targeting of melanoma heterogeneity with an AXL antibody-drug conjugate and BRAF/MEK inhibitors. *Nat. Med.* **2018**, *24*, 203–212. [CrossRef]
51. Rambow, F.; Rogiers, A.; Marin-Bejar, O.; Aibar, S.; Femel, J.; Dewaele, M.; Karras, P.; Brown, D.; Chang, Y.H.; Debiec-Rychter, M.; et al. Toward Minimal Residual Disease-Directed Therapy in Melanoma. *Cell* **2018**, *174*, 843–855.e19. [CrossRef]
52. Jiang, W.; Li, S.; Chen, X.; Zhang, W.; Chang, Y.; He, Y.; Zhang, S.; Su, X.; Gao, T.; Li, C.; et al. Berberine protects immortalized line of human melanocytes from H2O2-induced oxidative stress via activation of Nrf2 and Mitf signaling pathway. *J. Dermatol. Sci.* **2019**, *94*, 236–243. [CrossRef]
53. Hua, J.; Chen, H.; Chen, Y.; Zheng, G.; Li, F.; Qu, J.; Ma, X.; Hou, L. MITF acts as an anti-oxidant transcription factor to regulate mitochondrial biogenesis and redox signaling in retinal pigment epithelial cells. *Exp. Eye Res.* **2018**, *170*, 138–147. [CrossRef]
54. Nassour, H.; Wang, Z.; Saad, A.; Papaluca, A.; Brosseau, N.; Affar, E.B.; Alaoui-Jamali, M.A.; Ramotar, D. Peroxiredoxin 1 interacts with and blocks the redox factor APE1 from activating interleukin-8 expression. *Sci. Rep.* **2016**, *6*, 29389. [CrossRef]
55. Jessen, C.; Kreß, J.K.C.; Baluapuri, A.; Hufnagel, A.; Schmitz, W.; Kneitz, S.; Roth, S.; Marquardt, A.; Appenzeller, S.; Ade, C.P.; et al. The transcription factor NRF2 enhances melanoma malignancy by blocking differentiation and inducing COX2 expression. *Oncogene* **2020**, *39*, 6841–6855. [CrossRef]
56. Hsiao, J.J.; Fisher, D.E. The roles of microphthalmia-associated transcription factor and pigmentation in melanoma. *Arch. Biochem. Biophys.* **2014**, *563*, 28–34. [CrossRef]
57. Thomas, N.E.; Kricker, A.; Waxweiler, W.T.; Dillon, P.M.; Busman, K.J.; From, L.; Groben, P.A.; Armstrong, B.K.; Anton-Culver, H.; Gruber, S.B.; et al. Comparison of clinicopathologic features and survival of histopathologically amelanotic and pigmented melanomas: A population-based study. *JAMA Dermatol.* **2014**, *150*, 1306–1314. [CrossRef]
58. Brożyna, A.A.; Poliseno, L.; Slominski, A.T. Editorial: Role of pigmentation in melanoma. *Front. Oncol.* **2022**, *12*, 1084717. [CrossRef] [PubMed]
59. Carreira, S.; Goodall, J.; Denat, L.; Rodriguez, M.; Nuciforo, P.; Hoek, K.S.; Testori, A.; Larue, L.; Goding, C.R. Mitf regulation of Dia1 controls melanoma proliferation and invasiveness. *Genes Dev.* **2006**, *20*, 3426–3439. [CrossRef] [PubMed]
60. Hoek, K.S.; Schlegel, N.C.; Brafford, P.; Sucker, A.; Ugurel, S.; Kumar, R.; Weber, B.L.; Nathanson, K.L.; Phillips, D.J.; Herlyn, M.; et al. Metastatic potential of melanomas defined by specific gene expression profiles with no BRAF signature. *Pigment Cell Res.* **2006**, *19*, 290–302. [CrossRef] [PubMed]
61. Sarna, M.; Zadlo, A.; Hermanowicz, P.; Madeja, Z.; Burda, K.; Sarna, T. Cell elasticity is an important indicator of the metastatic phenotype of melanoma cells. *Exp. Dermatol.* **2014**, *23*, 813–818. [CrossRef] [PubMed]
62. Slominski, A.; Zbytek, B.; Slominski, R. Inhibitors of melanogenesis increase toxicity of cyclophosphamide and lymphocytes against melanoma cells. *Int. J. Cancer* **2009**, *124*, 1470–1477. [CrossRef] [PubMed]
63. Slominski, A.; Kim, T.-K.; Brożyna, A.A.; Janjetovic, Z.; Brooks, D.L.P.; Schwab, L.P.; Skobowiat, C.; Jóźwicki, W.; Seagroves, T.N. The role of melanogenesis in regulation of melanoma behavior: Melanogenesis leads to stimulation of HIF-1α expression and HIF-dependent attendant pathways. *Arch. Biochem. Biophys.* **2014**, *563*, 79–93. [CrossRef] [PubMed]
64. Alonso-Curbelo, D.; Riveiro-Falkenbach, E.; Pérez-Guijarro, E.; Cifdaloz, M.; Karras, P.; Osterloh, L.; Megías, D.; Cañón, E.; Calvo, T.G.; Olmeda, D.; et al. RAB7 controls melanoma progression by exploiting a lineage-specific wiring of the endolysosomal pathway. *Cancer Cell* **2014**, *26*, 61–76. [CrossRef] [PubMed]
65. Williams, K.C.; Coppolino, M.G. Phosphorylation of membrane type 1-matrix metalloproteinase (MT1-MMP) and its vesicle-associated membrane protein 7 (VAMP7)-dependent trafficking facilitate cell invasion and migration. *J. Biol. Chem.* **2011**, *286*, 43405–43416. [CrossRef] [PubMed]
66. Peinado, H.; Alečković, M.; Lavotshkin, S.; Matei, I.; Costa-Silva, B.; Moreno-Bueno, G.; Hergueta-Redondo, M.; Williams, C.; García-Santos, G.; Ghajar, C.; et al. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat. Med.* **2012**, *18*, 883–891. [CrossRef] [PubMed]

67. Guo, D.; Lui, G.Y.L.; Lai, S.L.; Wilmott, J.S.; Tikoo, S.; Jackett, L.A.; Quek, C.; Brown, D.L.; Sharp, D.M.; Kwan, R.Y.Q.; et al. RAB27A promotes melanoma cell invasion and metastasis via regulation of pro-invasive exosomes. *Int. J. Cancer* **2019**, *144*, 3070–3085. [CrossRef] [PubMed]
68. Weinberg, F.; Chandel, N.S. Mitochondrial metabolism and cancer. *Ann. N. Y. Acad. Sci.* **2009**, *1177*, 66–73. [CrossRef] [PubMed]
69. Trachootham, D.; Alexandre, J.; Huang, P. Targeting cancer cells by ROS-mediated mechanisms: A radical therapeutic approach? *Nat. Rev. Drug Discov.* **2009**, *8*, 579–591. [CrossRef]
70. Netto, L.E.; Antunes, F. The Roles of Peroxiredoxin and Thioredoxin in Hydrogen Peroxide Sensing and in Signal Transduction. *Mol. Cells* **2016**, *39*, 65–71. [CrossRef]
71. Benfeitas, R.; Uhlen, M.; Nielsen, J.; Mardinoglu, A. New Challenges to Study Heterogeneity in Cancer Redox Metabolism. *Front. Cell Dev. Biol.* **2017**, *5*, 65. [CrossRef] [PubMed]
72. Chang, X.Z.; Li, D.Q.; Hou, Y.F.; Wu, J.; Lu, J.S.; Di, G.H.; Jin, W.; Ou, Z.L.; Shen, Z.Z.; Shao, Z.M. Identification of the functional role of peroxiredoxin 6 in the progression of breast cancer. *Breast Cancer Res.* **2007**, *9*, R76. [CrossRef] [PubMed]
73. Lu, W.; Fu, Z.; Wang, H.; Feng, J.; Wei, J.; Guo, J. Peroxiredoxin 2 is upregulated in colorectal cancer and contributes to colorectal cancer cells' survival by protecting cells from oxidative stress. *Mol. Cell. Biochem.* **2014**, *387*, 261–270. [CrossRef] [PubMed]
74. Egler, R.A.; Fernandes, E.; Rothermund, K.; Sereika, S.; de Souza-Pinto, N.; Jaruga, P.; Dizdaroglu, M.; Prochownik, E.V. Regulation of reactive oxygen species, DNA damage, and c-Myc function by peroxiredoxin 1. *Oncogene* **2005**, *24*, 8038–8050. [CrossRef] [PubMed]
75. Ding, C.; Fan, X.; Wu, G. Peroxiredoxin 1—An antioxidant enzyme in cancer. *J. Cell. Mol. Med.* **2017**, *21*, 193–202. [CrossRef] [PubMed]
76. Kang, S.W.; Rhee, S.G.; Chang, T.S.; Jeong, W.; Choi, M.H. 2-Cys peroxiredoxin function in intracellular signal transduction: Therapeutic implications. *Trends Mol. Med.* **2005**, *11*, 571–578. [CrossRef] [PubMed]
77. Neumann, C.A.; Fang, Q. Are peroxiredoxins tumor suppressors? *Curr. Opin. Pharmacol.* **2007**, *7*, 375–380. [CrossRef]
78. Wei, Q.; Jiang, H.; Xiao, Z.; Baker, A.; Young, M.R.; Veenstra, T.D.; Colburn, N.H. Sulfiredoxin-Peroxiredoxin IV axis promotes human lung cancer progression through modulation of specific phosphokinase signaling. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 7004–7009. [CrossRef]
79. Ito, T.; Kimura, S.; Seto, K.; Warabi, E.; Kawachi, Y.; Shoda, J.; Tabuchi, K.; Yamagata, K.; Hasegawa, S.; Bukawa, H.; et al. Peroxiredoxin I plays a protective role against UVA irradiation through reduction of oxidative stress. *J. Dermatol. Sci.* **2014**, *74*, 9–17. [CrossRef]
80. Nawarak, J.; Huang-Liu, R.; Kao, S.H.; Liao, H.H.; Sinchaikul, S.; Chen, S.T.; Cheng, S.L. Proteomics analysis of A375 human malignant melanoma cells in response to arbutin treatment. *Biochim. Biophys. Acta* **2009**, *1794*, 159–167. [CrossRef]
81. Sinha, P.; Poland, J.; Kohl, S.; Schnölzer, M.; Helmbach, H.; Hütter, G.; Lage, H.; Schadendorf, D. Study of the development of chemoresistance in melanoma cell lines using proteome analysis. *Electrophoresis* **2003**, *24*, 2386–2404. [CrossRef] [PubMed]
82. Stone, W.L.; Krishnan, K.; Palau, V.E.; Lightner, J.W.; Brannon, M.F. AKT1 Activation Up Regulates Peroxiredoxin 1 in Human Melanoma Cells. *Free. Radic. Biol. Med.* **2016**, *100*, S130. [CrossRef]
83. Furuta, J.; Nobeyama, Y.; Umebayashi, Y.; Otsuka, F.; Kikuchi, K.; Ushijima, T. Silencing of Peroxiredoxin 2 and aberrant methylation of 33 CpG islands in putative promoter regions in human malignant melanomas. *Cancer Res.* **2006**, *66*, 6080–6086. [CrossRef] [PubMed]
84. Choi, H.S.; Shim, J.S.; Kim, J.A.; Kang, S.W.; Kwon, H.J. Discovery of gliotoxin as a new small molecule targeting thioredoxin redox system. *Biochem. Biophys. Res. Commun.* **2007**, *359*, 523–528. [CrossRef]
85. Chen, J.; Wang, C.; Lan, W.; Huang, C.; Lin, M.; Wang, Z.; Liang, W.; Iwamoto, A.; Yang, X.; Liu, H. Gliotoxin Inhibits Proliferation and Induces Apoptosis in Colorectal Cancer Cells. *Mar. Drugs* **2015**, *13*, 6259–6273. [CrossRef]
86. Hubmann, R.; Sieghart, W.; Schnabl, S.; Araghi, M.; Hilgarth, M.; Reiter, M.; Demirtas, D.; Valent, P.; Zielinski, C.; Jäger, U.; et al. Gliotoxin Targets Nuclear NOTCH2 in Human Solid Tumor Derived Cell Lines In Vitro and Inhibits Melanoma Growth in Xenograft Mouse Model. *Front. Pharmacol.* **2017**, *8*, 319. [CrossRef]
87. Hung, M.L.V.; Song, Y.W.; Cho, S.K. Effects of the Combination of Gliotoxin and Adriamycin on the Adriamycin-Resistant Non-Small-Cell Lung Cancer A549 Cell Line. *Mar. Drugs* **2018**, *16*, 105. [CrossRef] [PubMed]
88. Kweon, Y.O.; Paik, Y.H.; Schnabl, B.; Qian, T.; Lemasters, J.J.; Brenner, D.A. Gliotoxin-mediated apoptosis of activated human hepatic stellate cells. *J. Hepatol.* **2003**, *39*, 38–46. [CrossRef]
89. Vigushin, D.M.; Mirsaidi, N.; Brooke, G.; Sun, C.; Pace, P.; Inman, L.; Moody, C.J.; Coombes, R.C. Gliotoxin is a dual inhibitor of farnesyltransferase and geranylgeranyltransferase I with antitumor activity against breast cancer in vivo. *Med. Oncol.* **2004**, *21*, 21–30. [CrossRef]

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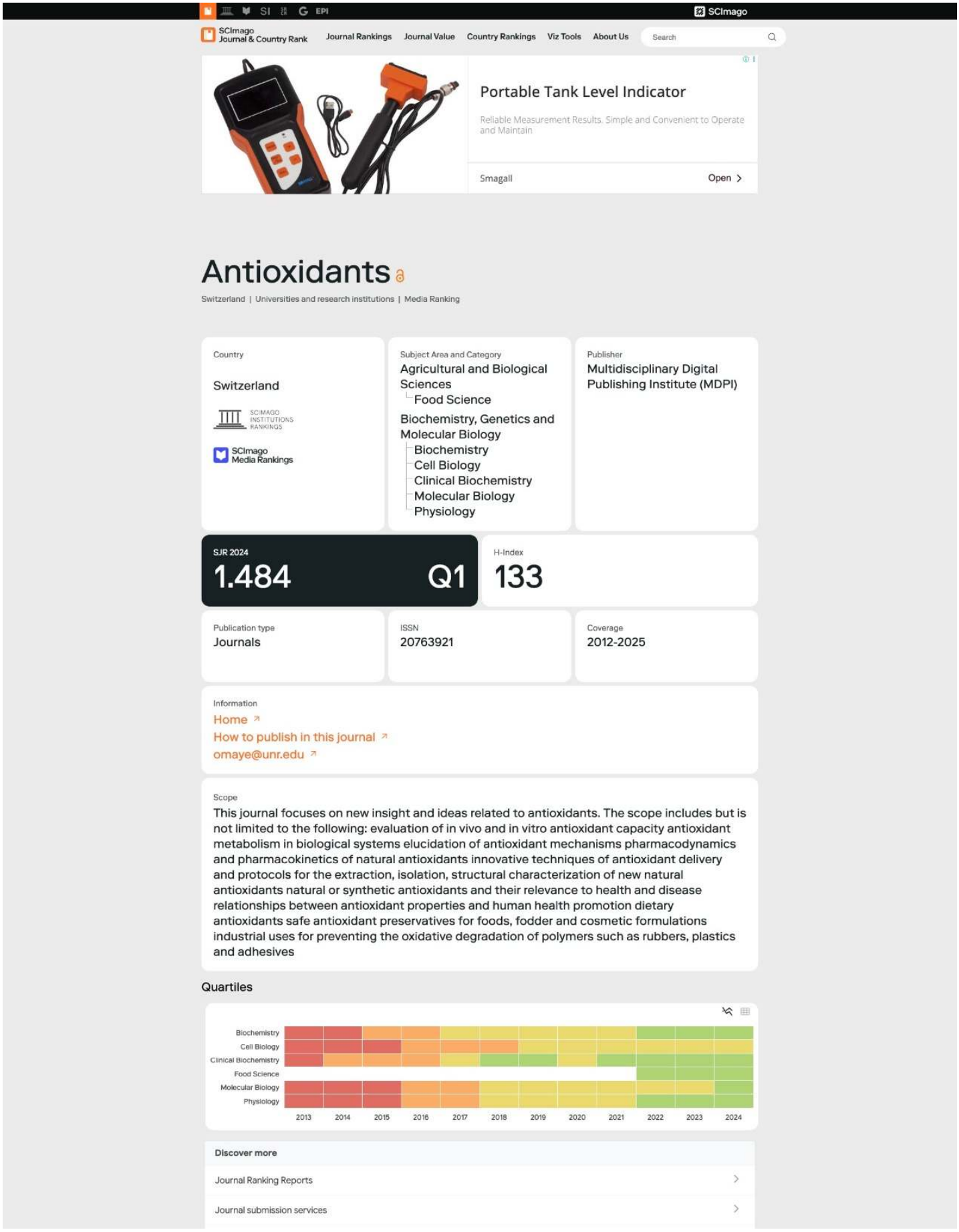
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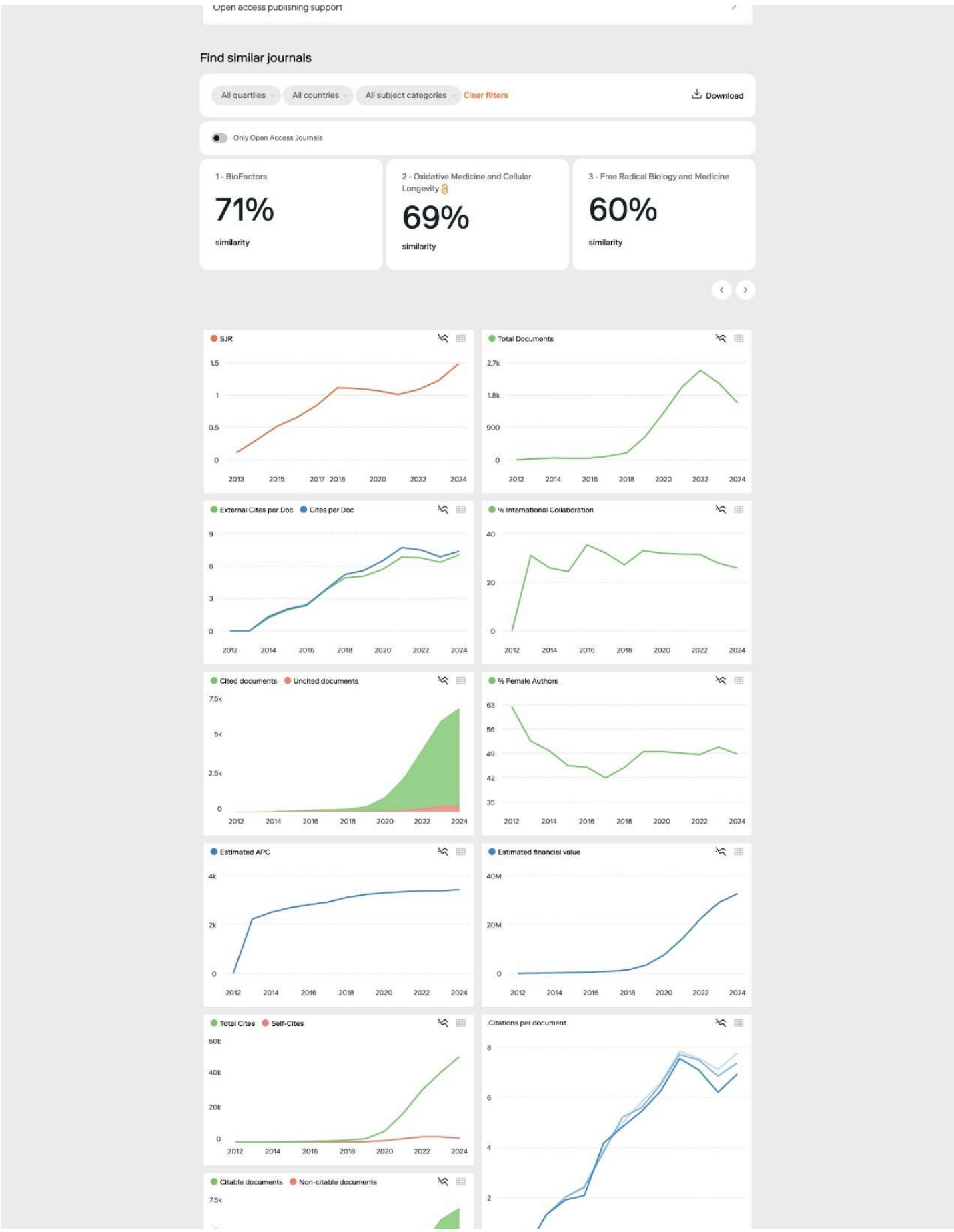
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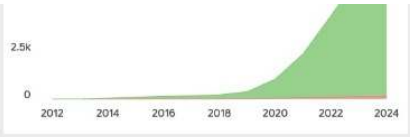
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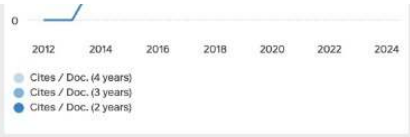
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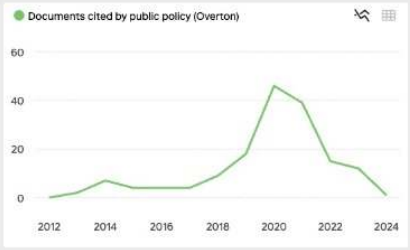
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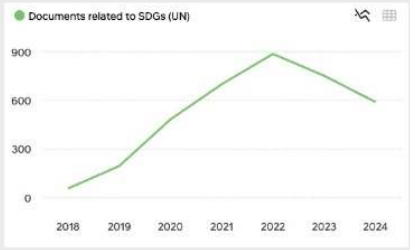
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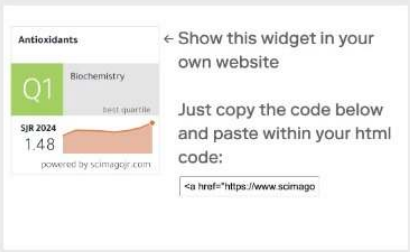
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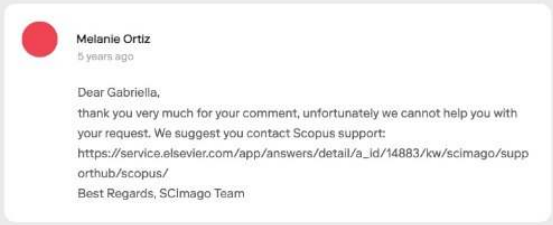
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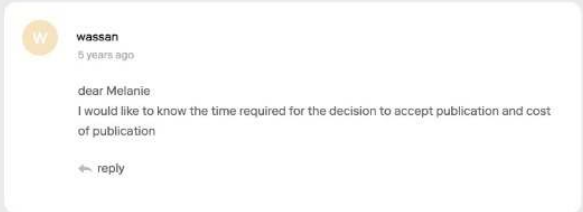
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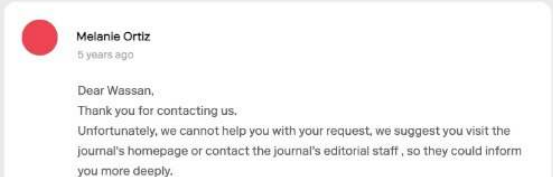
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S **Sam Danial**
5 years ago

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Regads
Sam

reply

Melanie Ortiz
5 years ago

Dear Sam,
Thank you for contacting us.
As you probably already know, our data come from Scopus, they annually send us an update of the data. This update is sent to us around April / May every year.
The calculation of the indicators is performed with the copy of the Scopus database provided to us annually. However, the methodology used (concerning the distribution by Quartiles) by Scopus is different from the one used by SCImago, even if, since the past year, the scientometrics indicators' calculation procedure changed in order to resemble SciVal.
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Best Regards,
SCImago Team

S **Sameh S. Zaghlool**
6 years ago

What is the antioxidants journal quartiles (Q) in the field of pharmacology and toxicology at 2019?

reply

Melanie Ortiz
6 years ago

Dear Sameh, thank you very much for your comment. SJR has been updated on June 1, 2019. Each year Scopus provides us an update database and, according to that new information, indicators are calculated. The publication of articles referring to 2019 is not over yet (we are in actual december), so the 2019 indicators will not be available until June 2020. Best Regards, SCImago Team

I **Ibrahim Salim**
7 years ago

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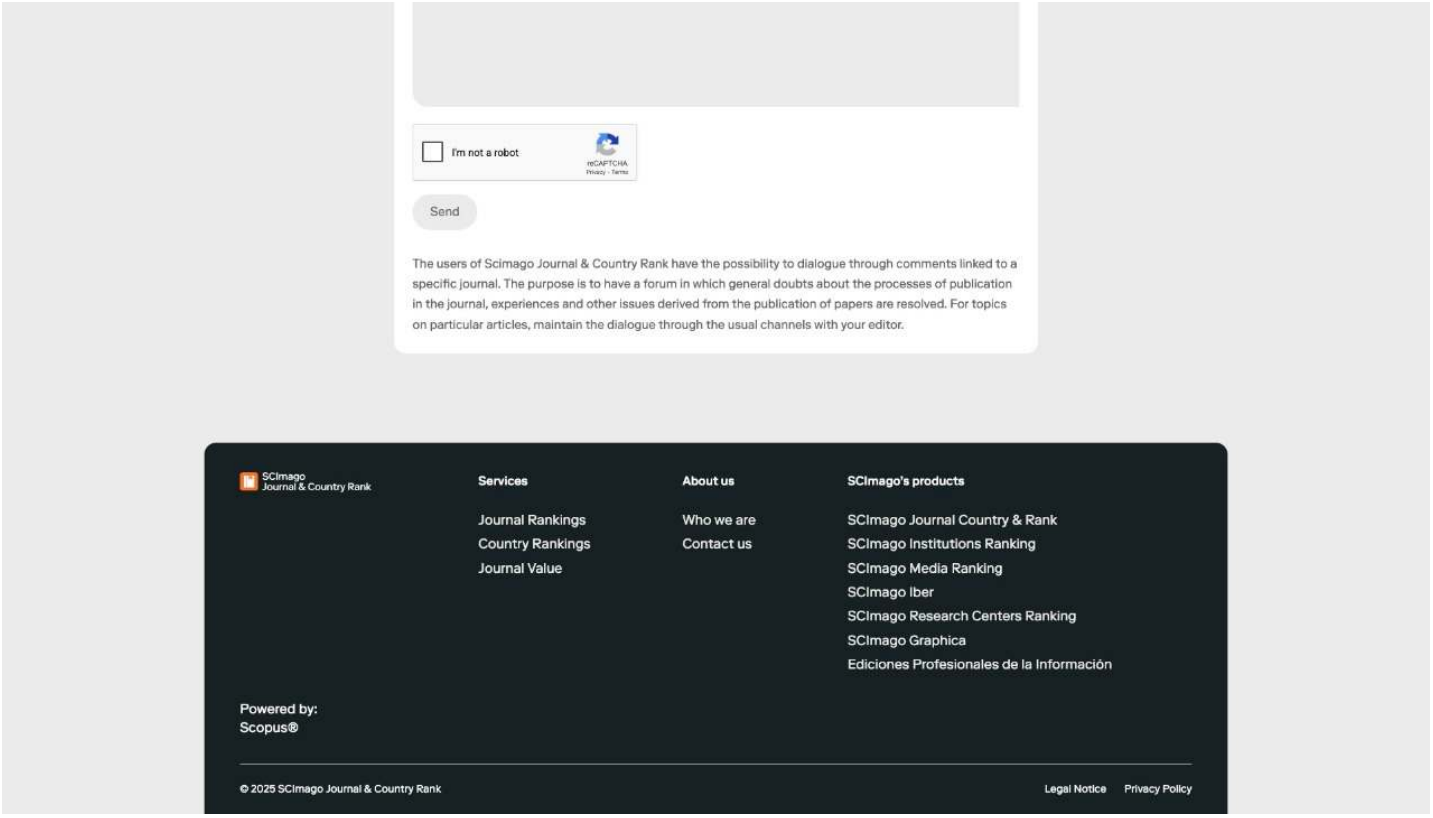
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14-weeks combined exercise epigenetically modulated 118 genes of menopausal women with prediabetes

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Background: Pre-diabetes precedes Diabetes Mellitus (DM) disease and is a critical period for hyperglycemia treatment, especially for menopausal women, considering all metabolic alterations due to hormonal changes. Recently, the literature has demonstrated the role of physical exercise in epigenetic reprogramming to modulate the gene expression patterns of metabolic conditions, such as hyperglycemia, and prevent DM development. In the present study, we hypothesized that physical exercise training could modify the epigenetic patterns of women with poor glycemic control.

Methods: 48 post-menopause women aged 60.3 ± 4.5 years were divided according to their fasting blood glucose levels into two groups: Prediabetes Group, PG (n=24), and Normal Glucose Group, NGG (n=24). All participants performed 14 weeks of physical exercise three times a week. The Infinium

Methylation EPIC BeadChip measured the participants' Different Methylated Regions (DMRs).

Results: Before the intervention, the PG group had 12 DMRs compared to NGG. After the intervention, five DMRs remained different. Interestingly, when comparing the PG group before and after training, 118 DMRs were found. The enrichment analysis revealed that the genes were related to different biological functions such as energy metabolism, cell differentiation, and tumor suppression.

Conclusion: Physical exercise is a relevant alternative in treating hyperglycemia and preventing DM in post-menopause women with poor glycemic control.

KEYWORDS

physical exercise, DNA methylation, combined physical exercise, epigenomics, older women

Introduction

Diabetes Mellitus (DM) is characterized by chronic hyperglycemia resulting from deficiencies in insulin secretion, insulin action, or both. Type 2 Diabetes (T2D) results from a progressive loss of pancreatic islet function followed by long-term insulin resistance and elevated insulin secretion (1) Prediabetes is an intermediate condition that precedes TD2. These conditions could occur during menopause and are related to sex hormone levels and aging changes. Postmenopausal women tend to have fluctuations in the hormone's estrogen and progesterone, these changes affect how cells respond to insulin. The process of hormonal changes after menopause causes fluctuations in blood sugar levels in women (2). During menopause, women could also experience predictable symptoms, such as increased total cholesterol and triglycerides (TG), decreased high-density lipoprotein cholesterol (HDL-c), increased android fat distribution, and impaired vascular function (2). Preventive action to manage hyperglycemia should be appropriately implemented in the prediabetes stage since glycemic control is the common factor determining complications from DM, especially in menopause (2, 3).

In this context, studies have emphasized the importance of epigenetic regulation considering its role in activating or deactivating genes that impact metabolic outcomes (4). Studies using animal models demonstrated epigenetic variations associated with the susceptibility to the development of metabolic diseases, such as DM (5).

Another factor that could influence hyperglycemia and metabolic alterations is exposure to heavy metals in the environment. Some metals, such as lead (Pb^{208}), can increase oxidative stress-inducing metabolic changes related to DM (6). Correlations between heavy metals and glycosylated hemoglobin (HbA1c), an indicator of hyperglycemia and long-term alterations in glucose metabolism, have been found in cross-sectional studies in France and Saudi Arabia (7).

Exercise training can promote health and improve metabolism through different mechanisms. The impact of exercise training on epigenetic programming has been generating exciting results (8). Yahaya (9) demonstrated that myocyte enhancer factor 2 (*MEF2)* migrates to the nucleus during exercise after being phosphorylated, interacting with peroxisome proliferative active receptor gamma coactivator 1A *(PPARγC1A)*, and histone acetyltransferases (HATs).

Physical exercise can increase the production of reactive oxygen species, both in skeletal muscle and in immune cells, leading to the development of oxidative stress, lipid, protein, and DNA modifications (10). Therefore, physical exercise can cause changes in DNA methylation (9, 11). Studies show that the response to physical exercise depends on genetic code and on the epigenetic signature, which can cause hypo or hypermethylation, of genes involved in muscle metabolism (9, 12). One study showed that chronic physical exercise caused hypomethylation of the promoter region of the nuclear receptor factor, fatty acid transporter (SLC27A4) and GLUT4 in patients with obesity and diabetes (13). Many studies involving physical exercise and DNA methylation, as Sailani et al. (14), showed that DNA methylation was lower in 714 promoter regions in physically active men compared to inactive men. Furthermore, it suggests that physical exercise is associated with the DNA methylation pattern increasing insulin sensitivity (14).

These modifications resulted in the acetylation of type 4 glucose transporter (*GLUT4)*, improving the expression of the gene and insulin tolerance in the muscles (15). Exercise training can also ameliorate the effects of heavy metal accumulation by increasing

urinary elimination (16). Due to all these benefits, exercise training could prevent hyperglycemia and its complications. In the present study, we hypothesized that physical exercise training could modify the epigenetic patterns of women with poor glycemic control. Thus, we aimed to analyze if the 14-week combined physical exercise intervention could modify epigenetic variables and improve the glucose serum levels.

Materials and methods

Ethical aspects, study design, and participants

The present study was approved by the Ethics Committee for Research with Humans of the School of Physical Education and Sport of Ribeirão Preto, University of São Paulo (EEFERP-USP). The registration number is CAAE: 79582817.0.0000.5656. The study was also registered in the Brazilian Registry of Clinical Trials under RBR-3g38dx. Participants were recruited in the Physical Education Program for the Elderly at EEFERP – USP. The inclusion criteria were women between 50 and 70 years with a medical clearance certificate to perform physical exercise and who were sedentary at the baseline. The exclusion criteria included diagnostic limitations to physical training or physical test, current smokers, caffeine consumption in the last 24 hours before any blood draw, and more than 25% combined training absence.

Forty-eight post-menopause women participated in the study. They were divided into two groups according to the fasting blood glucose levels determined by the American Diabetes Society (2020): Pre-diabetes Group (PG), composed of 24 women with a fasting blood glucose level higher than 100mg/dL and the Normal Glucose Group (NGG), composed of 24 women with a fasting blood glucose level lower than 100mg/dL (9).

Assessments

The anthropometric evaluation was assessed before and after 14 weeks of combined training. The height was measured using a vertical stadiometer - Balmak - EST-223 and weight using the G-Tech digital scale - Balgl200, G-Tech. Subsequently, the body mass index was calculated using the formula (weight/(height*height)) (17). Waist and hip circumferences were measured following the recommendations of Malachias et al. (17). Participants' systolic and diastolic blood pressure was measured using an Automatic Digital Upper Arm Blood Pressure Monitor Meter (SBH, 2010 - OMRON®, model HEM-7113) (17).

The biochemical analyses included uric acid, total cholesterol, high-density lipoproteins (HDL-C), low-density lipoproteins cholesterol (LDL-C), triglycerides (TG), blood glucose, and insulin. We also calculated the HORMAR IR (Blood (mmol) x Insulin (Ul/mL) ÷ 22. 5).

To investigate the physical benefits of 14 weeks of combined training, the elbow flexion and extension, sitting and standing up from a chair, agility dynamics, and the six-minute walk test was adopted. Also, the 6MWT (m) methodology was used to measure aerobic capacity (18).

We also determined the total serum concentration of metals (Hg202, Al27, As75, Cd111, Pb208, Ni60, Li7) through inductively coupled plasma mass spectrometry (ICP-MS), following the protocol described by Batista et al. (19).

Combined training

Combined training was performed for 14 weeks, three times a week, lasting 60 minutes. The training was divided into strength exercises (30 minutes) and aerobic training (treadmill or bicycle, with a total duration of 30 minutes) (14).

The training periodization followed the first two weeks. The participants performed two sets of 15 to 17 maximum repetitions and 50% of the aerobic training in the reserve heart rate. The participants started to do 10 to 12 maximum repetitions in the remaining weeks, and aerobic training became 70% of the reserve heart rate. The participants-maintained values between three and six, a score presented on Borg's subjective effort scale. The score we adopted on a scale from 0 to 10 represents moderate intensity. For the selection of exercises for strength training, the need to train the main muscles was considered, totaling five exercises for the muscles of the upper limbs and three exercises for the muscles of the lower limbs. Our group previously detailed the protocol (14).

Large-scale DNA methylation array (Infinium-EPIC bead chips)

The DNA methylation was analyzed at the baseline and after the 14 weeks of training. Salting-out technique (20) was used to extract DNA from leukocytes. Biodrop spectrophotometry was used to assess DNA quality and concentration using. 1% agarose gel test was used to guarantee genomic DNA integrity. The run time was 90 minutes at 80W (21).

Following the manufacturer's recommendations, the bisulfite method was applied using the DNA EZ methylation kit (Zymo Research, CA, USA). Illumina BeadChip EPIC Infinium Methylation BeadChip 850k was used for the methylation experiment. All recommendations for the Infinium® HD Assay Methylation Protocol Guide were followed (22).

Bioinformatics analysis

The raw data files were exported from iScan. A Samplesheet file was created to associate the collected information with

raw data (23). The RStudio software (version 4.0.2) was used to process the methylation data. The data was organized as the participants' codes, age, anthropometric, physical, biochemical, and metal information. The Sentrix Position column informs which row and column each sample was located in. The Sentrix_ID column provides the corresponding code of the BeadChip (23). The Bioconductor ChAMP data package was used for data normalization through the MyNorm function (24).

Differentially methylated regions (DMRs) were analyzed using the champ.DMR() function to compare PG vs. NGG groups. The bumphunter method was used to aggregate all probes into small groups. The random permutation method was applied to estimate DMRs with $p < 0.05$. Based on text mining and literature review, all DMRs were functionally annotated (24).

After identifying the DMRs inter and intra-group, the singular value decomposition (SVD) analysis method was used to evaluate the number and nature of significant components, correlating with various components of interest (24). Methylation data were normalized *via* minfi's ssNoob (25). DNAmAge was calculated using the Methylclock package through the DNAmAge function (26). We also investigated the epigenetic clock of participants from both groups for intrinsic epigenetic age acceleration (IEAA) and extrinsic epigenetic age acceleration (EEAA). The epigenetic clock is a biomarker for age-related epigenetic changes as well as disease-specific changes (27). This clock has been recently developed by using specific DNA methylation sites to describe biological age at the level of the cell, tissue, and organ (28). The multi-tissue algorithm by Horvath generates age prediction, which highly correlates with chronological age above r=0.9 (29). In this study, DNAm Age was calculated using Horvath's epigenetic clock algorithm using 353 CpGs (3).

In SVD analysis, the champ algorithm first does SVD deconvolution on the beta dataset. Then, the Random Theory Matrix method in the "isva" package is used to calculate the numbers of the latent variable and the top components of the SVD result. This analysis is comparable to a Principal Components Analysis (24)

Statistical analysis

Two researchers verified the resulting data to maintain the values' reliability and accuracy. The Statistica 7.0 software (StatSoft Statistica®) was used to perform statistical comparisons. The Shapiro-Wilk test tested the normality. The Levene test assessed the equality of variance between groups. Then, repeated-measures ANOVA was performed, considering $p < 0.05$. Fisher's *post hoc* compared all means and controlled the error rate at the significance level. Then, a Spearman correlation was performed for the non-parametric data and a Pearson correlation for the parametric data to identify possible correlations. The Student's t-test compared means in the descriptive analysis.

Results

Effects of combined training in blood pressure, biochemical variables, and improvement of physical skills

The mean age of participants in the PG was 61.3 ± 5.2 years, while in the NGG was 59.9 ± 4.8 years (Table 1). The 14 weeks of combined training had an effect of time in systolic blood pressure ($F=24.304$; $p<0.001$), diastolic blood pressure ($F=6.180$; $p=0.016$), total cholesterol ($F=7.191$); $p=0.010$), HDL-C ($F=8.168$; $p=0.006$), TG ($F=11.176$; $p=0.001$), and glucose ($F=25.281$; $p<0.001$). No undesirable effects were noted, but we did not observe group-time effect or group-time interaction (Table 1).

We observed the effect of time on elbow flexion and extension ($F=17.028$; $p<0.001$) for upper limb strength; sitting and standing up from a chair ($F=20.264$; $p<0.001$) for lower limb; agility test ($F=51.248$; $p<0.001$); and six-minute walk test ($F=23.872$; $p<0.001$) for aerobic fitness. The effect of time in both groups demonstrated an improvement in physical skills after 14 weeks of combined training. No group-time effect or group-time interaction was observed (Table 2).

Combined training decreases metals in both groups

Analyzing metal levels and combined training together, we observed the effect of time decreasing mercury (Hg) ($F=5.028$; $p=0.030$) and lead (Pb) ($F=12.100$; $p=0.001$) in both groups. No effect or group-time interaction was observed (Table 3). There was a positive correlation between the arterial blood pressure and Pb^{208} in the PG_{pre} (PAS: $r=0.389$, $p=0.003$; PAD: $r=0.337$, $p=0.010$). After the intervention, the correlation was not significant. The epigenetic clock was not different between groups and did not change after the 14-week physical intervention (Figure 1).

Differentially Methylated Regions after the 14 weeks of exercise compared with groups

Before the physical exercise training, we found 12 DMRs between the PG_{pre} and the NGG_{pre} (Table 4). After the 14 weeks of exercise, five regions remained Differentially Methylated between the two groups (Table 5).

After the 14-week of exercise, we found 118 DMRs in the PG_{post} compared to the PG_{pre} (Table 6), and we performed a

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TABLE 1 Mean ± standard deviation of anthropometric, blood pressure, and biochemical variables pre and post-14 weeks of combined training (PG: n = 24; NGG: n = 24).

Variables	Group	PRE	POST	p (group)	p (time)	p (group x time)
Age (years)	PG	61.3±5.2		0.184		
	NGG	59.9±4.8				
Anthropometrics						
Stature (m)	PG	1.6±0.1		0.199		
	NGG	1.6±0.1				
BM (kg)	PG	74.2±12.3	74.2±11.6	0.229	0.858	0.848
	NGG	70.3±8.2	70.2±8.0			
BMI (kg/m2)	PG	30.7±4.2	30.1±4.2	0.223	0.188	0.892
	NGG	28.2±3.2	27.8±2.8			
WC (cm)	PG	95.2±11.1	98.3±12.6	0.278	0.728	0.081
	NGG	94.1±10.3	92.2±9.4			
HC (cm)	PG	108.5±8.8	106.2±10.0	0.166	0.386	0.139
	NGG	103.6±7.3	104.2±6.2			
Blood pressure						
SBP (mmHg)	PG	140.9±20.1	126.7±15.1	<0.001#	<0.001*	0.192
	NGG	123.7±9.6	115.5±9.0			
DBP (mmHg)	PG	82.0±12.3	77.7±8.5	0.003#	0.016*	0.48
	NGG	74.0±6.7	71.6±5.4			
Biochemistries						
Uric acid (mg/dL)	PG	4.3±1.2	4.5±1.3	0.234	0.187	0.928
	NGG	4.0±1,1	4.1±1.0			
Cholesterol (mg/dL)	PG	220.2±37.0	207.2±38.1	0.614	0.010*	0.909
	NGG	212.8±33.1	201.9±29.1			
Glucose (mg/dL)	PG	115.1±24.0	104.9±12.0	<0.001#	0.23	0.05
	NGG	92.7±4.6	95.4±7.4			
HDL (mg/dL)	PG	50.7±9.4	48.9±8.9	0.026#	0.006*	0.114
	NGG	59.9±14.1	53.3±11.7			
LDL (mg/dL)	PG	135.6±37.1	131.1±37.3	0.299	0.198	0.848
	NGG	127.4±30.6	119.9±40.8			
Triglycerides (mg/dL)	PG	152.9±49.1	131.6±36.0	0.073	0.001*	0.654
	NGG	128.3±50.5	112.1±38.0			
Insulin UI/ml	PG	14.1±11.9	14.9±10.0	0.052	0.108	0.694
	NGG	8.8±4.9	10.5±5.1			
HOMAR IR	PG	71.5±63.8	71.7±54.3	0.018#	0.316	0.338
	NGG	36.4±19.6	44.7±22.7			

BMI, body mass index; WC, waist circumference; HC, hip circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoproteins LDL, low-density lipoproteins; CM, centimeters; mmHg, millimeters of mercury; kg, kilogram; m, meters; PG, prediabetes group; NGG, normal glucose group. *statistical difference time; #statistical difference group; $p < 0.05$ for pre versus post (repeated measures two-way ANOVA was used).

Singular Value Decomposition Analysis (SVD) to verify if the covariables of interest could explain the DNA methylation patterns (Figure 2).

We also performed a Linear Regression of significant traits (Pb and glucose) and the DNA methylation targets of the PG group. We found three DMRs for Pb and 25 DMRs for glucose (Figure 3). *BRCA1* was hypomethylated after a 14-week physical exercise intervention and was associated with glucose in the PG group. There was also an interesting overlap between the DMRs related to Pb and glucose enriched in the gene *SLFN12*. Notably, this gene region was differently methylated in the PG_{pre} group than NGG_{pre} and associated with Pb in the PG. After the intervention, a decrease in Pb was observed, and the methylation patterns of *SLFN12* in PG_{post} became similar to NGG_{post}.,,

TABLE 2 Mean ± standard deviation of the PRE AND POST motor variables 14 weeks of combined training (PG: n 24; NGG: n 24).

Variables	Group	PRE	POST	p (group)	p (time)	p (group x time)
EFE (repetitions)	PG	18.5 ± 4.3	21.8 ± 4.4	0.705	<0.001*	0.929
	NGG	18.2 ± 3.7	21.3 ± 3.4			
SAS (repetitions)	PG	14.5 ± 4.5	18.4 ± 6.1	0.363	<0.001*	0.880
	NGG	13.7 ± 3.5	17.3 ± 4.2			
Agility (sec)	PG	26.6 ± 3.2	23.4 ± 3.6	0.253	<0.001*	0.285
	NGG	25.9 ± 2.3	21.7 ± 2.7			
6MWT (m)	PG	540.3 ± 67.1	572.4 ± 60.3	0.905	<0.001*	0.257
	NGG	532.3 ± 38.8	584.2 ± 60.7			

EFE, elbow flexion and extension; SAS, sit and stand; sec, seconds; m, meters; PG, prediabetes group; NGG, normal glucose group. *statistical difference time; $p < 0.05$ for pre versus post (repeated measures two-way ANOVA was used).

TABLE 3 Mean ± standard deviation of PRE and POST metal values 14 weeks of combined training (PG: n 24; NGG: n 24).

Variables (µg/L)	Group	PRE	POST	p (group)	p (time)	p (group x time)
Hg	PG	0.06 ± 0.1	0.03 ± 0.1	0.651	0.030*	0.651
	NGG	0.05 ± 0.1	0.02 ± 0.1			
Al	PG	184.3 ± 150.7	159.5.1 ± 154.8	0.46	0.476	0.907
	NGG	152.2 ± 177.5	135.0 ± 59.0			
As	PG	2.9 ± 0.4	2.9 ± 0.2	0.386	0.818	0.181
	NGG	2.7 ± 0.2	3.0 ± 0.0			
Cd	PG	0.2 ± 0.8	0.03 ± 0.0	0.391	0.212	0.452
	NGG	0.8 ± 0.0	0.02 ± 0.0			
Pb	PG	2.1 ± 1.6	1.1 ± 0.3	0.385	0.001*	0.33
	NGG	1.7 ± 0.8	1.1 ± 0.0			
Ni	PG	6.3 ± 1.0	5.3 ± 1.3	0.175	0.44	0.879
	NGG	4.4 ± 2.4	3.0 ± 0.8			
Li	PG	0.6 ± 0.3	0.5 ± 0.1	0.492	0.104	0.934
	NGG	0.6 ± 0.3	0.5 ± 0.2			

Hg, mercury; Al, aluminum; As, arsenic; Cd, cadmium; Pb, lead; Ni, nickel; Li, lithium; PG, prediabetes group; NGG, normal glucose group. *statistical difference time; $p < 0.05$ for pre versus post (repeated measures two-way ANOVA.

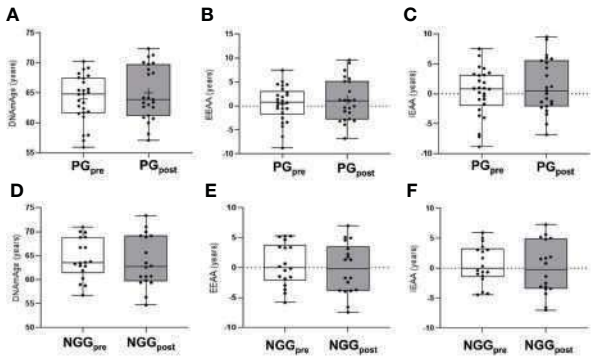

FIGURE 1
Differences in epigenetic age in PRE and POST comparisons 14 weeks of combined training. PG, prediabetes group; NGG, normal glucose group; EEAA, extrinsic epigenetic age acceleration; IEAA, intrinsic epigenetic age acceleration; Pre and pos-intervention comparison for the epigenetic clock in the PG group (panels **A–C**). Pre and post-intervention comparison for the epigenetic clock in the NGG group (panels **D–F**). (repeated measures two-way ANOVA was used). The presented values were not statically different between groups.

TABLE 4 Differentially Methylated Regions (DMRs) of the Prediabetes Group (PG) compared to the Normal Glucose Group (NGG) before 14 weeks of physical activity intervention.

NGG_{pre}	PG_{pre}	Gene	$\Delta\beta$	p-value
0.534	0.723	*IGR*	-0.19	<0.001
0.5	0.579	*RWDD3*	-0.08	0.013
0.25	0.317	*PM20D1*	-0.07	0.023
0.743	0.806	*WFIKKN2*	-0.06	0.015
0.747	0.784	*AURKC*	-0.04	0.026
0.199	0.231	*LOC441666*	-0.03	0.005
0.565	0.595	*HLA-DPB1*	-0.03	<0.001
0.104	0.131	*SLFN12*	-0.03	0.014
0.06	0.07	*CRYZ*	-0.01	0.007
0.045	0.035	*NPY*	0.01	0.004
0.059	0.049	*MIR596*	0.01	0.008
0.451	0.364	*DIP2C*	0.09	0.005

Discussion

Combined training demonstrated benefits in physical and biochemical variables, providing positive results to cardiovascular health

One of the aims of this study was to investigate the effect of combined training on epigenetic patterns in the prediabetes (PG) and normal glucose group (NGG). Combined training is an alternative program involving aerobic and strength exercises in the same training session (30). Its effects are widely recognized in the literature for the numerous benefits to the cardiorespiratory system, prevention of oxidative stress, and critical metabolic alterations in the glycemic balance (31). According to Bellazi et al. (12), the regulating factor of blood glucose concentration is achieved due to the power of action of the three variables: insulin, adequate meals, and physical exercise (32). Indeed, after 14 weeks of training, we found a decrease in glycemic values in the PG group, and in both groups, we observed decreased levels of total cholesterol and TG levels, indicating an improvement in metabolic indicators of cardiovascular health.

Moreover, the combined training improved both groups' systolic and diastolic blood pressure. A study by Schroeder et al. (13) observed that the combined training protocol was the only one that promoted significant changes in blood pressure values, with positive reductions in diastolic pressure (33). They demonstrated that the combined training had positive cumulative results in all cardiovascular outcomes (33).

Combined training also demonstrated benefits in functional capacity in our study, providing a time effect for the PG and NGG groups. These findings corroborated with Medeiros et al. (14), demonstrating an increase in maximal and functional strength after 12 weeks of training in 58 women aged 50-75 years (34).

Regarding heavy metal concentration, we observed a decrease in mercury and lead levels after 14 weeks of combined training. Another finding of our study was the positive correlation between lead and systolic and diastolic blood pressure values in the PG_{pre} group. Lead is widespread in the environment, and even low blood levels of this toxic metal may raise the risk of cardiovascular diseases, stroke, and chronic kidney disease. It is estimated that in adult populations lead exposures account for 5% of the population attributable risk for high blood pressure. Further, single toxicant exposures provide some evidence for developmental nephrotoxicity demonstrated by altered estimated glomerular filtration rate (eGFR) and other kidney biomarkers, increasing a risk for nephropathy in individuals with T2D (35). In a cross-sectional study carried

TABLE 5 Differentially Methylated Regions (DMRs) of the Prediabetes Group (PG) compared to the Normal Glucose Group (NGG) before 14 weeks of physical activity intervention.

NGG_{post}	PG_{post}	Gene	$\Delta\beta$	p-value
0.699	0.495	*IGR*	-0.2	<0.001
0.259	0.188	*LOC441666*	-0.07	0.012
0.078	0.064	*CRYZ*	-0.01	0.004
0.592	0.585	*HLA-DPB1*	-0.01	0.001
0.036	0.048	*NPY*	0.01	<0.001

TABLE 6 Differentially Methylated Regions (DMRs) from the PG_{post} compared to the PG_{pre} after 14-week physical intervention activity.

$\Delta\ \beta\ PG_{post}$ - PG_{pre}	Gene	p-value	$\Delta\ \beta\ PG_{post}$ - PG_{pre}	Gene	p-value
0.043	*CALD1*	0.004	-0.009	*HNRNPH1*	0.008
0.011	*MSI2*	0.024	-0.009	*ICK*	0.021
0.003	*RNF121*	0.024	-0.009	*AGL*	0.02
0	*SHFM1*	0.022	-0.009	*ANKRD11*	0.005
-0.001	*IDI1*	0.035	-0.009	*APH1A*	0.011
-0.001	*NUDT6*	0.024	-0.009	*ZSWIM7*	0.024
-0.001	*NDUFA4*	0.014	-0.009	IGR	0.02
-0.001	*APRT*	0.006	-0.009	*BCKDHA*	0.031
-0.001	*ATP6V1A*	0.01	-0.009	*NUP43*	0.028
-0.002	*WDR7*	0.01	-0.01	*RBPJ*	0.022
-0.002	*PLD3*	0.03	-0.01	*BDP1*	0.004
-0.003	*ZNHIT2*	0.02	-0.01	*COX8A*	0.011
-0.003	*ZNRD1*	0.019	-0.01	*GABPB2*	0.022
-0.003	*SLFN11*	0.013	-0.01	*TOM1L2*	0.026
-0.003	*FDPS*	0.008	-0.01	*PIGL*	0.016
-0.004	*FANCF*	0.005	-0.01	*HMGN4*	0.015
-0.004	*SNW1*	0.02	-0.01	*MOBKL3*	0.021
-0.004	*YIPF4*	0.008	-0.01	*HIST1H2BN*	0.004
-0.005	*ATP5A1*	0.012	-0.011	*CASP8AP2*	0.032
-0.005	*CHCHD8*	0.023	-0.011	*TAF15*	0.019
-0.005	*CEP97*	0.009	-0.011	*CENPK*	0.014
-0.005	*CCDC28A*	0.007	-0.012	*NECAP1*	0.007
-0.005	*PNPT1*	0.008	-0.012	*RANGAP1*	0.017
-0.005	*RBBP5*	0.011	-0.012	*EXOSC10*	0.013
-0.005	*NCRNA00188*	0.011	-0.012	*PSMB5*	0.009
-0.005	*GNAI3*	0.021	-0.012	*ZMYND11*	0.015
-0.005	*CWF19L1*	0.016	-0.013	*KAZALD1*	0.017
-0.006	*METTL3*	0.015	-0.013	*ERH*	0.023
-0.006	*ANAPC1*	0.028	-0.013	*FNTB*	0.007
-0.006	*LOC552889*	0.01	-0.013	*C7orf55*	0.024
-0.006	*NBR2/BRCA1*	0.01	-0.013	*GLIPR1L1*	0.008
-0.006	*CBY1*	0.004	-0.013	*GPATCH4*	0.012
-0.006	*C14orf101*	0.026	-0.013	*SRP14*	0.027
-0.007	*SLC4A1AP*	0.004	-0.014	*NRBF2*	0.022
-0.007	*AARS*	0.013	-0.014	*L3MBTL3*	0.027
-0.007	*C11orf65*	0.012	-0.014	*RND3*	0.032
-0.007	*DPY30*	0.025	-0.015	*ZNF684*	0.015
-0.007	*UBL7*	0.002	-0.015	*SNHG3/RCC1*	0.006
-0.007	IGR	0.015	-0.015	*MYOF*	0.002
-0.007	*BHLHE40*	0.029	-0.015	*TNFRSF10A*	0.017
-0.007	*COPZ1*	0.015	-0.015	*IDH3B*	0.024
-0.007	*H3F3B*	0.003	-0.015	*UFC1*	0.015
-0.007	*MCM2*	0.01	-0.017	*PCNP*	0.004
-0.007	*RPS27A*	0.003	-0.017	*MTMR12*	0.007
-0.008	IGR	0.007	-0.019	*RPL23A*	0.009
-0.008	*NCRNA00167*	0.005	-0.019	*C6orf52*	0.005
-0.008	*DBR1*	0.011	-0.019	*UFD1L*	0.011
-0.008	*C11orf60*	0.005	-0.02	*TNKS2*	0.011

(Continued)

TABLE 6 Continued

$\Delta\ \beta\ PG_{post}$ - PG_{pre}	Gene	p-value	$\Delta\ \beta\ PG_{post}$ - PG_{pre}	Gene	p-value
-0.008	*SLC25A19*	0.025	-0.02	*ILF2*	0.004
-0.008	*SIDT1*	0.006	-0.02	*COX5B*	0.01
-0.008	*LEO1*	0.018	-0.02	*GNB2L1*	0.011
-0.009	*MED28*	0.004	-0.02	*C4orf14*	0.012
-0.009	*VWA5A*	0.018	-0.022	*WDTC1*	0.004
-0.009	*NR1H2*	0.027	-0.022	*HIST1H4C*	0.011
-0.009	*PES1*	0.028	-0.022	*PFDN2*	0.011
-0.009	*WDR62*	0.011	-0.024	*SNORD27*	0.01
-0.009	*MOCS3/DMP1*	0.009	-0.028	*CMTM6*	0.003
-0.009	*LSM5*	0.005	-0.033	*CNIH4*	0.008
-0.009	*COPS4*	0.02	-0.048	*KLF14*	0.018

out by Asgary et al. (15), the authors demonstrated that lead, mercury, and cadmium levels were higher in patients with coronary artery disease (36).

Differentially methylated regions in the prediabetes group are related to inflammation, insulin signaling, and cancer and are associated with a higher risk of T2DM

Our findings demonstrated that genes in differentially methylated regions of the PG group were related to inflammation and insulin signaling. Physical activity has improved glycemic control in adults with type 2 diabetes (27). Moreover, it can induce metabolic adaptations and changes in DNA methylation due to energy requirements (14). These changes in DNA methylation allowed for increased insulin sensitivity, expression of genes involved in energy metabolism, myogenesis, contractile properties, and oxidative stress (14). In the Figure 4 is shown some metabolic pathways functionally enriched from the obtained DMRs.

Figure 5 shows hypermethylated pathways that are potentially altered after 14-week physical exercise intervention. Here we described specific hypermethylated genes' role in PG observed in our study. *HLA-DPB1* plays a central role in the immune system by presenting peptides derived from extracellular proteins. Also, it is linked with inflammation and systemic insulin resistance (37). *RWDD3* gene also plays an immune function. The isoforms RWDD3 positively regulate the

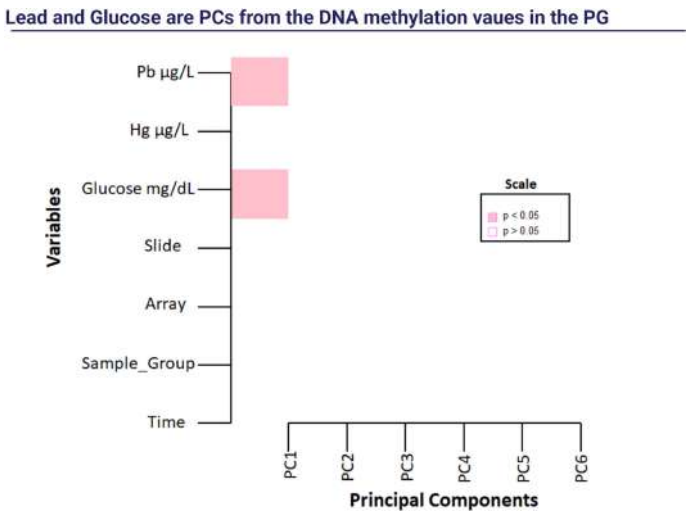

FIGURE 2
Singular Value Decomposition Analysis (SVD). Note: Pb: lead; Hg: mercury.

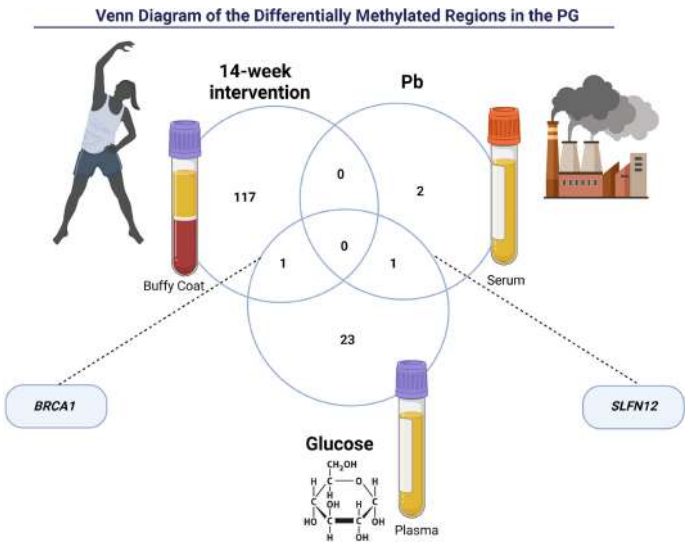

FIGURE 3
The intersection of Differently Methylated Regions of the prediabetes group (PG) between post-intervention, lead (Pb), and glucose. *BRCA1* gene is related to 14-week intervention and glucose. *SLFN12* gene is related to glucose and lead (Pb).

NF-kappa-β signaling pathway by enhancing the NF-kappa-β inhibitor, promoting its stabilization, and regulating the hypoxia-inducible factor-1 alpha (38). Other genes are found hypermethylated in PG group-related metabolism and genomic stability. The gene *TYW3/CRYZ* is related to levels of resistin. Previous studies reported associations between elevated circulating resistin levels and increased risk of cardiovascular disease (28). PM20D1 is an enzyme that condenses fatty acids and amino acids. The *PM20D1* locus shows genome-wide association studies (GWAS) with body mass index (BMI) and weaker associations with obesity-related conditions like type 2 diabetes and HDL-C levels (39). Aurora C kinases (AURKC) are essential kinases for cell division *via* regulating mitosis, especially the process of chromosomal segregation, associated with malignant cell transformation and genomic instability (16). PDE3A binding to SLFN12 results in cell killing and optimizing

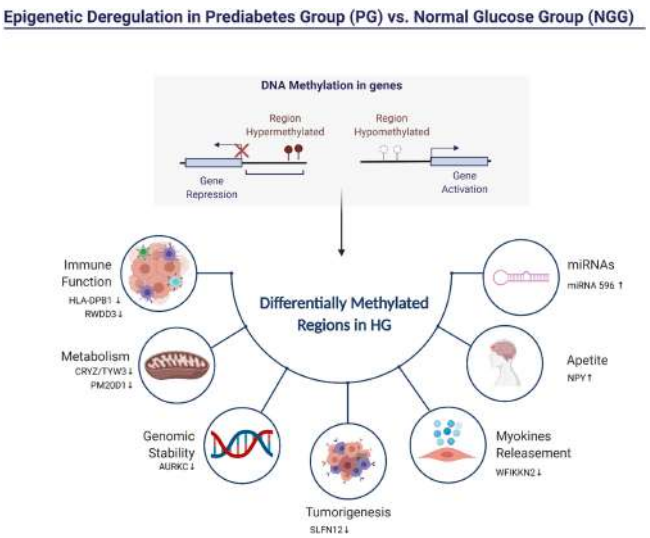

FIGURE 4
Representation of the pathways possibly influenced by differentially methylated regions (DMRs) in PG vs. NGG.

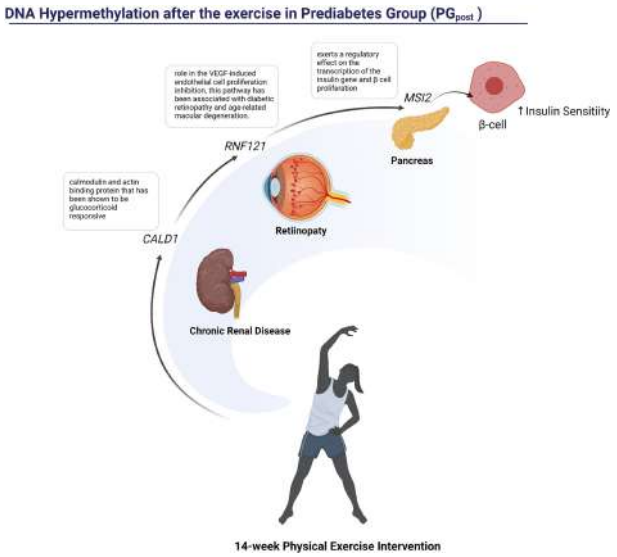

FIGURE 5
Representation of the pathways influenced possibly by hypermethylated regions in PG after the 14-week physical exercise intervention.

cancer therapeutics (40). WFIKKN2 is a follistatin domain-containing protein that binds GDF8/GDF11 proteins with high affinity, both of which have been implicated in DM development (41). The hypermethylation of these genes in individuals of the PG group could indicate a compromised metabolic function which could be associated with a higher risk of T2DM (42).

The epigenetic reprogramming of genes after a 14-week physical exercise intervention can promote many alterations at a cellular level that regulates cellular metabolism in human metabolic diseases such as diabetes, obesity, and cancer

After the 14-week physical exercise intervention, 118 DMRs were detected. Three of these genes were hypermethylated: *CALD1*, *RNF121*, and *MSI2*. The *CALD1* was previously related to diabetes nephropathy (43), while the role of RNF121 in the VEGF-induced endothelial cell proliferation inhibition has been associated with diabetic retinopathy and age-related macular degeneration (44). The *MSI2* hypomethylation has already been described as an epigenetic biomarker for hyperglycemic individuals by the Korean Genome Epidemiology Study (KoGES) (45).

The additional 115 genes were hypomethylated and involved in many biological functions (Figure 6). Regarding the effects on general metabolism, the FDPS (*Farnesyl pyrophosphate synthase*) is a crucial enzyme for several classes of essential metabolites, including sterols, dolichols, carotenoids, and ubiquinones. The BHLHE40 Class E basic helix-loop-helix protein 40 is also a target that may influence the general metabolism due to its role as a transcriptional repressor in

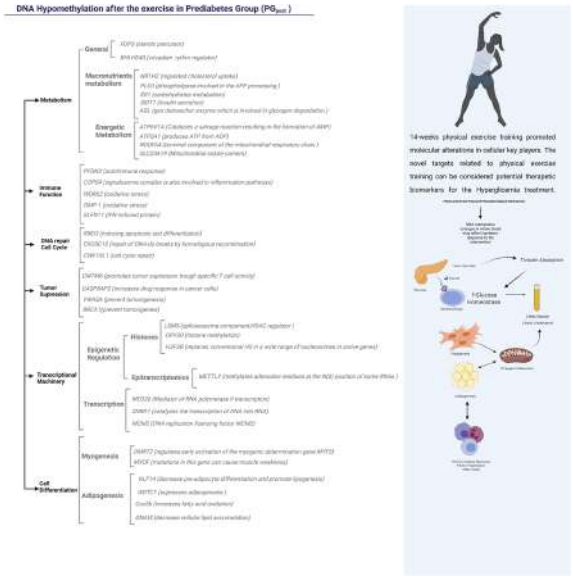

FIGURE 6
Representation of the functions possibly influenced by hypomethylated regions in PG after the 14-week physical exercise intervention. The Functional Annotation was done using the Genecards Database and literature text mining.

regulating the circadian rhythm and negatively regulating the activity of the clock genes and clock-controlled genes (46).

The mechanisms related to metabolism also included targets related to macronutrients, such as lipids and carbohydrates, which are important in the etiology of hyperglycemia (47). The NR1H2 (Oxysterols receptor LXR-beta; Nuclear receptor) gene was differentially methylated and can regulate cholesterol uptake through the ubiquitination of LDLR, VLDLR, and LRP8. Indeed, the PLD3 - Phospholipase D3 is an important key in lipids metabolism and may be involved in the Amyloid Precursor Protein (APP), already been related to Alzheimer´s Disease (AD). Three genes were differentially methylated in carbohydrate metabolism: IDI1, AGL, and SIDT1. ID1 has already been related to nephropathy, a condition connected to advanced stages of T2D (48). The AGL gene encodes the glycogen debrancher enzyme involved in glycogen degradation (49). The SIDT1 gene is associated with insulin secretion, and its expression was decreased in individuals with hyperglycemia (50).

Four differentially methylated genes were associated with energetic metabolism: ATP6V1A, ATP5A1, NDUFA4, and SLC25A19. The ATP6V1A is responsible for acidifying a variety of intracellular molecules, leading to HIF1A hydroxylation and catalyzing AMP formation (51). The ATP5A1 produces ATP from ADP (52). NDUFA4 is a cytochrome c oxidase subunit NDUFA4 and catalyzes oxygen reduction in water (53). The SLC25A19 mitochondrial thiamine pyrophosphate carrier is a transporter mediating thiamine pyrophosphate (ThPP) entrance into mitochondria (54).

The most interesting genes involved with the immune function were the COPS4 signalosome complex subunit 4, involved in phosphorylation of p53/TP53, c-jun/JUN, IkappaBalpha/NFKBIA, which leads to modulation of the inflammasome (55); theWDR62 and DMP-1 related to oxidative stress; and the SLFN11, interferon (IFN)-induced target (16).

The tumor suppression mechanisms, insulin resistance, and metabolic diseases have a common path (56). The CMTM6 depletion significantly decreases tumor-specific T cell activity (57). Indeed, the hypermethylation of two CpG sites upstream of the CASP8AP2 promoter influenced gene expression and treatment outcomes in lymphoblastic leukemia (58). In the present study, CASP8AP2 was hypomethylated after the intervention, suggesting that physical exercise training may decrease the risk of developing cancer in this population. The VWA5A plays a role in tumorigenesis as a tumor suppressor (59). BRCA1 is another important tumor suppressor, and studies have shown that individuals with hyperglycemia had this gene naturally more methylated than normoglycemic individuals (60).

Physical exercise can promote many alterations at a cellular level. Liu et al., 2020, demonstrated that miRNAs could epigenetically modulate the myogenesis and adipogenesis processes after a 12-week physical exercise intervention in older individuals (61). In the present study, important differentially methylated regions in the PG were related to myogenesis and adipogenesis after the intervention. The DMRT2 directly regulates early activation of the myogenic determination. It is required to initiate or maintain the proper organization of the sclerotome, dermomyotome, and myotome (by similarity). Mutations in the *MYOF* gene can cause muscle weakness, which is involved in membrane regeneration and repair. Reduced *KLF14* expression increases pre-adipocyte proliferation and decreases lipogenesis. In mice, the deletion of *KLF14* partially reproduces the human phenotype of insulin resistance, dyslipidemia, and T2D (62). The deregulation of the KLF14/PLK1 cascade plays a crucial role in thrombin-induced endothelial dysfunction in T2DM patients (63).

There is substantial epidemiological evidence that exposure to low levels of various environmental chemicals can influence the development of chronic metabolic diseases, including diabetes (64). It has been well demonstrated that lead shows potential genotoxicity to various research subjects under different conditions (65, 66). A study with human lymphoblastoid TK6 cells found that lead exposure results in DNA damage *via* promoting oxidative stress and the promoter methylation of DNA repair genes, such as *BRCA1* (67).

The *BRCA1* plays a critical role in DNA repair. Mutations in the gene that lead to loss of protein function can accumulate damaged DNA within the cell tumor-suppressor gene, which is strongly linked to an increased risk of breast cancer development (68). Recent studies demonstrated that BRCA1 is expressed in skeletal muscle and is a critical regulator of metabolic function in cultured human myotubes (69). Also, deletion of *BRCA1* expression in vascular cells results in the reduced catabolic breakdown of free fatty acids, and it can influence the metabolic phenotype of cultured cells and potentially the cells' ability to respond to nutrient overload conditions (70).

Schlafens (*SLFN*) are intriguing proteins that play different roles in regulating cell proliferation, cell differentiation, immune cell growth and maturation, and inhibiting viral replication (17, 71).. However, it is still poorly understood how SLFNs interfere with the cell cycle machinery. It is a particularly tempting target for further research, as this gene is known to be down-regulated during T cell activation and up-regulated by type I interferons (IFNs) (18). It has also been suggested in cancer therapy since its expression was positively correlated with the growth inhibitory action of topoisomerase inhibitors on human cancer cells (19).

Acute exercise seems to be associated with active demethylation pathways. The mechanism proposed for active demethylation involves ten-eleven translocation (TET) proteins, suggesting that metabolites produced during exercise could alter the activity of TET proteins (20).

This study's strengths were demonstrating the modulation of biochemical and physical parameters after a physical exercise intervention between different groups. In addition to verifying differently methylated regions in the PG group compared to the NGG group using a large-scale DNA methylation array (Infinium-EPIC beadchips) and the effect of combined training.

The limitations were that analyzes of RNA expressions of the differentially methylated genes were not performed.

Conclusions

The 14-week combined physical exercise intervention led to the modulation of biochemical and physical parameters and the epigenetic reprogramming of genes involved in many biological functions such as metabolism, cell differentiation, tumor suppression, and immune function. Moreover, exercise training decreases mercury and lead serum concentrations. In future studies, longer intervention could be proposed to check the sustained effects of long-term combined physical exercise practice.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number can be found below:GEO, GSE199700.

Ethics statement

The present study was approved by the ethics committee for Research with Human of the School of Physical Education and Sport of Ribeirão Preto, University of São Paulo (EEFERP-USP). The registration number is CAAE: 79582817.0.0000.5656. The study was also registered in the Brazilian Registry of Clinical Trials under RBR-3g38dx. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization: NN and GR; methodology: NN, IN, and GR; formal analysis: NN, GR, CS-L, AC, and KR; resources: CBu and CN; data curation: GR, NN, and IN; performed experiments: NN, GR, LW, MP, DM, WS, and MA; writing—original draft preparation: NN, GR, IN, TP, CBr, and KR; writing—review and editing: TP, NN, GR, IN, and CBr; visualization: All authors; supervision: FB, CBu, and CN; funding acquisition: FB, CBu, and CN. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Association, AD. Diagnosis and classification of diabetes mellitus. *Diabetes Care* (2009) 32:S62–7. doi: 10.2337/dc09-S062

2. Kaaja RJ. Metabolic syndrome and the menopause. *Menopause Int* (2008) 14 (1):21–5. doi: 10.1258/mi.2007.007032

3. Luc K, Schramm-Luc A, Guzik TJ, Mikolajczyk TP. Oxidative stress and inflammatory markers in prediabetes and diabetes. *J Physiol Pharmacol* (2019) 70:809–24. doi: 10.26402/jpp.2019.6.01

4. Muka T, Asllanaj E, Avazverdi N, Jaspers L, Stringa N, Milic J, et al. Age at natural menopause and risk of type 2 diabetes: A prospective cohort study. *Diabetologia* (2017) 60:1951–60. doi: 10.1007/s00125-017-4346-8

5. Kato M, Natarajan R. Epigenetics and epigenomics in diabetic kidney disease and metabolic memory. *Nat Rev Nephrol* (2019) 15:327–45. doi: 10.1038/s41581-019-0135-6

6. Rosen ED, Kaestner KH, Natarajan R, Patti M-E, Sallari R, Sander M, et al. Epigenetics and epigenomics: Implications for diabetes and obesity. *Diabetes* (2018) 67:1923–31. doi: 10.2337/db18-0537

7. Javaid A, Akbar I, Javed H, Khan U, Iftikhar H, Zahra D, et al. Role of heavy metals in diabetes: Mechanisms and treatment strategies. *Crit Rev Eukaryot Gene Expr* (2021) 31:65–80. doi: 10.1615/CritRevEukaryotGeneExpr.2021037971

8. Cai J, Li Y, Liu S, Liu Q, Xu M, Zhang J, et al. Associations between multiple heavy metals exposure and glycated hemoglobin in a Chinese population. *Chemosphere* (2021) 287:132159. doi: 10.1016/j.chemosphere.2021.132159

9. Yahaya T. Role of epigenetics in the pathogenesis and management of type 2 diabetes mellitus. *Translation: Univ Toledo J Med Sci* (2019) 6:20–8. doi: 10.46570/utjms.vol6-2019-319

10. Denham J. Exercise and epigenetic inheritance of disease risk. *Acta Physiol (Oxf)* (2018) 222:1–20. doi: 10.1111/apha.12881

11. Tang S, Yu X, Wu C. Comparison of the levels of five heavy metals in human urine and sweat after strenuous exercise by ICP-MS. *J Appl Mathematics Phys* (2016) 4:183–8. doi: 10.4236/jamp.2016.42022

12. Bellazzi R, Nucci G, Cobelli C. The subcutaneous route to insulin-dependent diabetes therapy. *IEEE Eng Med Biol Mag* (2001) 20:54–64. doi: 10.1109/51.897828

13. Schroeder EC, Franke WD, Sharp RL, Lee D-C. Comparative effectiveness of aerobic, resistance, and combined training on cardiovascular disease risk factors: A randomized controlled trial. *PloS One* (2019) 14:e0210292. doi: 10.1371/journal.pone.0210292

14. Medeiros LHL, Sandbakk SB, Bertazone TMA, Bueno Júnior CR. Comparison of periodization models of concurrent training in recreationally active postmenopausal women. *J Strength Cond Res* (2020) 36:977–83. doi: 10.1519/JSC.0000000000003559

15. Asgary S, Movahedian A, Keshvari M, Taleghani M, Sahebkar A, Sarrafzadegan N. Serum levels of lead, mercury and cadmium in relation to coronary artery disease in the elderly: A cross-sectional study. *Chemosphere* (2017) 180:540–4. doi: 10.1016/j.chemosphere.2017.03.069

16. Anguita-Ruiz A, Bustos-Aibar M, Plaza-Díaz J, Mendez-Gutierrez A, Alcalá-Fdez J, Aguilera CM, et al. Omics approaches in adipose tissue and skeletal muscle addressing the role of extracellular matrix in obesity and metabolic dysfunction. *Int J Mol Sci* (2021) 22:2756. doi: 10.3390/ijms22052756

17. Malachias MVB. 7th Brazilian guideline of arterial hypertension: Presentation. *Arq Bras Cardiol* (2016) 107:XV–XIX. doi: 10.5935/abc.20160140

18. Rikli RE, Jones CJ. Development and validation of a functional fitness test for community-residing older adults. *J Aging Phys Act* (1999) 7(2):129–61. doi: 10.1123/japa.7.2.129

19. Batista BL, Rodrigues JL, Nunes JA, Souza VC, de O, Barbosa F. Exploiting dynamic reaction cell inductively coupled plasma mass spectrometry (DRC-ICP-MS) for sequential determination of trace elements in blood using a dilute-and-Shoot procedure. *Anal Chim Acta* (2009) 639:13–8. doi: 10.1016/j.aca.2009.03.016

20. Cabo R, Hernes S, Slettan A, Haugen M, Ye S, Blomhoff R, et al. Effects of polymorphisms in endothelial nitric oxide synthase and folate metabolizing genes on the concentration of serum nitrate, folate, and plasma total homocysteine after folic acid supplementation: A double-blind crossover study. *Nutrition* (2015) 31:337–44. doi: 10.1016/j.nut.2014.08.009

21. De Santis MA, Giuliani MM, Giuzio L, De Vita P, Lovegrove A, Shewry PR, et al. Differences in gluten protein composition between old and modern durum wheat genotypes in relation to 20th century breeding in Italy. *Eur J Agron* (2017) 87:19–29. doi: 10.1016/j.eja.2017.04.003

22. Li H, Chen R, Cai J, Cui X, Huang N, Kan H. Short-term exposure to fine particulate air pollution and genome-wide DNA methylation: A randomized, double-blind, crossover trial. *Environ Int* (2018) 120:130–6. doi: 10.1016/j.envint.2018.07.041

23. Masser DR, Stanford DR, Freeman WM. Targeted DNA methylation analysis by next-generation sequencing. *J Vis Exp* (2015) 96:52488. doi: 10.3791/52488

24. Tian Y, Morris TJ, Webster AP, Yang Z, Beck S, Feber A, et al. ChAMP: Updated methylation analysis pipeline for illumina BeadChips. *Bioinformatics* (2017) 33:3982–4. doi: 10.1093/bioinformatics/btx513

25. Fortin J-P, Triche TJ, Hansen KD. Preprocessing, normalization and integration of the illumina HumanMethylationEPIC array with minfi. *Bioinformatics* (2017) 33:558–60. doi: 10.1093/bioinformatics/btw691

26. Pelegí-Sisó D, de Prado P, Ronkainen J, Bustamante M, González JR. Methylclock: A bioconductor package to estimate DNA methylation age. *Bioinformatics* (2021) 37:1759–60. doi: 10.1093/bioinformatics/btaa825

27. Carbia-Nagashima A, Gerez J, Perez-Castro C, Paez-Pereda M, Silberstein S, Stalla GK, et al. RSUME, a small RWD-containing protein, enhances SUMO conjugation and stabilizes HIF-1alpha during hypoxia. *Cell* (2007) 131:309–23. doi: 10.1016/j.cell.2007.07.044

28. Qi Q, Menzaghi C, Smith S, Liang L, de Rekeneire N, Garcia ME, et al. Genome-wide association analysis identifies TYW3/CRYZ and NDST4 loci associated with circulating resistin levels. *Hum Mol Genet* (2012) 21:4774–80. doi: 10.1093/hmg/dds300

29. Benson KK, Hu W, Weller AH, Bennett AH, Chen ER, Khetarpal SA, et al. Natural human genetic variation determines basal and inducible expression of PM20D1, an obesity-associated gene. *PNAS* (2019) 116:23232–42. doi: 10.1073/pnas.1913199116

30. Lewis TA, de Waal L, Wu X, Youngsaye W, Wengner A, Kopitz C, et al. Optimization of PDE3A modulators for SLFN12-dependent cancer cell killing. *ACS Med Chem Lett* (2019) 10:1537–42. doi: 10.1021/acsmedchemlett.9b00360

31. Gudmundsdottir V, Zaghlool SB, Emilsson V, Aspelund T, Ilkov M, Gudmundsson EF, et al. Circulating protein signatures and causal candidates for type 2 diabetes. *Diabetes* (2020) 69:1843–53. doi: 10.2337/db19-1070

32. Kondás K, Szláma G, Trexler M, Patthy L. Both WFIKKN1 and WFIKKN2 have high affinity for growth and differentiation factors 8 and 11. *J Biol Chem* (2008) 283:23677–84. doi: 10.1074/jbc.M803025200

33. Félétou M, Galizzi J-P, Levens NR. NPY receptors as drug targets for the central regulation of body weight. *CNS Neurol Disord Drug Targets* (2006) 5:263–74. doi: 10.2174/187152706777452236

34. Wang J, Chen L, Qiang P. The role of IGF2BP2, an M6A reader gene, in human metabolic diseases and cancers. *Cancer Cell Int* (2021) 21:99. doi: 10.1186/s12935-021-01799-x

35. Chen Y, Gong W, Dai W, Pan Z, Xu X, Jiang H. MiR-596 down regulates SOX4 expression and is a potential novel biomarker for gastric cancer. *Trans Cancer Res* (2020) 9:1294–302. doi: 10.21037/tcr.2020.02.02

36. Wang Z, Wang Z, Zhou Z, Ren Y. Crucial genes associated with diabetic nephropathy explored by microarray analysis. *BMC Nephrol* (2016) 17:128. doi: 10.1186/s12882-016-0343-2

37. Maghsoudlou A, Meyer R, Rezazadeh K, Arafa E, Pudney J, Hartsough EJ, et al. RNF121 inhibits angiogenic growth factor signaling by restricting cell surface expression of VEGFR-2. *Traffic* (2016) 17:289–300. doi: 10.1111/tra.12353

38. Ahmed SAH, Ansari SA, Mensah-Brown EPK, Emerald BS. The role of DNA methylation in the pathogenesis of type 2 diabetes mellitus. *Clin Epigenet* (2020) 12:104. doi: 10.1186/s13148-020-00896-4

39. Sato F, Muragaki Y, Zhang Y. DEC1 negatively regulates AMPK activity *via* LKB1. *Biochem Biophys Res Commun* (2015) 467:711–6. doi: 10.1016/j.bbrc.2015.10.077

40. Chiu C-J, Taylor A. Dietary hyperglycemia, glycemic index and metabolic retinal diseases. *Prog Retin Eye Res* (2011) 30:18–53. doi: 10.1016/j.preteyeres.2010.09.001

41. Schisler JC, Charles PC, Parker JS, Hilliard EG, Mapara S, Meredith D, et al. Stable patterns of gene expression regulating carbohydrate metabolism determined by geographic ancestry. *PloS One* (2009) 4:e8183. doi: 10.1371/journal.pone.0008183

42. Perveen S, Gupta N, Kumar M, Kaur P, Chowdhury MR, Kabra M. Spectrum of amyloglucosidase mutations in Asian Indian patients with glycogen storage disease type III. *Am J Med Genet A* (2020) 182:1190–200. doi: 10.1002/ajmg.a.61547

43. Ottosson-Laakso E, Krus U, Storm P, Prasad RB, Oskolkov N, Ahlqvist E, et al. Glucose-induced changes in gene expression in human pancreatic islets: Causes or consequences of chronic hyperglycemia. *Diabetes* (2017) 66:3013–28. doi: 10.2337/db17-0311

44. Alzamora R, Al-Bataineh MM, Liu W, Gong F, Li H, Thali RF, et al. AMP-activated protein kinase regulates the vacuolar h+-ATPase *via* direct phosphorylation of the a subunit (ATP6V1A) in the kidney. *Am J Physiology-Renal Physiol* (2013) 305:F943–56. doi: 10.1152/ajprenal.00303.2013

45. Chomova M, Balazova M, Muchova J. Diabetes-induced abnormalities of mitochondrial function in rat brain cortex: The effect of n-3 fatty acid diet. *Mol Cell Biochem* (2017) 435:109–31. doi: 10.1007/s11010-017-3061-6

46. Pitceathly RDS, Rahman S, Wedatilake Y, Polke JM, Cirak S, Foley AR, et al. NDUFA4 mutations underlie dysfunction of a cytochrome c oxidase subunit linked to human neurological disease. *Cell Rep* (2013) 3:1795–805. doi: 10.1016/j.celrep.2013.05.005

47. Kang J, Samuels DC. The evidence that the DNC (SLC25A19) is not the mitochondrial deoxyribonucleotide carrier. *Mitochondrion* (2008) 8:103–8. doi: 10.1016/j.mito.2008.01.001

48. Bech-Otschir D, Kraft R, Huang X, Henklein P, Kapelari B, Pollmann C, et al. COP9 signalosome-specific phosphorylation targets P53 to degradation by the ubiquitin system. *EMBO J* (2001) 20:1630–9. doi: 10.1093/emboj/20.7.1630

49. Kaul K, Misri S, Ramaswamy B, Ganju RK. Contribution of the tumor and obese microenvironment to triple negative breast cancer. *Cancer Lett* (2021) 509:115–20. doi: 10.1016/j.canlet.2021.03.024

50. Burr ML, Sparbier CE, Chan Y-C, Williamson JC, Woods K, Beavis PA, et al. CMTM6 maintains the expression of PD-L1 and regulates anti-tumour immunity. *Nature* (2017) 549:101–5. doi: 10.1038/nature23643

51. Li Z-G, Jiao Y, Li W-J, Deng G-R, Cui L, Gao C, et al. Hypermethylation of two CpG sites upstream of CASP8AP2 promoter influences gene expression and treatment outcome in childhood acute lymphoblastic leukemia. *Leuk Res* (2013) 37:1287–93. doi: 10.1016/j.leukres.2013.07.018

52. Anghel SI, Correa-Rocha R, Correa-Rochal R, Budinska E, Boligan KF, Boliganl KF, et al. Breast cancer suppressor candidate-1 (BCSC-1) is a melanoma tumor suppressor that down regulates MITF. *Pigment Cell Melanoma Res* (2012) 25:482–7. doi: 10.1111/j.1755-148X.2012.01018.x

53. Karachanak-Yankova S, Dimova R, Nikolova D, Nesheva D, Koprinarova M, Maslyankov S, et al. Epigenetic alterations in patients with type 2 diabetes mellitus. *Balkan J Med Genet* (2016) 18:15–24. doi: 10.1515/bjmg-2015-0081

54. Liu H-W, Cheng H-C, Tsai S-H, Sun W-H. Effect of progressive resistance training on circulating adipogenesis-, myogenesis-, and inflammation-related MicroRNAs in healthy older adults: An exploratory study. *GER* (2020) 66:562–70. doi: 10.1159/000510148

55. Small KS, Todorčević M, Civelek M, El-Sayed Moustafa JS, Wang X, Simon MM, et al. Regulatory variants at KLF14 influence type 2 diabetes risk *via* a female-specific effect on adipocyte size and body composition. *Nat Genet* (2018) 50:572–80. doi: 10.1038/s41588-018-0088-x

56. Hao J-S, Zhu C-J, Yan B-Y, Yan C-Y, Ling R. Stimulation of KLF14/PLK1 pathway by thrombin signaling potentiates endothelial dysfunction in type 2 diabetes mellitus. *BioMed Pharmacother* (2018) 99:859–66. doi: 10.1016/j.biopha.2018.01.151

57. Zhu H, Ge K, Lu J, Jia C. Downregulation of GNAI3 promotes the pathogenesis of Methionine/Choline-deficient diet-induced nonalcoholic fatty liver disease. *Gut Liver* (2020) 14:492–9. doi: 10.5009/gnl19115

58. Jones OA, Maguire ML, Griffin JL. Environmental pollution and diabetes: A neglected association. *Lancet* (2008) 371:287–8. doi: 10.1016/S0140-6736(08)60147-6

59. Pasha Shaik A, Sankar S, Reddy SC, Das PG, Jamil K. Lead-induced genotoxicity in lymphocytes from peripheral blood samples of humans: *In vitro* studies. *Drug Chem Toxicol* (2006) 29:111–24. doi: 10.1080/01480540500408739

60. Nersesyan A, Kundi M, Waldherr M, Setayesh T, Mišík M, Wultsch G, et al. Results of micronucleus assays with individuals who are occupationally and environmentally exposed to mercury, lead and cadmium. *Mutat Res Rev Mutat Res* (2016) 770:119–39. doi: 10.1016/j.mrrev.2016.04.002

61. García-Lestón J, Méndez J, Pásaro E, Laffon B. Genotoxic effects of lead: An updated review. *Environ Int* (2010) 36:623–36. doi: 10.1016/j.envint.2010.04.011

62. Liu X, Wu J, Shi W, Shi W, Liu H, Wu X. Lead induces genotoxicity *via* oxidative stress and promoter methylation of DNA repair genes in human lymphoblastoid TK6 cells. *Med Sci Monit* (2018) 24:4295–304. doi: 10.12659/MSM.908425

63. Roy R, Chun J, Powell SN. BRCA1 and BRCA2: Different roles in a common pathway of genome protection. *Nat Rev Cancer* (2012) 12:68–78. doi: 10.1038/nrc3181

64. Jackson KC, Tarpey MD, Valencia AP, Iñigo MR, Pratt SJ, Patteson DJ, et al. Induced cre-mediated knockdown of Brca1 in skeletal muscle reduces mitochondrial respiration and prevents glucose intolerance in adult mice on a high-fat diet. *FASEB J* (2018) 32:3070–84. doi: 10.1096/fj.201700464R

65. Jackson KC, Gidlund E-K, Norrbom J, Valencia AP, Thomson DM, Schuh RA, et al. BRCA1 is a novel regulator of metabolic function in skeletal muscle. *J Lipid Res* (2014) 55:668–80. doi: 10.1194/jlr.M043851

66. Privat M, Radosevic-Robin N, Aubel C, Cayre A, Penault-Llorca F, Marceau G, et al. BRCA1 induces major energetic metabolism reprogramming in breast cancer cells. *PloS One* (2014) 9:e102438. doi: 10.1371/journal.pone.0102438

67. Vomhof-DeKrey EE, Lee J, Lansing J, Brown C, Darland D, Basson MD. Schlafen 3 knockout mice display gender-specific differences in weight gain, food efficiency, and expression of markers of intestinal epithelial differentiation, metabolism, and immune cell function. *PloS One* (2019) 14:e0219267. doi: 10.1371/journal.pone.0219267

68. Kovalenko PL, Basson MD. Schlafen 12 expression modulates prostate cancer cell differentiation. *J Surg Res* (2014) 190:177–84. doi: 10.1016/j.jss.2014.03.069

69. Katsoulidis E, Carayol N, Woodard J, Konieczna I, Majchrzak-Kita B, Jordan A, et al. Role of schlafen 2 (SLFN2) in the generation of interferon α-induced growth inhibitory responses. *J Biol Chem* (2009) 284:25051–64. doi: 10.1074/jbc.M109.030445

70. Li D, Chen J, Ai Y, Gu X, Li L, Che D, et al. Estrogen-related hormones induce apoptosis by stabilizing schlafen-12 protein turnover. *Mol Cell* (2019) 75:1103–1116.e9. doi: 10.1016/j.molcel.2019.06.040

71. Zoppoli G, Regairaz M, Leo E, Reinhold WC, Varma S, Ballestrero A, et al. Putative DNA/RNA helicase schlafen-11 (SLFN11) sensitizes cancer cells to DNA-damaging agents. *Proc Natl Acad Sci U.S.A.* (2012) 109:15030–5. doi: 10.1073/pnas.1205943109

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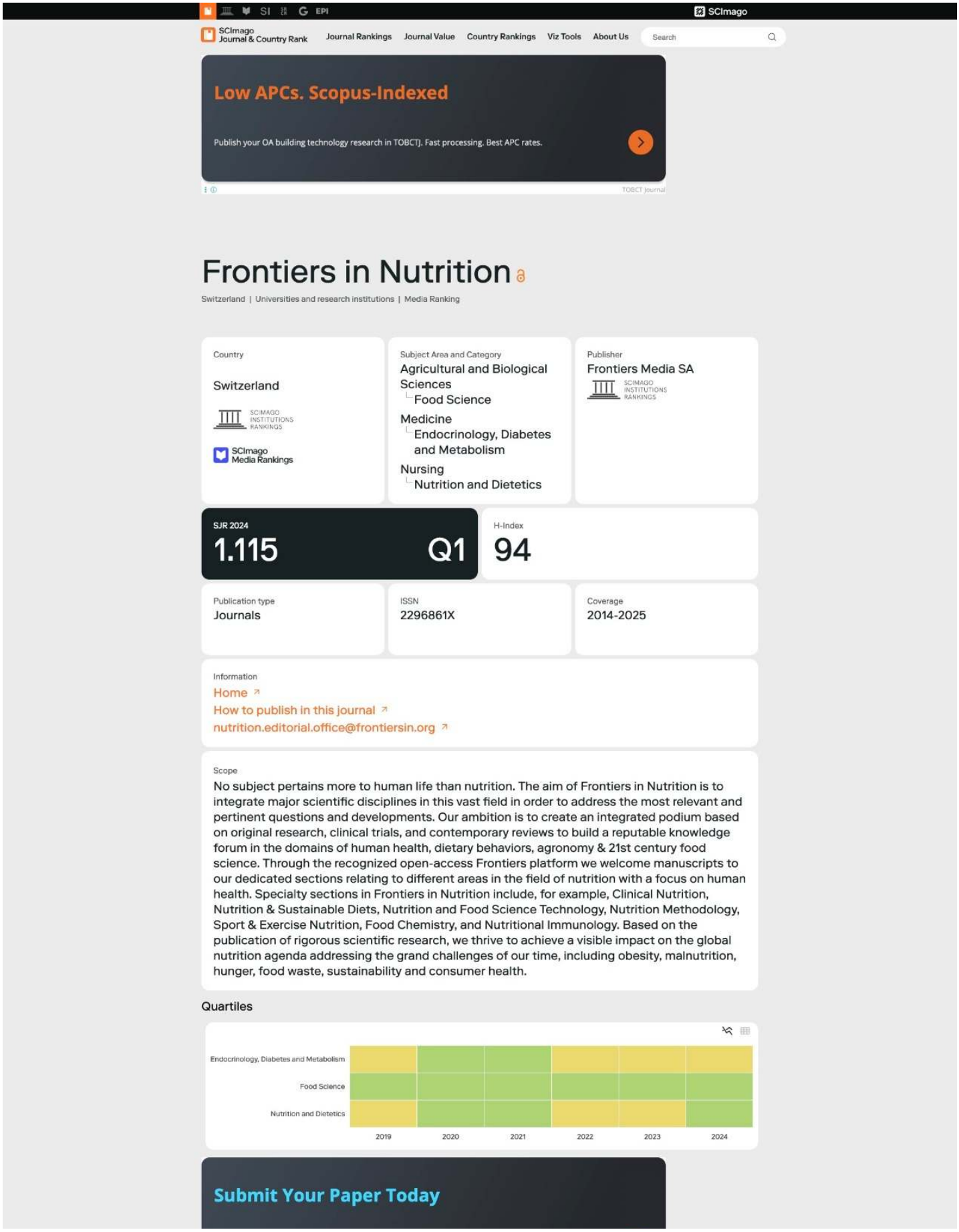
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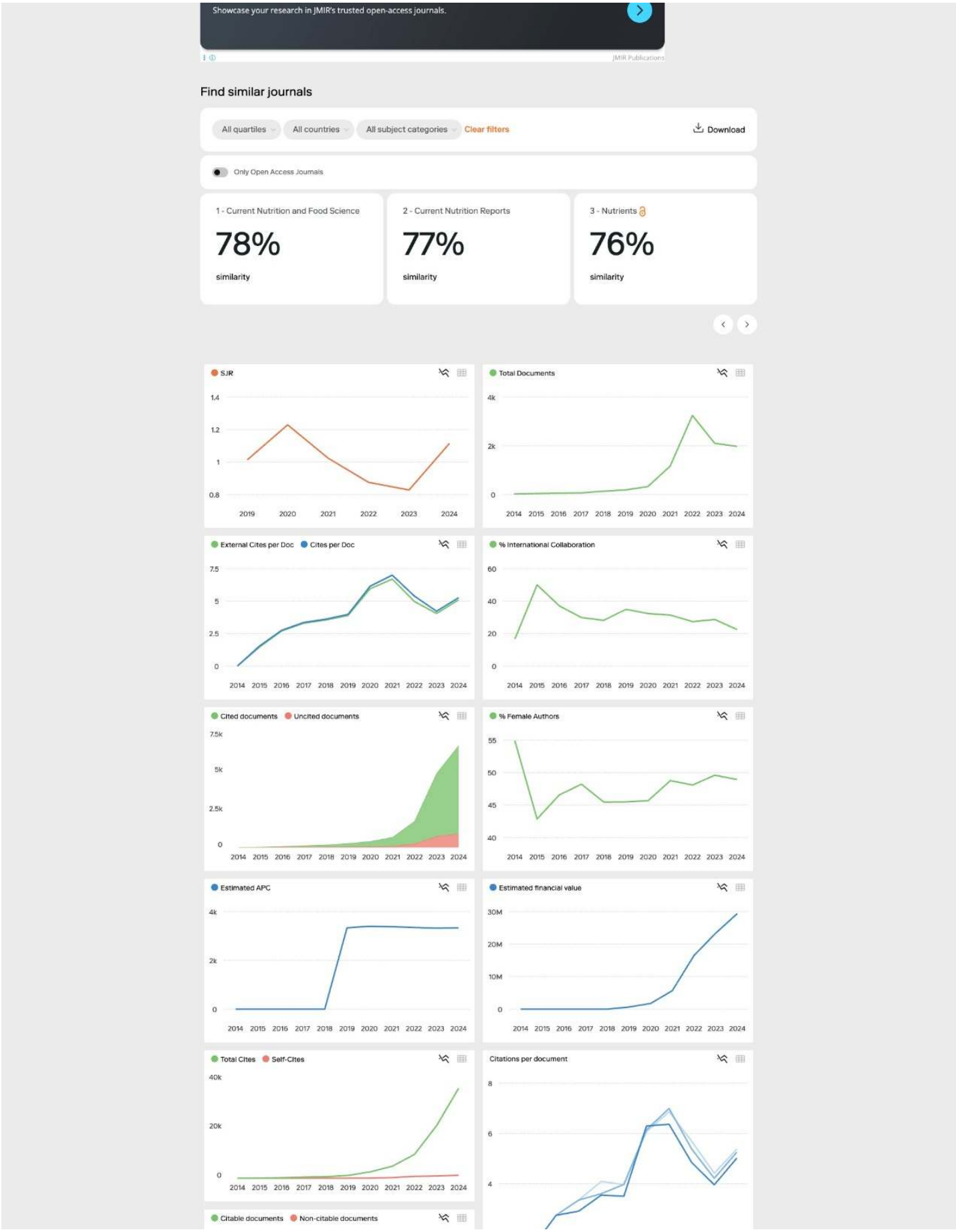
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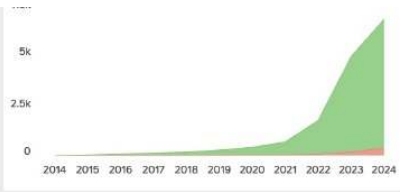
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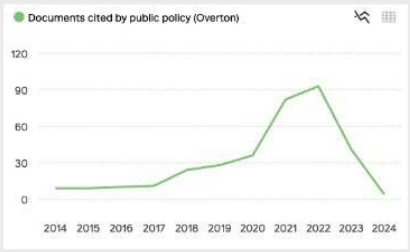
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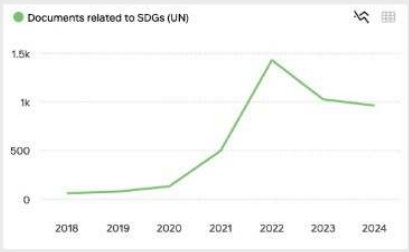
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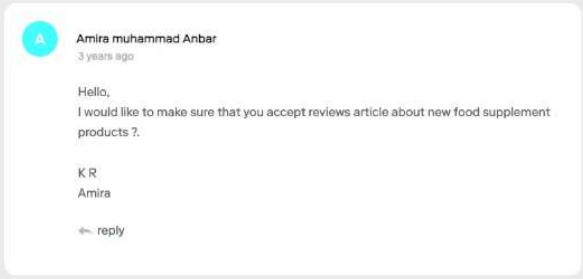
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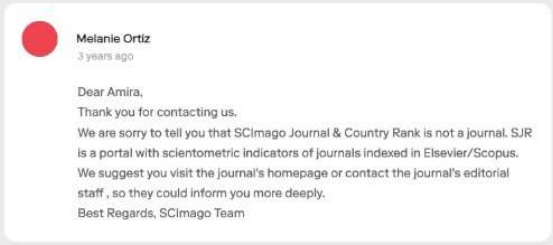
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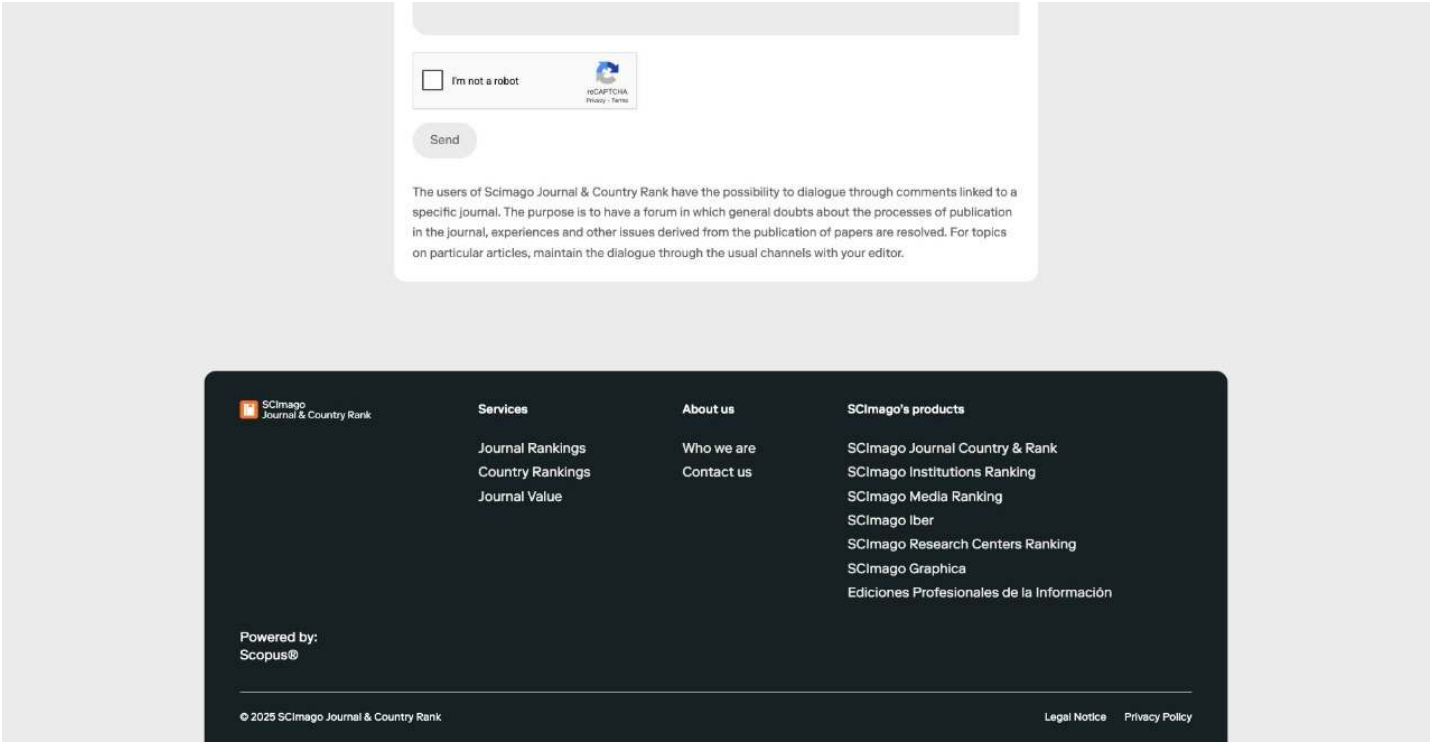
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Research Article

DNA methylation signatures of arsenic exposure and obesity

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Abstract

Emerging evidence suggests a link between environmental pollution and epigenetic alterations, prompting the need for comprehensive investigations into the relationship between pollutants and health conditions in human populations. This study investigates the interplay between obesity and exposure to toxic metals, examining clinical, serum metal concentrations, and epigenetic signatures. Our approach included serum metal concentration analysis by inductively coupled plasma mass spectrometry and epigenetic analysis using 450k Illumina BeadChips data. Singular value decomposition and linear regression models were used to identify metal associations with DNA methylation. Marked differences were evident in weight, body mass index, glycaemia, High Density Lipoprotein cholesterol (HDL-c), and triglycerides between patients with obesity and without obesity. Metal serum concentrations revealed higher arsenic levels in participants with obesity, while elevated mercury concentrations were found in individuals without obesity. Epigenetic analysis identified 2045 arsenic-associated differentially methylated positions (DMPs) in individuals with obesity, including 57 hypermethylated and 159 hypomethylated sites in promoter regions. These DMPs demonstrated direct associations of arsenic exposure, and traits such as insufficient sleep, smoking, and diseases such as gestational diabetes. Functional enrichment analysis (using traits, gene ontology, and KEGG pathways) highlighted pathways linking obesity and arsenic exposure, specifically the Wnt and TNF signalling pathways. Additionally, hypermethylated sites were linked with cancer, rheumatoid arthritis, and gestational diabetes, emphasizing the intricate relationship between these conditions. Notably, *ABCF1* and *BRCA1* showed significant differences in methylation associated with arsenic and obesity. The findings provide valuable insights into unravelling the connections between obesity and arsenic exposure, contributing to understand the potential molecular mechanisms and pathways in these intersecting fields.

Keywords: obesity; arsenic exposure; metal serum concentrations; DNA methylation; toxic metal

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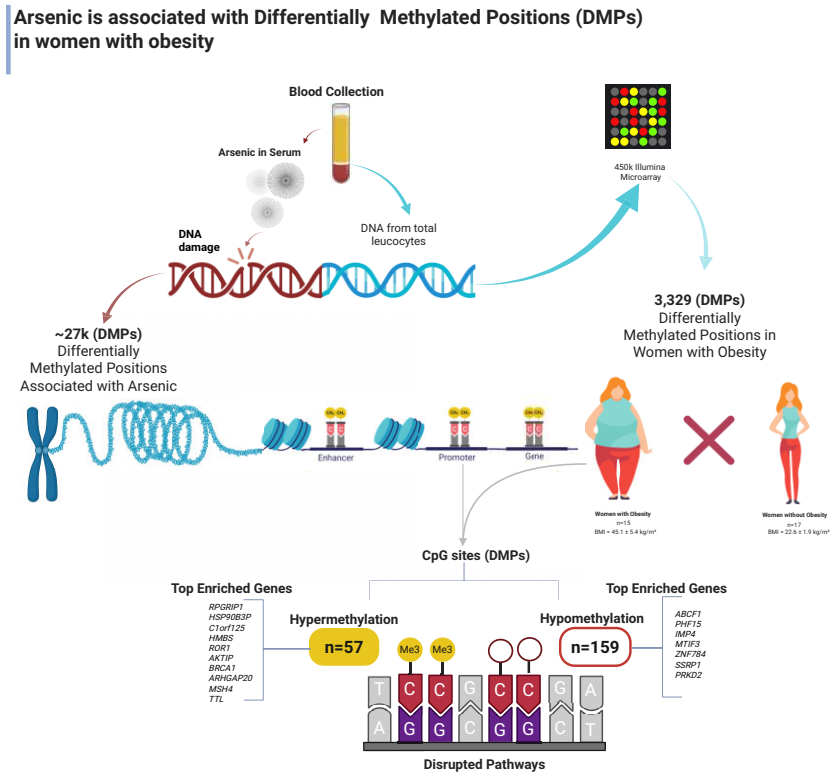

Introduction

Toxic metals, recognized as substances with potentially adverse effects on human health, pose significant risks, given their association with the development of diseases, including obesity, cancer, and neurodegenerative disorders [1]. The release of pollutants into the environment, a consequence of extensive anthropogenic activities, raises a global concern about their impact on public health [2]. Particularly in middle-income countries such as Brazil, limited economic capabilities make it harder to effectively remove toxic metals, amplifying susceptibility to their harmful effects, primarily through contaminated drinking water sources [3]. Emerging evidence suggests a link between environmental pollution and epigenetic alterations, prompting the need for comprehensive investigations into the interplay between pollutants and various health conditions in human populations [4]. Elevated concentrations of certain substances may disrupt DNA methylation, potentially influencing gene expression and triggering additional epigenetic modifications [5]. DNA methylation involves the addition of a methyl group to cytosine residues in cytosine-phosphate-guanine (CpG) dinucleotides, forming the basis for identifying differentially methylated positions (DMPs) in epigenetic studies. Clusters of CpG sites, known as CpG islands, are typically located at the promoters of genes. These regions, when differentially methylated, can act as regulatory regions and are analysed as DMPs in epigenome-wide studies [6]. The methylation patterns are dynamic, showing spatial and temporal variation during development and in response to environmental stimuli, which supports the investigation of DMPs in relation to environmental exposures such as toxic metals [7]. Notably, epigenome-wide studies have revealed epigenetic signatures associated with obesity, suggesting a potential link between environmental factors, DNA methylation, and metabolic conditions [8]. Despite this, a notable gap remains in the literature regarding the integrative effects of toxic metals, DNA methylation, and obesity [9–12], justifying the importance of investigating this correlation. This study aimed to address this gap by evaluating and integrating data on toxic metals and the methylome in a Brazilian sample, specifically exploring potential associations between toxic metal exposure and differential changes in DNA methylation between individuals with and without obesity.

Materials and methods

Ethical aspects

The Clinical Hospital of Ribeirão Preto of the University of São Paulo (HCRP-USP) ethics committee approved the study under license CAAE: 14275319.7.0000.5440. All participants received information and only those who freely consented joined the study. Helsinki declaration principles guided all the procedures adopted. The clinical study was registered under the Universal Trial Number U1111-1267-7108

Recruitment of participants

Participants were recruited in HCRP-USP, and we observed the highest rates of obesity in women identified as female biological sex [13]. We then selected participants with a body mass index (BMI) $\geq$30 kg/m^2 ($n = 15$) from the Metabolic Diseases outpatient clinic in HCRP-USP. We recruited participants without obesity participants with a BMI of 18.5 and 24.9 kg/m^2 ($n = 17$) from the other outpatient clinics in the same hospital, and the participants did not have serious diseases. For this study, we excluded pregnant and lactating women, smokers, and those who consumed alcohol. All participants gave written informed consent for the research.

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Blood collection, biochemical, and chemical analysis

Participants were instructed to arrive after 12 h of fasting. Blood samples were collected, and biochemical analysis was performed on plasma samples. We performed enzymatic and colorimetric methods to analyse total cholesterol and High Density Lipoprotein cholesterol (HDL-c). To calculate Low Density Lipoprotein cholesterol (LDL-c), we use the Friedewald equation (PMID: 4337382). We measured fasting blood glucose using the enzymatic system for determining blood glucose (liquefied glucose).

Toxic metal analysis was conducted on serum samples following a previously standardized protocol [14]. Total concentrations of metals (mercury, arsenic, and cadmium) were determined using inductively coupled plasma mass spectrometry (ICP-MS) (Perkin Elmer Sciex Norwalk, CTUSA). The spectrometer was an ICP-MS ELAN 6100 Sciex® (PerkinElmer Instruments, SP, Brazil). Samples were diluted in the ratio 1:50 with a solution containing Triton X-100 0.01% (v/v), HNO_3 0.05% (v/v), and 10 mg/L rhodium as an internal standard. The concentration of the analytical calibration standards ranged from 0 to 50 μg/L.

DNA extraction and methylation data analysis

Before the methylation experiment, we extracted leucocyte DNA using the Promega automated system (Madison, WI, USA), following the manufacturer's recommendations.

After extraction, we used nanodrop quantification analyses to check sample purity (Thermo Fisher, Waltham, MA, USA) as sample quality control.

Following the recommendations of the Infinium Human Methylation 450k protocol, the experiment was divided into 4 days: the first was for bisulfite conversion using the EZ DNA Methylation Kit (Zymo Research Corporation, Irvine, CA, USA), the second was for DNA amplification, and the last was for reading the intensities obtained using the iScan system (Illumina, Inc., San Diego, CA, USA).

After the readings, we created a sample sheet to identify the position of each sample in Rstudio and begin bioinformatics processing. We used the MyNorm function from the *ChAMP* package for data normalization (BMIQ method), probes with a detection *P* value > .01, probes with a beadcount <3 in at least 5% of samples, NoCG Start Only Keep CpGs, probes with Single-nucleotide polymorphisms (SNPs) as identified in Zhou's Nucleic Acids Research Paper 2016, probes that align to multiple locations (MultiHit Start), and probes located on X, Y chromosome were removed [15]. After applying the standard filtering steps implemented in the *ChAMP* pipeline, 409 887 probes were retained for analysis. The following filtering criteria were used: removal of unqualified probes (detection *P*-value > .01 in at least one sample, $n = 2012$), non-CG probes ($n = 3024$), probes containing SNPs at or near the CpG site ($n = 58\,682$), multihit probes mapping to multiple genomic locations ($n = 11$), and probes located on sex chromosomes (X and Y; $n = 9760$). These filters are applied to improve data quality and eliminate sources of technical and biological bias prior to differential methylation analysis.

The gene methylation profiling datasets (GSE166611) were acquired from the Gene Expression Omnibus at the National Center for Biotechnology Information. The dataset is accessible at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE166611. The sample set comprised 32 peripheral blood samples, 15 of which were sourced from individuals with obesity and 17 from those classified as without obesity (normal weight). Supplemental data file 1 contains all the considered CpG sites for this study.

Differential methylation analysis

To calculate DMPs, we adopted the Bioconductor *ChAMP* package. The output of champ.DMP() includes a data frame of *P*-values, *t*-statistics, and the difference in mean methylation between the groups compared (labelled as logFC, analogous to log fold change in gene expression analyses) [16]. In *ChAMP*, we used the myDMP function to perform the association analysis with toxic metals. The *limma* package is the default modelling framework for identifying DMPs. A threshold of $P < .05$ and false discovery rate (FDR) < 0.05 was applied to identify significant results. The analysis compared individuals with obesity to those without obesity, with the normal weight group set as the reference.

The *ChAM P* pipeline uses beta values for normalization and quality control steps and internally converts data to M-values during differential methylation analysis, as M-values provide better statistical properties for linear modelling.

Regarding covariate adjustment, age was assessed using singular value decomposition (SVD) and was not retained as it did not contribute significantly to technical variance. Cell-type composition was corrected using reference-based deconvolution. BMI was intentionally excluded as a covariate because it is a primary phenotypic variable of interest; adjusting for it would remove the biological differences under investigation.

To visualize the results, a volcano plot was generated to highlight hypomethylated and hypermethylated CpG sites, and a Manhattan plot was constructed to illustrate the most significant DMPs associated with arsenic exposure. In the package Champ, we used the myDMP function to perform the association analysis with the toxic metals. *Limma* package is the default function to determine DMPs. $P < .05$ and FDR < 0.05 were considered significant for this analysis. The differential methylation data compared the individuals with obesity against the group without obesity. In this analysis, the group without obesity (normal weight) was adopted as the reference group.

Functional enrichment

To perform functional enrichment analysis of DMPs associated with metals, we used the Epigenetic Wide Association Study (EWAS) Toolkit. We adopted EWAS as an enrichment tool because it is becoming increasingly significant in identifying associations between epigenetic variations and various biological characteristics.

After the terms of the CpG lists associated with metals were imputed in the EWAS Toolkit, adopting $P < .05$ as significant for the survey, this article included functional enrichment analyses using the trait, gene ontology (GO), and KEGG pathway categories from the EWAS Toolkit.

Gene expression

Gene expression data were obtained from the Gene Expression Omnibus database, specifically from dataset GSE83223, which used a subset of the same samples. In this analysis, we included 13 individuals with obesity and 9 individuals without obesity. Expression values were normalized through log2 transformation to ensure comparability across samples. Samples were categorized into two groups: obese and control, as defined by metadata annotations. A design matrix was constructed to model group-specific differences, enabling robust differential expression analysis. This was conducted using the limma package (version 3.54.0), which involved fitting a linear model to the normalized data and

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Table 1. Sample characterization[a]

Parameters	With obesity ($n = 15$)	Without obesity ($n = 17$)
Age (years)	38.6 ± 11.6	39.1 ± 13.4
Weight (kg)	116.1 ± 15.7[b]	59.9 ± 6.8
Height (m)	1.6 ± 0.1	1.6 ± 0.1
BMI (kg/m^2)	45.1 ± 5.4[b]	22.6 ± 1.9
Glycaemia (mg/dL)	100.7 + 33.8[b]	81.6 ± 9.8
TC (mg/dL)	190.8 ± 36.8	180.2 ± 35.5
HDL-c (mg/dL)	45.7 ± 7.9[b]	55.8 ± 17.7
LDL-c (mg/dL)	113.9 + 35.4	110.9 ± 29.0
TG (mg/dL)	145.5 + 40.7[b]	66.9 ± 29.2

[a]Values are shown in mean ± standard deviation.
[b]Unpaired *t*-test: $P < .05$ related to the control group.

creating a contrast matrix to assess differences between the two groups.

Statistical analysis

The statistical analysis was performed using SPSS (v 25.0, Chicago, IL, USA). The Shapiro–Wilk test was used to verify normality, and *t*-tests or Mann–Whitney tests were used to verify differences between groups. Significance was assumed with $P < .05$.

Results

Clinical variables

Table 1 shows the clinical variables for the patients included in the study. Five variables were different in women with obesity compared to women without obesity. The obesity group showed weight, BMI, glycaemia, HDL-c, and triglycerides (TGs). Also, it showed lower HDL-c. The observed differences between patients with and without obesity were expected.

Metals serum concentration

Table 2 presents the toxic metals serum conc. in both groups. Participants without obesity had significantly higher levels of mercury, while those with obesity showed significantly higher levels of arsenic ($P < .05$). Notably, the mean concentrations of cadmium, mercury, and arsenic in both groups remained within the population reference ranges previously reported for Brazilian samples.

Epigenetic analysis

Epigenetic Wide Association Study

The SVD analysis revealed a significant influence ($P < .05$) of cadmium, arsenic, mercury, and BMI on DNA methylation variability. Subsequently, a supervised analysis of DMPs was conducted using these findings. Individual linear regressions were performed to find DMPs associated with cadmium, arsenic, and mercury. Significant associations were found for cadmium and arsenic (Table 3). Arsenic had the highest number of associated DMPs (Fig. 1). The most associated CpGs for metals were located in the genic regions (GRs). The number of mapped genes was lower than the number of sites mapped in the respective GRs because some genes had more than one differentially associated DMP. All metals were associated with DNAse hypersensitive sites (DHS) and enhancers. Some sites were classified as DHS and enhancer sites (Table 3).

Figure 1 illustrates the Manhattan plot, depicting the distribution of differential DMPs associated with arsenic, highlighting CpG sites with significance levels above the threshold of $-\log10(p) = 10^{-6}$.

Differential methylation analysis between individuals with and without obesity

In our investigation, we identified 2045 DMPs associated with arsenic that were also differentially methylated in individuals with obesity (Fig. 2A). Among these, 1092 DMPs were hypomethylated and 953 DMPs were hypermethylated (Fig. 2B). Subsequent analyses showed that 922 sites were situated within promoter regions, specifically within the TSS1500 and TSS200 regions. Notably, among these, 57 sites exhibited hypermethylation (methylation change $\Delta\beta > 0.05$), while 159 sites demonstrated hypomethylation (methylation change $\Delta\beta < -0.05$); those sites were functionally enriched in Figs 3 and 4.

Figure 3 illustrates the functional enrichment analysis of hypomethylated DMPs in individuals with obesity. We have marked the mentioned pathways or terms in the figure with an asterisk to improve visualization. Hypomethylation was linked to insufficient sleep, smoking, colorectal laterally spreading tumours, gestational diabetes mellitus, and polycystic ovary syndrome. These findings suggest shared epigenetic alterations contributing to the complex relationship between obesity and these specific traits (Fig. 3A).

In the GO analysis (Fig. 3B), the hypomethylated group showed significant associations with GO terms related to norepinephrine uptake, suggesting a potential link between the ingestion and

Table 2. Toxic metals serum concentration[a]

Metals	With obesity ($n = 15$)	Without obesity ($n = 17$)	Reference[b] [17]
Cadmium (μg/L)	0.03 ± 0.02	0.21 ± 0.47	0.014–1.15
Mercury (μg/L)	0.26 ± 0.26[c]	0.63 ± 0.38	0.38–4.60
Arsenic (μg/L)	6.06 ± 1.10[c]	5.72 ± 0.54	3.39–11.84

[a]Values are shown in mean ± standard deviation. [b]Refer to the values of ranges observed in the literature, values for the Brazilian population. [c]Unpaired *t*-test: $P < .05$ related to the control group was considered significantly different.

Table 3. DMPs associated with metals[a]

Metal	DMPs	GR (%)	DHS	Enhancer	FDR1	Genes (n)
Cadmium	351	266 (75.7%)	118	25	3.0×10^{-5}	248
Arsenic	26 950	22 089 (81.9%)	4044	4920	3.1×10^{-3}	2545

[a]DMP obtained by linear regression analysis, $P < .05$, FDR < 0.05 (Benjamini–Hochberg analysis). FDR1: FDR of the associations. Genes (n) = number of mapped genes.

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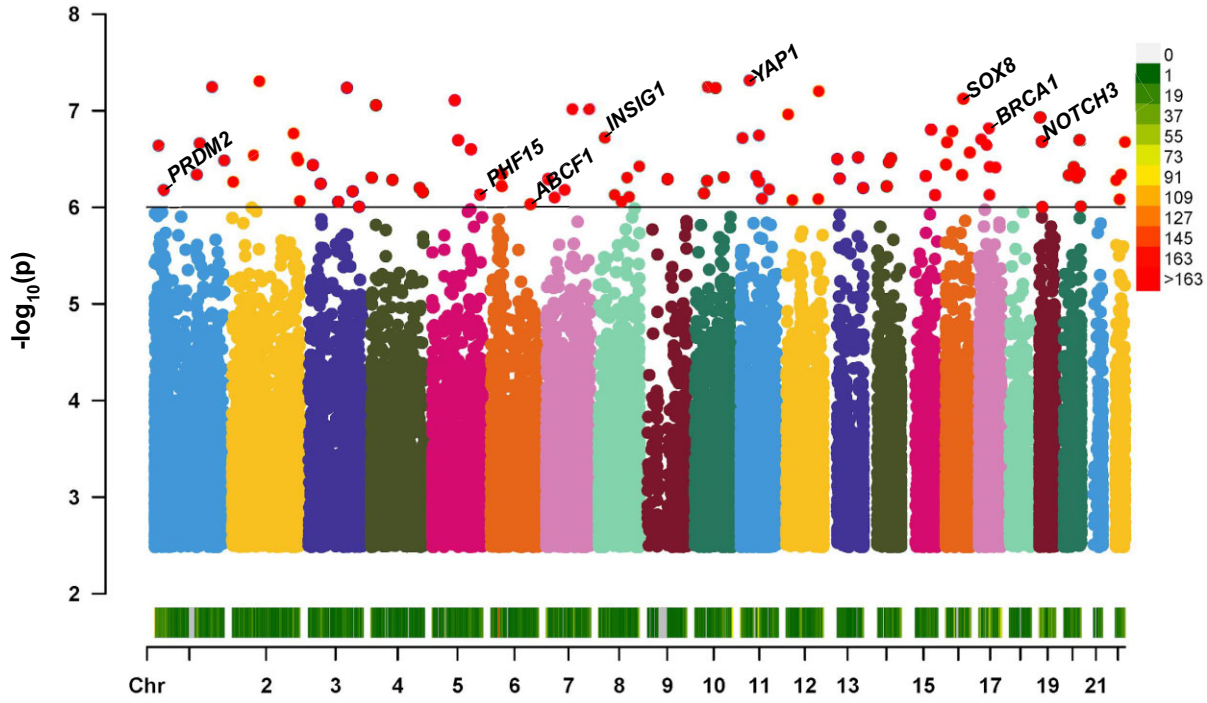

Figure 1. Manhattan plot from arsenic-associated DMPs.

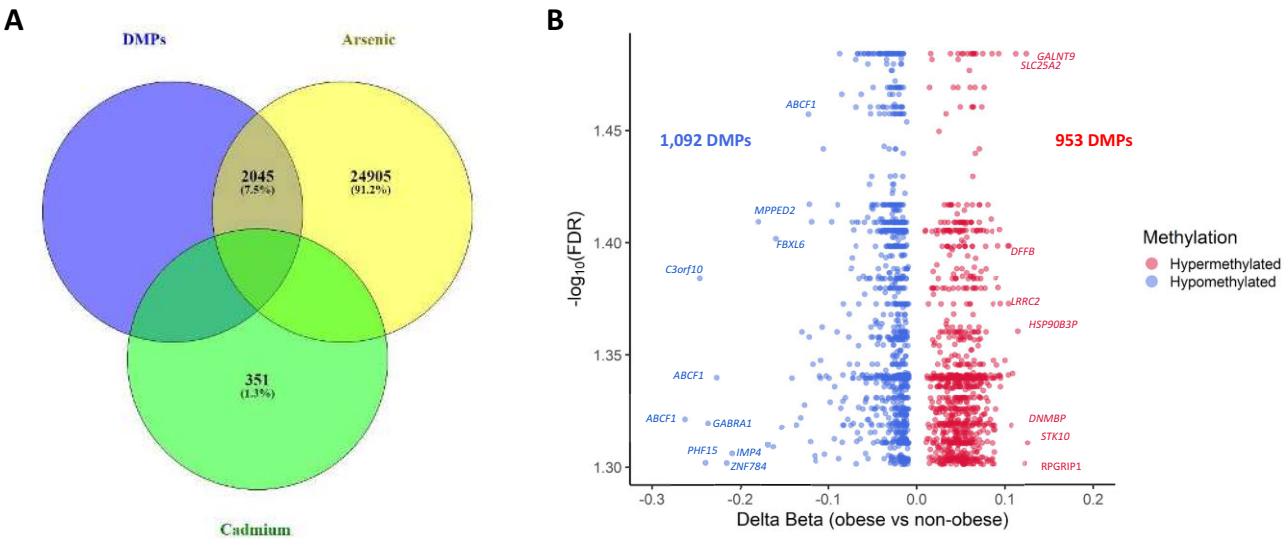

Figure 2. Differential methylation analysis. (A) Overlapping among signature DMPs for obesity, arsenic, and cadmium. (B) Volcano plot showing the unique overlap between obesity and arsenic. The Venn diagram visually represents the overlap and unique sets of differential methylated positions (DMPs) in individuals with obesity compared to normal-weight individuals (3329 DMPs were previously reported from the same dataset [9], and 2045 were differentially methylated and associated with arsenic). The intersection of DMPs and arsenic shows the CpG sites that are differentially methylated in individuals with obesity and were commonly and independently associated with arsenic (2045 CpG sites). Cadmium had no intersection with DMPs.

appetite synapse mechanisms and altered norepinephrine regulation, as well as obesity. The analysis also highlighted GO terms related to formyltransferase and IMP cyclohydrolase activity, highlighting molecular activities that potentially contribute to the complex landscape of arsenic-obesity-associated processes (Fig. 3B).

Significant functional enrichment was observed in KEGG pathways associated with obesity (Fig. 3C), including the Notch signalling pathway, Tumor necrosis factor (TNF) signalling pathway, thermogenesis, and Wnt signalling pathway. The Notch signalling pathway, known for its role in cellular differentiation and development, suggests potential implications for obesity-related processes. Concurrently, the TNF signalling pathway, which is central to inflammation and the immune response, aligns with the recognized link between obesity and chronic inflammation (Fig. 3C).

Functional enrichment analysis (trait category) of hypermethylated DMPs revealed associations with lifestyle-related traits, including exercise and meditation, as well as environmental fac-

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Figure 3. Enrichment analysis of hypomethylated sites. DMC: number of probe sites that are differentially methylated; DMG: number of genes differentially methylated in the respective pathways.

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Figure 4. Enrichment analysis of hypermethylated sites. DMC: number of probe sites that are differentially methylated; DMG: number of genes differentially methylated in the respective pathways.

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Table 4. Top 20 common CpGs associated with arsenic located in the promoter region[a]

CpG ID	Gene	Feature	Δβ[b]	P-value	P-adjusted value	Gene expression (FC)	P-value
cg14793544	*ABCF1*	TSS1500	−0.26	<.001	.007	10.25	.001
cg18015044	*ABCF1*	TSS1500	−0.23	<.001	.007		
cg00678158	*ABCF1*	TSS1500	−0.18	<.001	.007		
cg21752471	*PHF15*	TSS200	−0.24	<.001	.006	–	–
cg20670292	*IMP4*	TSS1500	−0.17	<.001	.006	−0.69	.345
cg22169206	*ZNF784*	TSS1500	−0.16	<.001	.006	0.04	.95
cg11306428	*ZNF784*	TSS1500	−0.14	<.001	.006	–	–
cg24780865	*MTIF3*	TSS1500	−0.16	<.001	.006	−0.01	.999
cg02688348	*SSRP1*	TSS200	−0.14	<.001	.006	−2.38	.032
cg04427462	*PRKD2*	TSS1500	−0.13	<.001	.006	−0.38	.330
cg18642567	*RPGRIP1*	TSS1500	0.13	<.001	.033	0.53	.672
cg04090794	*HSP90B3P*	TSS1500	0.12	<.001	.050	–	–
cg21943599	*C1orf125*	TSS1500	0.11	<.001	.042	–	–
cg20929545	*HMBS*	TSS1500	0.10	<.001	.039	−2.97	.776
cg00152515	*ROR1*	TSS1500	0.09	<.001	.046	−0.02	.971
cg07161888	*AKTIP*	TSS200	0.09	<.001	.048	−0.55	.448
cg27581762	*BRCA1*	TSS1500	0.09	<.001	.042	−0.76	.543
cg26135012	*ARHGAP20*	TSS200	0.09	<.001	.048	–	–
cg20932762	*MSH4*	TSS1500	0.09	<.001	.045	–	–
cg01217204	*TTL*	TSS1500	0.09	<.001	.050	−3.91	.445

[a]CpG ID: CpG Illumina identification number. Δβ: delta beta is the difference of beta values, with normal weight as a reference; negative values: mean hypomethylated sites; positive values: mean hypermethylated sites. [b]All differentially methylated CpGs had $P \leq .0001$ and P-adjusted value < .05. The top independently associated methylated sites refer to the first 10 sites that are hypermethylated or hypomethylated; an analysis was done with independent linear regression.

tors such as air pollution. Hypermethylated sites showed significant links to pathways associated with cancer, rheumatoid arthritis, gestational diabetes, prenatal arsenic exposure, in-utero arsenic exposure, and dilated cardiomyopathy. These findings suggest a multifaceted relationship between hypermethylation patterns and various lifestyle factors, environmental exposures, and disease pathways (Fig. 4A).

Functional enrichment analysis (GO category) revealed that hypermethylated DMPs are associated with obesity. They exhibit significant links to pathways such as Wnt signalling, cellular response to DNA damage stimulus, feeding behaviour, and T-cell differentiation. These findings suggest a potential connection between obesity and arsenic exposure, indicating that epigenetic alterations may contribute to shared molecular mechanisms (Fig. 4B). Functional enrichment analysis (KEGG category) also identified noteworthy associations between hypermethylated DMPs, the Notch pathway, certain cancers, taste transduction, and the JAK-STAT signalling pathway (Fig. 4C).

To provide more detailed insight, we focused on the top 20 DMPs associated with arsenic in individuals with obesity. Table 4 provides a comprehensive summary of these sites, highlighting their methylation status in the promoter regions. These findings highlight the most significant associations between arsenic exposure and DNA methylation alterations in individuals with obesity, particularly in key regulatory regions near the transcription start site (TSS). Gene expression analysis revealed significant findings for several key genes, with *ABCF1* standing out due to its markedly elevated expression levels (fold change = 10.25, $P = .001$).

Discussion

Our case–control study identified differences between groups in serum metal concentrations and patterns of metal-related DMPs. Our study brings novel information of public interest, combining data on metal exposure, obesity, and DNA methylation. Toxic metals may also represent risk factors for the development of obesity and comorbidities. In this study, we observed higher levels of arsenic in participants with obesity and higher levels of mercury in individuals without obesity.

Eick and Steinmaus [18] assert that while experimental studies indicate arsenic's potential involvement in obesity pathogenesis, such associations have yet to be substantiated in human research. A cross-sectional study with 524 adults in Bangladesh showed that chronic arsenic exposure was associated with increased adiposity (measured via waist circumference, triceps skinfold thickness, and serum leptin), despite no change in BMI. The excess fat accumulation was accompanied by reduced muscle mass and increased risk of insulin resistance, hypertension, and hyperglycaemia. These findings suggest that arsenic may promote sarcopenic obesity, a novel pathway for cardiometabolic risk [19]. According to research by Rothenberg *et al.* [11], blood mercury levels were 22% lower in adults with obesity than those classified as overweight or having a normal weight. Research conducted by Yang *et al.* [20] suggests that elevated mercury levels in individuals without obesity may potentially exert metabolic effects and be associated with non-alcoholic liver disease in the KoNEHS cohort.

The SVD analysis revealed that toxic metals contributed to the variability in DNA methylation data. A similar approach was used to show associations between lead and DNA methylation [21]. This study highlights that other toxic metals can also have significant effects in the SVD analysis. Considering all associated sites with metals, we observed a common association in genic and regulatory regions such as DHS and enhancer regions, suggesting that these compounds might have functional relevance.

Arsenic, a non-mutagenic human carcinogen, elicits tumour formation through alternative mechanisms. An expanding body of research indicates that its carcinogenic potential may stem from epigenetic modifications, particularly alterations in DNA methylation patterns [22]. This study evaluated whether exposure to metals could overlap with differential DNA methylation changes between individuals with and without obesity. We observed associations between arsenic and obesity-related DMPs, but not with cadmium. It is known that arsenic may be related to obesity and diabetes [23] and that individuals with obesity can

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be more susceptible to arsenic-related cancer later in life [24]. Arsenic-related interactions with cancer and metabolic outcomes were confirmed in this study through functional enrichment analysis.

In this study, we decided to functionally enrich only differentially methylated sites individuals with obesity and associated with arsenic, instead of all observed associations. The functional enrichment analysis (trait, GO, and KEGG categories) (Figs 3 and 4) revealed intricate pathways, epigenetic factors, immunological responses, and environmental influences that may be related to obesity and arsenic exposure. These findings highlight the complex interplay between epigenetic effects, environmental factors, and lifestyle choices in the development and progression of various correlated diseases and disorders.

Islam *et al.* [13] summarized in their review that arsenic's carcinogenic effects are related to both hypo- and hypermethylation patterns, and Martin *et al.* [25] referred that they concluded, based on their review, that arsenic can be a diabetogen and has different associations with DNA methylation.

Figure 3 summarizes the main findings for the 159 hypomethylated sites in promoter regions in individuals with obesity that were also associated with arsenic. In Fig. 3A, traits related to cancer and metabolic diseases were significantly enriched. In Fig. 3C, besides thermogenesis, some immune and cancer-related pathways, interestingly, we find associations of the enriched DMPs with cellular differentiation pathways such as notch and Wnt, suggesting that the differential effects of arsenic in individuals with obesity may be related to intricate molecular events. The functional enrichment analysis also revealed that hypomethylation is a site related to cellular differentiation pathways and may be a mechanism interlinking adipogenesis and arsenic exposure. Cheng *et al.* [26] reported that *in vitro*, arsenic trioxide treatment was able to disrupt the adipogenesis/osteogenesis pathways in mesenchymal stem cells, while the exposure *in vivo* showed initially a decrease and then an increase in gene expression of adipogenesis-related targets [27]

The hypermethylated DMPs located in promoter regions were also enriched, and Fig. 4 summarizes the analysis findings. Figure 4A shows the diverse spectrum of conditions retrieved in the study, including different kinds of cancer, gestational diabetes mellitus, polycystic ovary syndrome, meditation practice, and exercise, revealing significant associations with metabolic traits, cancer, and lifestyle. Notably, prenatal and *in utero* arsenic exposure emerge as crucial factors, potentially linking obesity and connecting the phenotype with possible transgenerational effects. Arsenic has been documented by its capacity to induce DNA methylation alterations, which disrupt endocrine function [28] and promote carcinogenesis [29]. As suggested by Boekelheide *et al.* [30], arsenic has epigenetic effects in early-life and later-life exposure.

The enrichment of hypermethylated sites from this study suggests two traits related to early-life exposure to arsenic, indicating that the prenatal and *in utero* stages are critical for arsenic-related epigenetic effects. The Wnt/β-catenin pathway has a central role in inhibiting adipocyte differentiation. The activation of canonical Wnt signalling prevents the expression of PPARγ and C/EBPα, two transcription factors essential for adipogenesis [31]. Our identification of hypomethylated DMPs in Wnt-related genes suggests that arsenic may disrupt this inhibitory pathway, promoting uncontrolled adipocyte formation and contributing to obesity. Prior *in vitro* studies have demonstrated that arsenic trioxide exposure can inhibit Wnt signalling and shift mesenchymal stem cells towards adipogenic lineage [26]. These findings suggest that arsenic may deregulate the adipogenesis–osteogenesis balance, which could contribute to metabolic dysfunction.

TNF-α is a key pro-inflammatory cytokine involved in chronic, low-grade inflammation associated with obesity. It promotes insulin resistance by phosphorylating IRS-1, reducing its activity, and disrupting downstream insulin signalling [32]. Our study revealed an enrichment of TNF signalling pathway genes among the arsenic-associated DMPs in individuals with obesity, suggesting that arsenic may potentiate inflammatory pathways that are already elevated in obesity. Arsenic has been shown to upregulate *TNF-α* expression and activate *NF-κB* signalling, further intensifying systemic inflammation in cancer [13].

Table 4 shows the top 20 differentially methylated sites in the promoter regions, both the top 10 hypomethylated and hypermethylated sites. We observed that individuals with obesity had lower methylation levels of the DMPs located in the promoter region of the *ABCF1* gene when compared to individuals without obesity. This points to a differential response between groups. The mechanisms explaining such differences are still unknown. Still, they may be related to the previously reported alterations in ABC transporters in patients with obesity, which are independently triggered by high-fat diets in adipose tissue. This can be linked to fat and bone mass remodelling [14]. These findings suggested that the diet should be evaluated as a covariate in metal mixture effect studies to better understand macronutrient consumption, environmental exposures, and obesity.

On the other hand, the hypermethylated sites were associated with homologous recombination, Fanconi anaemia, and breast cancer. Selmin *et al.* [33] demonstrated that arsenic exposure in MCF7 breast cancer cells leads to promoter hypermethylation of *BRCA1*, contributing to gene silencing and disruption of the FA/BRCA DNA repair pathway. Similarly, Peremartí *et al.* [34] showed that chronic low-level arsenic exposure induces *BRCA1* promoter hypermethylation and silencing in the same cell line model. Our study also found BRCA1 hypermethylation patterns in peripheral blood DNA from individuals with obesity exposed to arsenic. This expands prior evidence by suggesting that *BRCA1* may be a systemic epigenetic target of arsenic, not limited to *in vitro* cancer models, and potentially relevant in metabolic contexts such as obesity-related dysregulation.

Domingo-Relloso *et al.* [16] in their population-based cohort study found associations between blood arsenic levels and methylation changes at specific CpG sites, particularly in genes involved in inflammation and cardiometabolic health. However, the study did not focus on obesity or stratify by obesity status. Our study extends these findings by evaluating obesity as a differential context, identifying over 2000 arsenic-associated DMPs specifically in obese individuals, and linking these to both promoter hypomethylation (e.g. *ABCF1*) and enrichment of pathways such as Wnt and TNF signalling, which are directly implicated in adipogenesis and inflammation. This supports the idea that obesity may amplify the epigenetic effects of arsenic exposure, a direction not explored in previous studies.

The relationship between arsenic exposure, obesity, and DNA methylation reveals a complex multiple biological mechanisms. Arsenic, a known environmental pollutant, has been linked to epigenetic modifications that may contribute to obesity-related traits. This study identified significant hypomethylation in DMPs from pathways such as Wnt and Notch signalling, both of which are critical for cellular differentiation. These findings suggest that arsenic may disrupt adipogenesis, potentially contributing to obesity-related phenotypes by altering differentiation and inflammatory responses. On the other hand, hypermethylated DMPs

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in genes such as *BRCA1* highlight potential disruptions in DNA repair mechanisms, which may contribute to cancer risks and metabolic disorders. These signatures not only reflect arsenic's role as an epigenetic modifier but also emphasize its broad impact on metabolic health. We also observed enrichment of hypermethylated DMPs linked to prenatal and *in utero* arsenic exposure, supporting the idea that early-life environmental exposures can induce persistent epigenetic changes with potential effects across the life course [35]. These findings align with prior work suggesting epigenetic inheritance of environmental risks [36] and highlight the need for future longitudinal cohort studies in pregnant populations or children living in contaminated areas [37].

Given the association between arsenic and metabolic dysregulation, our findings reinforce the need for preventive interventions in regions with arsenic exposure, particularly in middle-income countries such as Brazil, where infrastructure limitations pose challenges to water safety. Screening individuals in such areas for epigenetic markers of exposure, and coupling this with nutritional or behavioural interventions, may reduce the impact of chronic exposure on obesity-related diseases.

The study was limited by its constrained sample size, which hindered the ability to make extensive adjustments. However, we specifically focused on including only women to mitigate this limitation. This decision was based on the predominance of female patients in our outpatient clinic and their higher compliance with clinical follow-up and medical procedures, which is consistent with previous findings on sex differences in healthcare utilization and engagement [38, 39]. Additionally, sex-based biological differences, particularly in DNA methylation patterns, could introduce confounding variability in small samples, especially in the context of complex environmental exposures such as arsenic. By restricting the sample to one sex, we aimed to reduce biological heterogeneity and enhance the statistical power and interpretability of our epigenetic analyses [40]. Although this reduces the generalizability of our results to male populations, it strengthens internal validity and establishes a robust foundation for future studies with larger, sex-diverse cohorts. Age was not significant in the batch effect analysis, so we standardized all data by accounting for cell-type estimation.

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Author contributions

Natália Yumi Noronha (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Investigation [equal], Writing—original draft [equal], Writing—review & editing [equal]), Guilherme da Silva Rodrigues (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Methodology [equal], Writing—original draft [equal], Writing—review & editing [equal]), Lígia Moriguchi Watanabe (Conceptualization [equal], Formal analysis [equal], Methodology [equal], Writing—original draft [equal], Writing—review & editing [equal]), Isabella Harumi Yonehara Noma (Conceptualization [equal], Investigation [equal], Methodology [equal], Writing—original draft [equal], Writing—review & editing [equal]), Jean-Baptiste Cazier (Funding acquisition [equal], Investigation [equal], Project administration [equal], Writing—original draft [equal], Writing—review & editing [equal]), Chanachai Sae-Lee (Data curation [equal], Formal analysis [equal], Validation [equal], Writing—original draft [equal], Writing—review & editing [equal]), Pitaksin Chitta (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Writing—original draft [equal], Writing—review & editing [equal]), Vanessa Aparecida Batista Pereira (Conceptualization [equal], Data curation [equal], Methodology [equal], Writing—original draft [equal], Writing—review & editing [equal]), Marcela Augusta de Souza Pinhel (Formal analysis [equal], Project administration [equal], Writing—original draft [equal], Writing—review & editing [equal]), Luísa Maria Diani (Data curation [equal], Writing—original draft [equal], Writing—review & editing [equal]), Fernando Barbosa (Funding acquisition [equal], Investigation [equal], Project administration [equal], Supervision [equal], Writing—original draft [equal], Writing—review & editing [equal]), Torsten Plösch (Funding acquisition [equal], Investigation [equal], Project administration [equal], Supervision [equal], Writing—original draft [equal], Writing—review & editing [equal]), and Carla Barbosa Nonino (Funding acquisition [equal], Methodology [equal], Project administration [equal], Supervision [equal], Writing—original draft [equal], Writing—review & editing [equal]).

Supplementary data

Supplementary data is available at *EnvEpig* online.

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Data availability

The data underlying this article are available in the article and in its online supplementary material.

References

1. Dutta S, Gorain B, Choudhury H *et al.* Environmental and occupational exposure of metals and female reproductive health. *Environ Sci Pollut Res* 2022;**29**:62067–92.
2. Zhou J, Meng X, Deng L *et al.* Non-linear associations between metabolic syndrome and four typical heavy metals: data from NHANES 2011–2018. *Chemosphere* 2022;**291**:132953.
3. Chowdhury S, Mazumder MAJ, Al-Attas O *et al.* Heavy metals in drinking water: occurrences, implications, and future needs in developing countries. *Sci Total Environ* 2016;**569–570**:476–88.
4. Ruiz-Hernandez A, Kuo CC, Rentero-Garrido P *et al.* Environmental chemicals and DNA methylation in adults: a systematic review of the epidemiologic evidence. *Clin Epigenetics* 2015;**7**:55.
5. Li E, Zhang Y. DNA methylation in mammals. *Cold Spring Harb Perspect Biol* 2014;**6**:a019133.
6. Bird A. DNA methylation patterns and epigenetic memory. *Genes Dev* 2002;**16**:6–21.

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7. Dutta S, Ruden DM. Heavy metals in umbilical cord blood: effects on epigenetics and child development. *Cells* 2024;**13**:1775.
8. Wahl S, Drong A, Lehne B *et al.* Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity. *Nature* 2017;**541**:81–86.
9. Park SS, Skaar DA, Jirtle RL *et al.* Epigenetics, obesity and early-life cadmium or lead exposure. *Epigenomics* 2017;**9**:57–75.
10. Seki Y, Williams L, Vuguin PM *et al.* Minireview: epigenetic programming of diabetes and obesity: animal models. *Endocrinology* 2012;**153**:1031–38.
11. Rothenberg SE, Korrick SA, Fayad R. The influence of obesity on blood mercury levels for U.S. non-pregnant adults and children: NHANES 2007–2010. *Environ Res* 2015;**138**:173–80.
12. Noronha NY, da Silva Rodrigues G, de Souza Pinhel MA *et al.* Sample preparation to bioinformatics analysis of DNA methylation: association strategy for obesity and related trait studies. *J Vis Exp* 2022;62598.
13. Islam R, Zhao L, Wang Y *et al.* Epigenetic dysregulations in arsenic-induced carcinogenesis. *Cancers* 2022;**14**:4502.
14. Montalvany-Antonucci CC, Zicker MC, Ferreira AVM *et al.* High-fat diet disrupts bone remodeling by inducing local and systemic alterations. *J Nutr Biochem* 2018;**59**:93–103.
15. Li M, Zou D, Li Z *et al.* EWAS Atlas: a curated knowledgebase of epigenome-wide association studies. *Nucleic Acids Res* 2019;**47**:D983–8.
16. Domingo-Relloso A, Makhani K, Wang W *et al.* Arsenic exposure, blood DNA methylation, and cardiovascular disease. *Circ Res* 2023;**132**:271–85. https://doi.org/10.1161/CIRCRESAHA.122.320991
17. Almeida Lopes ACB, Martins AC, Urbano MR *et al.* Blood reference values for metals in a general adult population in southern Brazil. *Environ Res* 2020;**183**:109178.
18. Eick SM, Steinmaus C. Arsenic and obesity: a review of causation and interaction. *Curr Environ Health Rep* 2020;**7**:343–51.
19. Jubayar AM, Khan S, Sadi J *et al.* Novel evidence of arsenic-related excess adiposity and its implication in the risk of cardiometabolic diseases. *Environ Res* 2025;**228**:121059.
20. Yang YJ, Yang EJ, Park K *et al.* Association between blood mercury levels and non-alcoholic fatty liver disease in non-obese populations: the Korean National Environmental Health Survey (KoNEHS) 2012–2014. *Int J Environ Res Public Health* 2021;**18**:6412.
21. Yumi Noronha N, da Silva Rodrigues G, Harumi Yonehara Noma I *et al.* 14-weeks combined exercise epigenetically modulated 118 genes of menopausal women with prediabetes. *Front Endocrinol* 2022;**13**:895489.
22. Saintilnord WN, Fondufe-Mittendorf Y. Arsenic-induced epigenetic changes in cancer development. *Semin Cancer Biol* 2021;**76**:195–205.
23. Farkhondeh T, Samarghandian S, Azimi-Nezhad M. The role of arsenic in obesity and diabetes. *J Cell Physiol* 2019;**234**:12516–29.
24. Steinmaus C, Castriota F, Ferreccio C *et al.* Obesity and excess weight in early adulthood and high risks of arsenic-related cancer in later life. *Environ Res* 2015;**142**:594–601.
25. Martin EM, Stýblo M, Fry RC. Genetic and epigenetic mechanisms underlying arsenic-associated diabetes mellitus: a perspective of the current evidence. *Epigenomics* 2017;**9**:701–10.
26. Cheng H, Qiu L, Zhang H *et al.* Arsenic trioxide promotes senescence and regulates the balance of adipogenic and osteogenic differentiation in human mesenchymal stem cells. *Acta Biochim Biophys Sin* 2011;**43**:204–209.
27. Shearer JJ, Figueiredo Neto M, Umbaugh CS *et al.* In vivo exposure to inorganic arsenic alters differentiation-specific gene expression of adipose-derived mesenchymal stem/stromal cells in C57BL/6J mouse model. *Toxicol Sci* 2017;**157**:172–82.
28. Bommarito PA, Martin E, Fry RC. Effects of prenatal exposure to endocrine disruptors and toxic metals on the fetal epigenome. *Epigenomics* 2017;**9**:333–50.
29. Zhou Q, Xi S. A review on arsenic carcinogenesis: epidemiology, metabolism, genotoxicity and epigenetic changes. *Regul Toxicol Pharmacol* 2018;**99**:78–88.
30. Boekelheide K, Blumberg B, Chapin RE *et al.* Predicting later-life outcomes of early-life exposures. *Environ Health Perspect* 2012;**120**:1353–61.
31. Ross SE, Hemati N, Longo KA *et al.* Inhibition of adipogenesis by Wnt signaling. *Science* 2000;**289**:950–53.
32. Hotamisligil GS, Peraldi P, Budavari A *et al.* IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α–and obesity-induced insulin resistance. *Science* 1996;**271**:665–68.
33. Selmin OI, Donovan MG, Skovan BA *et al.* Arsenic-induced BRCA1 CpG promoter methylation is associated with the downregulation of ERα and resistance to tamoxifen in MCF7 breast cancer cells. *Int J Oncol* 2019;**55**:681–92.
34. Peremartí J, Felip I, Martínez-Cardús A *et al.* Chronic exposure to low levels of arsenic induces BRCA1 promoter hypermethylation and silencing in MCF7 breast cancer cells. *Toxicol Sci* 2014;**142**:378–88.
35. Broberg K, Ahmed S, Engström K *et al.* Arsenic exposure in early pregnancy alters genome-wide DNA methylation in cord blood, particularly in boys. *J Dev Orig Health Dis* 2014;**5**:288–98. https://doi.org/10.1017/S2040174414000221
36. Nohara K, Suzuki T, Okamura K. Gestational arsenic exposure and paternal intergenerational epigenetic inheritance. *Toxicol Appl Pharmacol* 2020;**398**:115007. https://doi.org/10.1016/j.taap.2020.115007
37. Perera F, Herbstman J. Prenatal environmental exposures, epigenetics, and disease. *Reprod Toxicol* 2011;**31**:363–73. https://doi.org/10.1016/j.reprotox.2010.09.009
38. Bertakis KD, Azari R, Helms LJ *et al.* Gender differences in the utilization of health care services. *J Fam Pract* 2000;**49**:147–52.
39. Courtenay WH. Constructions of masculinity and their influence on men's well-being: a theory of gender and health. *Soc Sci Med* 2000;**50**:1385–401.
40. Cavalli G, Heard E. Advances in epigenetics link genetics to the environment and disease. *Nature* 2019;**571**:489–99.

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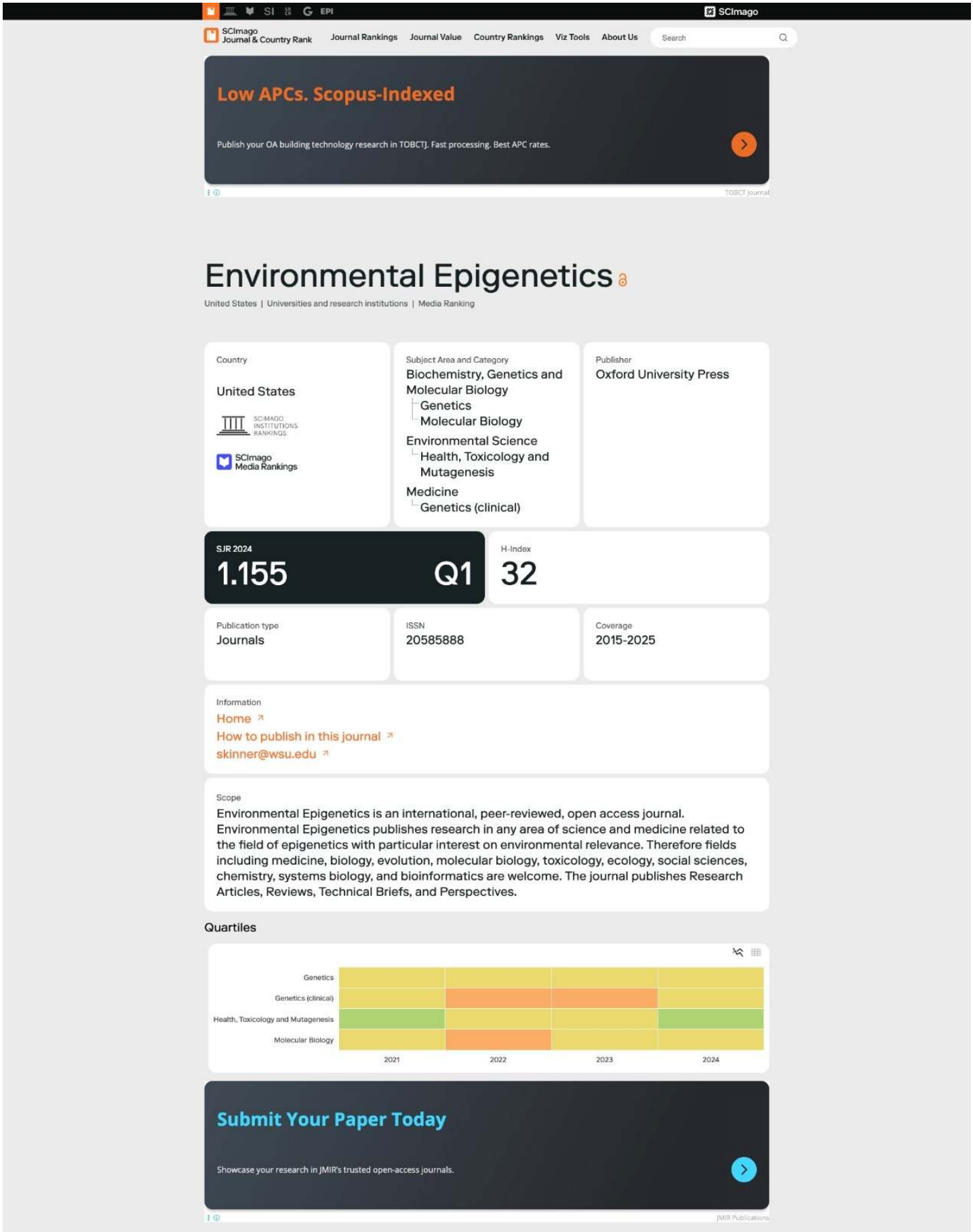
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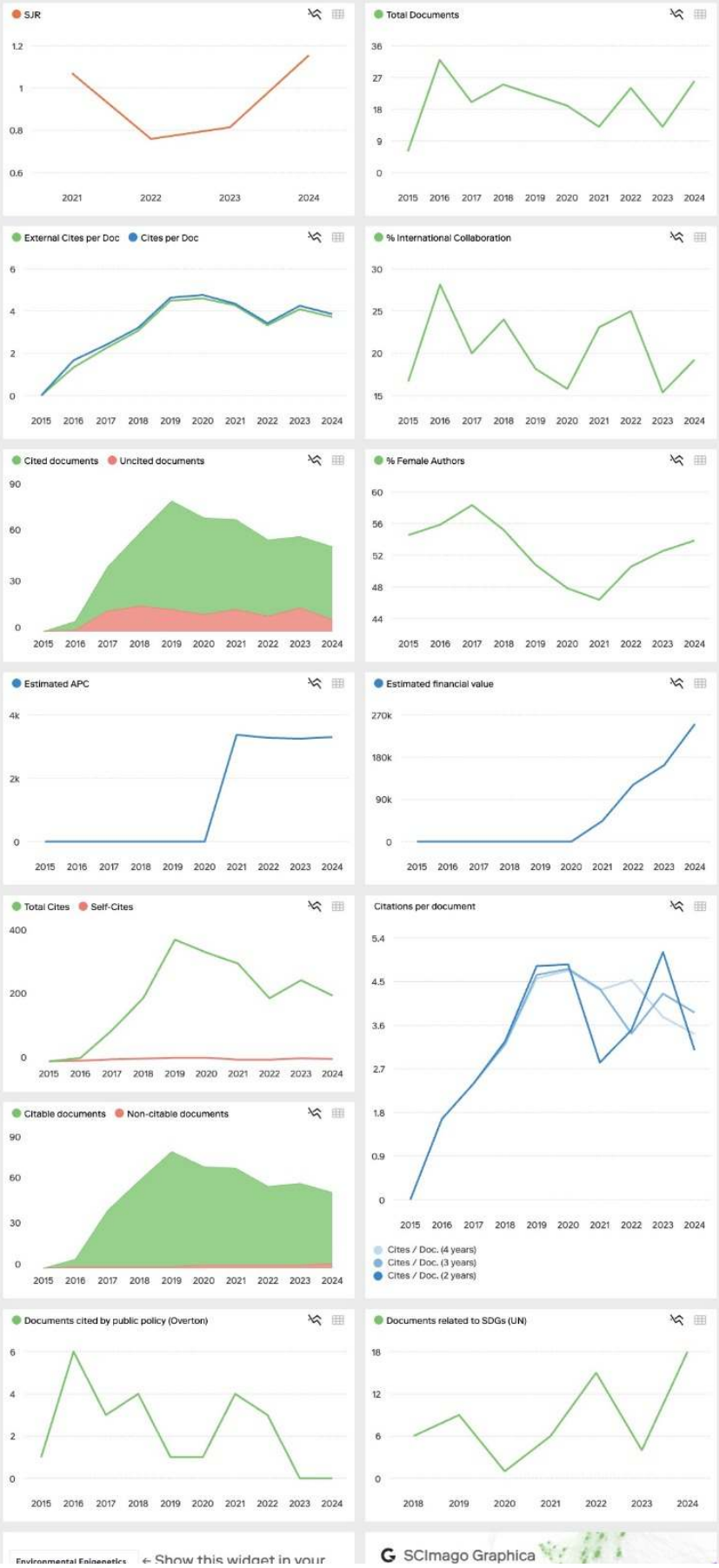
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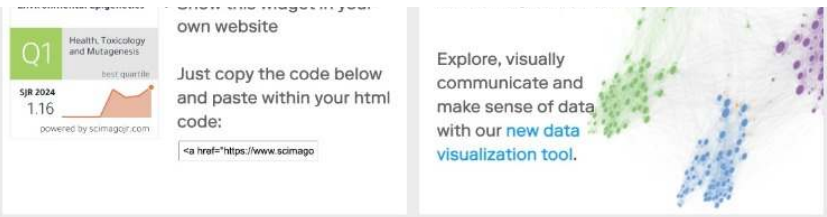

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14-Week exercise training modifies the DNA methylation levels at gene sites in non-Alzheimer's disease women aged 50 to 70 years

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ABSTRACT

Exercise training emerges as a key strategy in lifestyle modification, capable of reducing the risk of developing Alzheimer's disease (AD) due to risk factors such as age, family history, genetics and low level of education associated with AD. We aim to analyze the effect of a 14-week combined exercise training (CT) on the methylation of genes associated with AD in non-alzheimer's disease women. CT sessions lasted 60 min, occurring three times a week for 14 weeks. Forty non-Alzheimer's disease women aged 50 to 70 years (60.7 ± 4.1 years) with a mean height of 1.6 ± 0.1 m, mean weight of 73.12 ± 9.0 kg and a mean body mass index of 29.69 ± 3.5 kg/m2, underwent two physical assessments: pre and post the 14 weeks. DNA methylation assays utilized the EPIC Infinium Methylation BeadChip from Illumina. We observed that 14 weeks of CT led to reductions in systolic ($p = 0.001$) and diastolic ($p = 0.017$) blood pressure and improved motor skills post-intervention. Among 25 genes linked to AD, CT induced differentially methylated sites in 12 genes, predominantly showing hypomethylated sites (negative β values). Interestingly, despite hypomethylated sites, some genes exhibited hypermethylated sites (positive β values), such as *ABCA7*, *BDNF*, and *WWOX*. A 14-week CT regimen was adequate to induce differential methylation in 12 CE-related genes in healthy older women, alongside improvements in motor skills and blood pressure. In conclusion, this study suggest that combined training can be a strategy to improve physical fitness in older individuals, especially able to induce methylation alterations in genes sites related to development of AD. It is important to highlight that training should act as protective factor in older adults.

1. Introdution

Alzheimer's disease (AD) stands as the most prevalent form of dementia among the older population, representing 60 to 80 % of diagnosed cases in the world (Duthey, 2013). Global prevalence estimates exceed 45 million individuals. With an aging global population, a threefold rise in disease prevalence by 2050 is anticipated (Jakovljevic et al., 2021; El-Hayek et al., 2019). The economic impact of AD is substantial, evidenced by an estimated cost of $818 billion due to clinical and caregiving expenses (El-Hayek et al., 2019).

Therefore, it is essential to allocate efforts to research and find effective interventions for disease prevention. Exercise training emerges as a key strategy in lifestyle modification, capable of reducing the risk of developing AD arising from risk factors such as age, familial history, genetics, and low educational attainment associated with AD (Crous-Bou et al., 2017). Neurological and vascular adaptations during exercise

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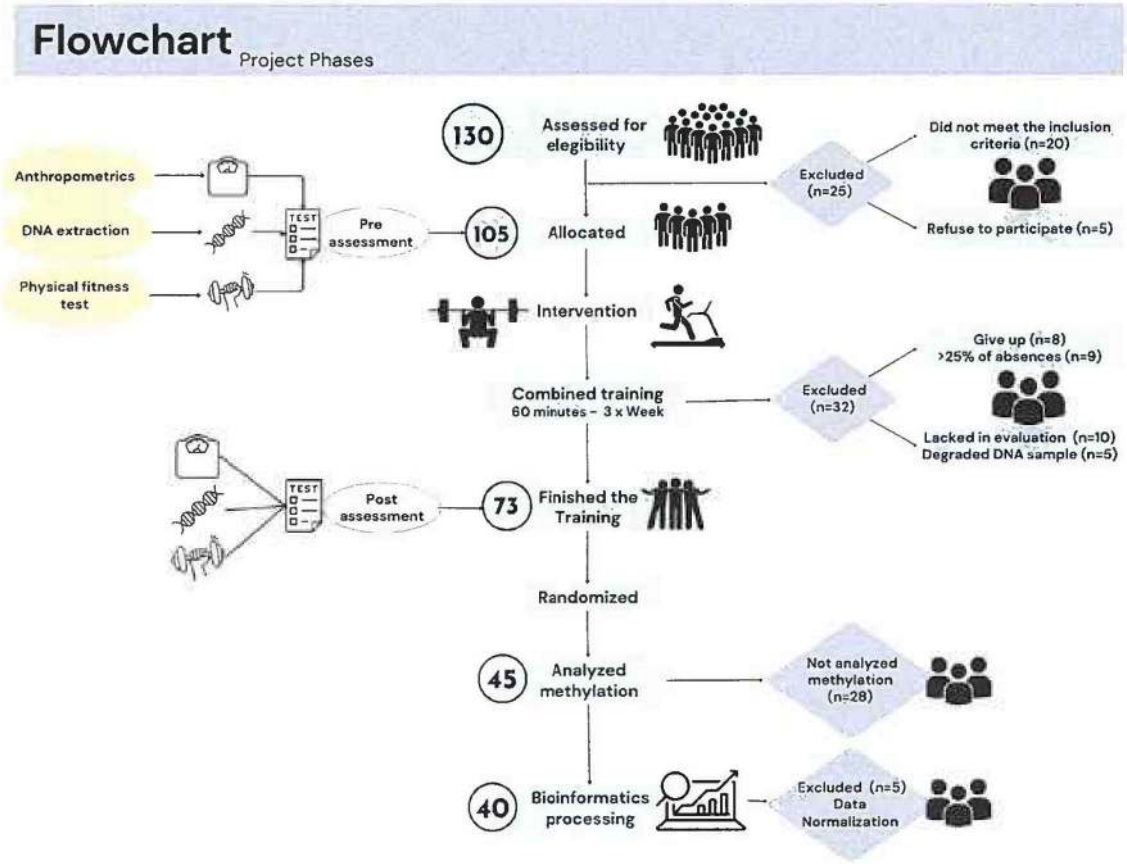

Fig. 1. Flowchart and experimental design.

training foster neurogenesis, and synaptic plasticity, diminish inflammatory processes, and mitigate cellular damage due to oxidative stress, important processes in prevention AD prevention (Foster et al., 2011).

A recent meta-analysis highlights that engaging in moderate-intensity aerobic and resistance exercises for at least 45 min, multiple times weekly, enhances cognition in adults aged 50 and above (Northey et al., 2018). Additionally, exercise training contributes to AD prevention even in cases of high genetic risk, an unmodifiable factor (Hamer and Chida, 2009). The meta-analysis that analyzed 16 studies (n = 160,000 participants) found a 45 % reduction in the risk of developing AD due to the regular practice of physical activity (Hamer and Chida, 2009). After 3.5 years of monitoring 716 older subjects, one study reported that individuals with low daily physical activity levels were 53 % more likely to suffer AD than those who reported more active lives (Buchman et al., 2012). It is still estimated, that 3 % of dementia cases could be prevented by increasing levels of free-living physical activity (Liang et al., 2020; Livingston et al., 2020).

It is well know that exercise training and others external factors such as dietary habits, smoking, or alcohol consumption interact with the genome and affect the phenotype via modifications in epigenetic mechanisms (DNA methylation, histone modifications, chromatin remodeling and non-coding RNA), which can suppress or increase gene expression (Wu et al., 2023).

Potentially, DNA methylation is the epigenetic mechanism that has the greatest impact on the regulation of gene expression, being related to the aging process, health parameters, and the increase or reduction in the risk of developing cardiovascular and neurological diseases, cancers, among other complications (Wu et al., 2023; Barrón-Cabrera et al., 2019). Current dementia research increasingly delves into the role of epigenetics in AD development, a crucial mechanism in memory formation and regulation (Higgins-Chen et al., 2021).

Recently, it was presented in the literature that eight weeks of combined exercise training showed changes in methylation patterns compared to baseline values, as well as rejuvenating the epigenetic clock by two years in older women (da Silva et al., 2023a). Conversely, a year-long aerobic training study in 320 healthy older women revealed no influence of exercise training on DNA methylation in specific breast cancer-related genes (Boyne et al., 2018). To date, evidence remains lacking regarding whether any form of exercise training can modify methylation levels in genes linked to AD among healthy seniors. A recent meta-analysis delineating AD risk loci implicated in Aβ, tau, immunity, and lipid processing identified 25 genes associated with AD development, emphasizing the need to elucidate how exercise training impacts the epigenetics of these genes to bolster AD prevention in conjunction with training (Verheijen and Sleegers, 2018).

It is important to investigate alternative ways of preventing AD. The literature shows that exercise can be used as a tool, but it is still not clear how exercise acts on DNA methylation in healthy older people when we observe genetic sites related to AD, being able to understand whether the exercise would actually act as a protective factor for the development of AD in healthy women aged 50 to 70 years.

Our investigation aims to examine the effects that combined exercise training carried out for 14 weeks has on DNA methylation in genes associated with AD in healthy non-Alzheimer's disease women aged 50 to 70 years, understanding how exercise would act in an epigenetic way to prevent AD.

2. Methods

The research project underwent review by the Research Ethics Committee involving Human Subjects at EEFERP-USP (CAAE: 79582817.0.0000.5659), adhering to Resolution 466/12 by the Ministry of Health, as regulated by the National Health Council. This study was registered in the Brazilian Clinical Trials Registry (REBEC) under registration number RBR-3g38dx.

2.1. Eligibility criteria

Women aged between 50 and 70 years old were included, with medical authorization to practice physical exercise and identified as physically inactive using the Modified Baecke Questionnaire (QBMI) for the Elderly. The QBMI measures the three domains of physical activity (domestic, sporting, and free time) usually carried out by the elderly, scores lower than 9.11 consider those assessed to be physically inactive (Florindo et al., 2003). Another criterion chosen for eligibility not to include people with cognitive impairment was achieving >26 points on the Montreal Cognitive Assessment (Wang et al., 2022), the minimum score for cognitively normal older adults in this cognitive test. This instrument was developed as a brief instrument for screening mild cognitive impairment. The Montreal Cognitive Assessment stands out for evaluating different cognitive domains such as: attention and concentration, executive function, memory, language, visual-constructive capacity, abstract summary, calculation, and orientation (Wang et al., 2022).

Participants were excluded based on criteria such as physical limitations that make it difficult to perform exercises or motor tests used in the study, current smokers or those who smoked in the last six months, degraded DNA at the time of bioinformatics processing, individuals who consumed caffeine within 24 h prior to blood collection and those diagnosed with Alzheimer's disease (Fig. 1).

2.2. Participants

The sample size required for the experiment was calculated using the GPower software. We used tests for independent samples to analyze the results of post-intervention changes. With an alpha error probability of 0.05 and power adjusted to 0.8 %, we estimated that a minimum of 35 participants would be necessary to detect a difference between the means.

We recruited more participants than necessary due to dropouts during the intervention process and exclusion of participants after normalization in the processing of methylation data, we calculated a sample with 28 % more, totaling 40 participants in the final group (Fig. 1).

2.3. Combined training protocol

The combined exercise training protocol consisted of sessions lasting 60 min, conducted three times a week over a period of 14 weeks. The training sessions were a combination of cardiovascular exercises (treadmill or bicycle) lasting 30 min and muscular strength exercises, also lasting 30 min (Rodrigues et al., 2023; da Silva et al., 2023b).

We adopted eight exercises for strength training, comprising five exercises for the upper limbs (inclined bench press, leg curl, leg extension, leg press 45, triceps bar, rower, arm curl, and lat pull-down) and three for the lower limbs (leg curl, leg extension, and leg press at 45°) (da Silva et al., 2023b). During the initial two weeks, participants performed two sets of 15 to 17 maximum repetitions for strength exercises and spent 30 min on a treadmill or stationary bicycle using 50 % of their heart rate reserve. From weeks 3 to 14, two sets of 10 to 12 maximum repetitions were completed, along with 30 min on a treadmill or stationary bicycle at 70 % of the heart rate reserve (Rodrigues et al., 2023). Training intensities were also assessed using the Borg Rating of Perceived Exertion (Borg and Noble, 1974). The Polar Team2 from Finland was utilized for heart rate measurements (Rodrigues et al., 2021).

2.4. Assessment

Participants underwent two rounds of physical assessments: before and after the 14 weeks of combined training. We assessed anthropometric variables (height (cm), weight (kg), body mass index (kg/m^2), waist circumference (cm) and hip circumference (cm)) (Rodrigues et al., 2023), and systolic and (SBP) diastolic blood (DBP) pressure pressures were measured using a previously calibrated automatic digital blood pressure gauge (OMRON®, Jundiaí, Brazil, model HEM-7113, SBH, 2010).

The motor variables evaluated included arm curl, chair stand, agility, 6-Min walk test (Rikli and Jones, 1999), maximum strength of the upper limbs measured by inclined bench press, and maximum strength of the lower limbs assessed by the leg press 45° (Izquierdo et al., 2002).

2.5. DNA extraction and methylation analysis

Peripheral blood samples were collected in tubes with EDTA at two different time points: before and after 14 weeks of training. Subsequently, genomic DNA was isolated from peripheral blood using the salting-out method (Shokrzadeh and Mohammadpour, 2018). The quality and integrity of the DNA were assessed by spectrophotometry (Bulla et al., 2016).

DNA methylation assays were conducted using the array technique, employing the EPIC Infinium Methylation BeadChip from Illumina. The fluorescence of the Infinium BeadChip was detected using the Illumina iScan system (Noronha et al., 2022). Intensities were converted into Beta values of methylation and exported as raw data. Subsequently, data processing was carried out using Rstudio software (R version 3.6.3) (Noronha et al., 2022), analyzing methylation patterns following the recommendations of the chAMP package from Bioconductor (Tian et al., 2017; Correia et al., 2023).

In the DMP function, we modified the command to pheno = myLoad $pd.$Sample_Group and conducted compare group to analyze the pre- and post-intervention moments. We adopted a significance threshold of $p < 0.05$ and subsequently filtered hypermethylation and hypomethylation values in the gene list, considering the beta values (Correia et al., 2023), as a method of adjusting the p value, the default = BH (Benjamini-Hochberg) recommended by ChAMP was adopted (Li and Barber, 2019).

2.6. Enrichment analysis (String)

We enriched our gene list using the STRING software. It's worth noting that the desired specificity cutoff for functional associations in STRING approximately corresponds to the annotation granularity of KEGG pathway maps (Kanehisa et al., 2017). We performed cytosine-phosphate-guanine enrichment through the epigenome-wide association study (EWAS), as it is becoming increasingly significant in identifying associations between epigenetic variations and the different biological characteristics that can be presented (Li et al., 2019).

2.7. Statistical analysis

The motor and anthropometric data collected were entered into an Excel file by two researchers and subsequently cross-checked to eliminate potential errors. We utilized GraphPad Prism 8 for statistical analyses, employing the Shapiro-Wilk test for normality assessment.

To compare values before and after the intervention, we used the Student's t-test for symmetrically distributed data. For asymmetrically distributed data, we employed the Mann-Whitney test. Across all statistical analyses, we set the significance level at $p < 0.05$.

Table 1
Means and standard deviations of the variables adopted for sample characterization ($n = 40$).

Variables	Pre	Post	*P*-value
YEARS OF EDUCATION (years)	10.6 ± 3.3		
AGE (years)	60.7 ± 4.1		
HEIGHT (m)	1.6 ± 0.1		
BW (Kg)	73.12 ± 9.0	73.46 ± 8.5	0.887
BMI (Kg/m2)	29.69 ± 3.5	29.35 ± 3.1	0.714
WC (cm)	94.56 ± 8.0	96.35 ± 9.0	0.467
HC (cm)	106.8 ± 7.5	106.1 ± 6.8	0.718
SBP (mmHg)	134.1 ± 14.7	121.0 ± 10.6	0.001*
DBP (mmHg)	80.15 ± 8.4	75.17 ± 5.5	0.017*

Note - BW: body weight; BMI: body mass index; WC: waist circumference; HC: hip circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; Normality test: Shapiro-Wilk; Student's t-test $p < 0.05$.

3. Results

3.1. Anthropometric data and blood pressure

Below are presented the results as mean and standard deviation of the 40 participants, regarding anthropometric comparisons and pre- and post-training blood pressure. We observed that 14 weeks of combined training lead to reductions in both SBP ($p = 0.001$) and DBP ($p = 0.017$) women with an average age of 60.7 ± 4.1 years, but no significant differences were found in anthropometric variables (Table 1).

3.2. Physical fitness tests

Physical improvements were observed after 14 weeks across all motor tests employed in this study. Significant enhancements and a considerable increase in strength were evident in the arm curl (Fig. 2A), chair stand (Fig. 2B), upper limb maximum repetition (Fig. 2E), and lower limb maximum repetition (Fig. 2F) tests when comparing the initial assessment to the final evaluation. The physical outcomes demonstrate that the combined training regimen yielded improvements in both strength tests and the assessment of aerobic fitness (6-min walk, Fig. 2D), as well as agility (Fig. 2C).

3.3. Genetic enrichment

We conducted enrichment analysis of the list of 25 genes used in this study to comprehend their potential relationships (Fig. 3). Following comparisons between the two assessment points, we observed differentially methylated sites in 12 genes (Fig. 4).

In the comparison of beta values, we observed that the vast majority of genes exhibited hypomethylated sites (negative β values). Despite these hypomethylated sites, some genes still displayed hypermethylated sites (positive β values), such as *ABCA7*, *BDNF*, and *WWOX* (Table 2).

We performed Cytosine-phosphate-Guanine enrichment of the 12 genes that were differentially methylated after 14 weeks of training to identify which gene ontology they were linked to (Fig. 5).

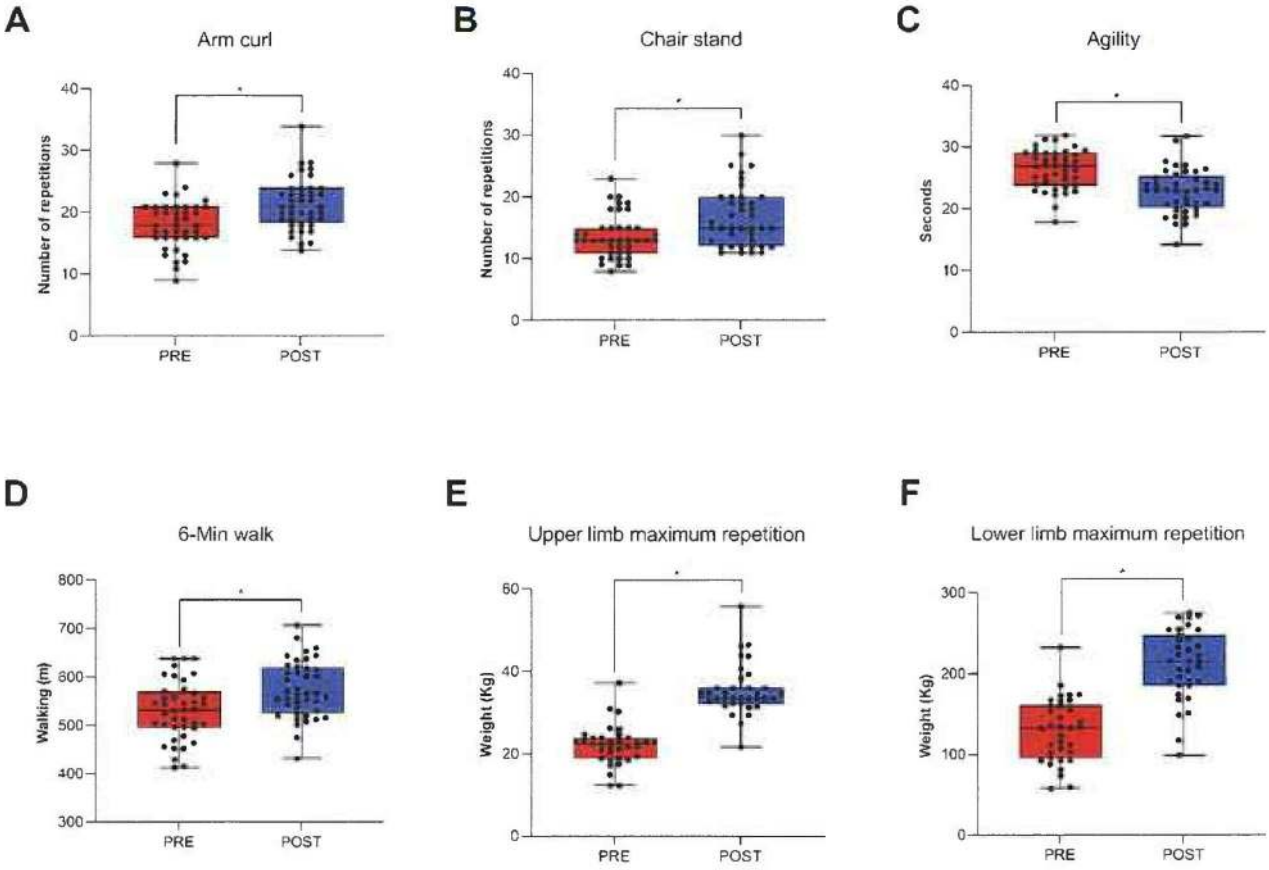

Fig. 2. Pre- and Post-Exercise Intervention Comparisons for Motor Variables ($n = 40$).
Note - Normality test: Shapiro-Wilk; Mann-Whitney test $p < 0.05$.

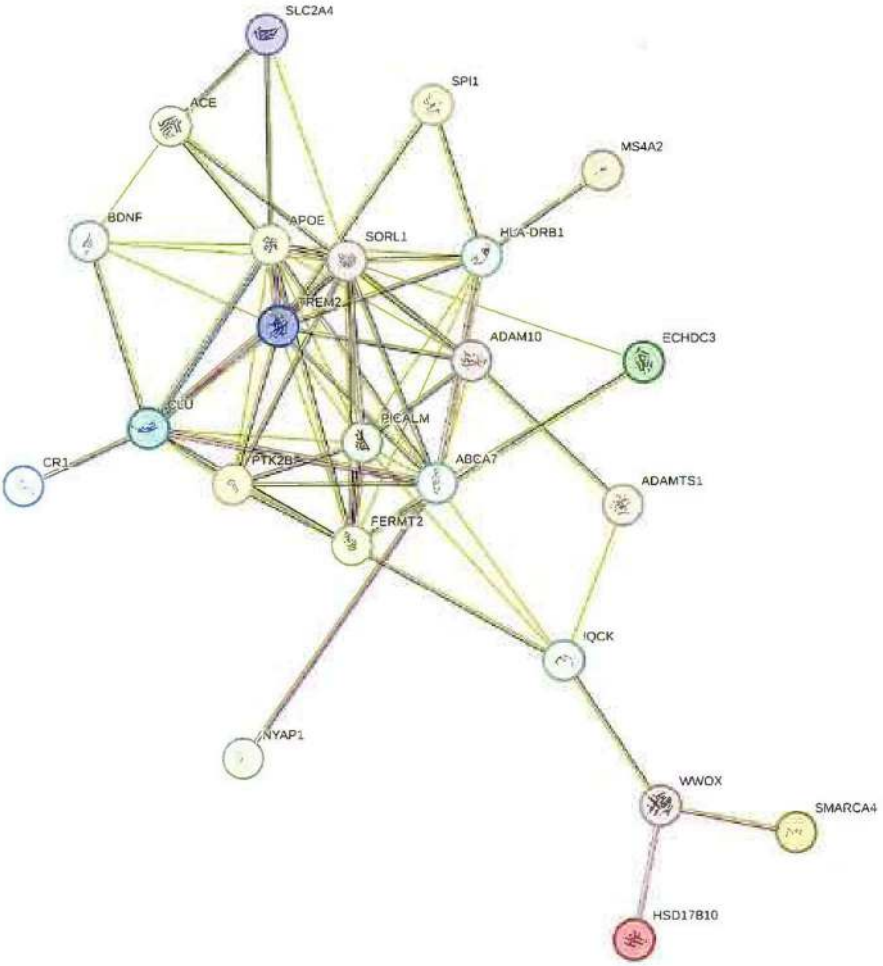

Fig. 3. Genetic enrichment of the list of genes adopted in the study.

4. Discussion

The present study aimed to investigate the effect that 14 weeks of combined training has on three distinct evaluation moments at sites of genes related to AD in women aged 50 to 70 years. Our data presented that 14 weeks of combined training were insufficient to obtain significant differences in anthropometric variables. The combined training is effective in improving health, however some recent studies have shown it may not be as effective in improving anthropometric data in older people (Rodrigues et al., 2023; da Silva et al., 2023b). These findings align with a 2020 study conducted by Brandão et al. (Brandao et al., 2020) that assessed the effects of an 8-week combined training program in overweight or obese older women. Although combined training was effective in improving cardiorespiratory fitness and muscular strength of participants, there was no significant change in body mass index (BMI) and waist circumference (Brandao et al., 2020).

The literature shows that similar training periods also did not result in changes in anthropometric data, such as the 2023 (Rodrigues et al., 2023) study comparing fourteen weeks of multicomponent training versus combined training in physically inactive older women, which did not show significant changes in anthropometric data like BMI and body fat percentage (Rodrigues et al., 2023). Therefore, other intervention strategies (such as nutritional ones) may be necessary in association to enhance the response magnitude of these data in older people (Amati et al., 2010).

According to our observations, combined training is an effective strategy to reduce systolic and diastolic blood pressure in older people, achieving significant results in 14 weeks of training in this study. When compared with the literature, we found a 2019 study by Leando et al. (Leandro et al., 2019) that assessed the effects of an eight-week combined training program on the blood pressure of hypertensive older women. The study showed that combined training significantly reduced systolic and diastolic blood pressure compared to a control group that did not undergo physical training. Furthermore, a 2019 study conducted by Ruangthai and colleagues (Ruangthai and Phoemsapthawee, 2019) evaluated the effects of a 12-week combined training program on the blood pressure of pre-hypertensive older women and men. The results indicated that the hypotensive and antioxidant effects of combined training were significantly high. The combined training is effective in reducing systolic and diastolic blood pressure, as well as providing other health benefits, similar to our findings. Our study had a longer training period than the studies presented above, which may have potentiated the results due to the broader range of stimuli.

There is an association between epigenetic modifications and the loss of phenotypic plasticity. Epigenetic patterns contribute to the etiology of Alzheimer's disease (AD) and other neurodegenerative disorders (Fuso, 2013). Various external factors, including stressors (physical and behavioral), nutritional elements, pollutants, pesticides, chemicals, metals, physical exercise, mental activity, and lifestyle, can exert effects that might contribute to the onset and progression of numerous diseases.

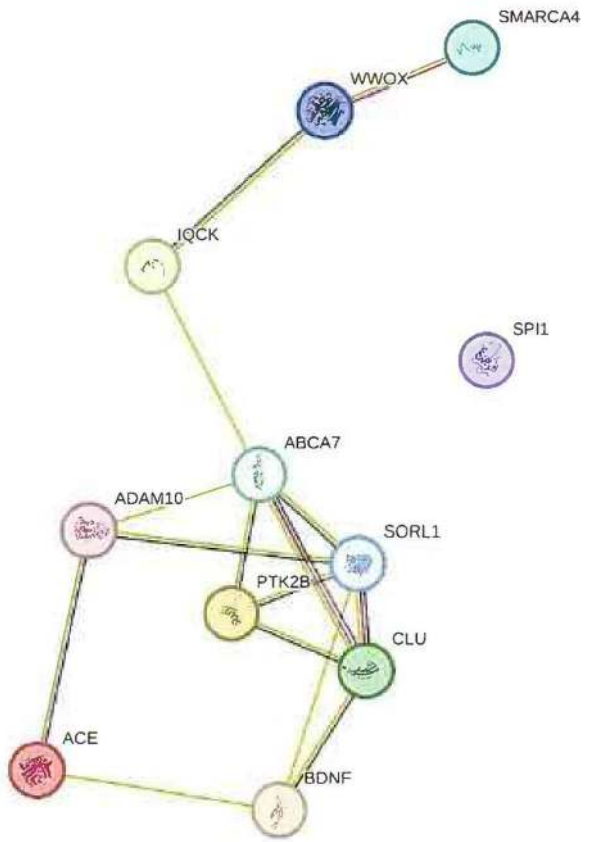

Fig. 4. Enrichment of genes with differentially methylated sites in training comparisons.

Physical exercise has been linked to a reduced risk and protective effects against mild cognitive impairment and AD (Nicolia et al., 2015). Studies have shown that moderate physical activity in animal models induces positive physiological and cognitive effects by altering transcriptional profiles through epigenetic modifications (Kaliman et al., 2011). A meta-analysis of 13 studies has confirmed the impact of physical activities on positive cognitive effects and their protective role against AD (Jia et al., 2019).

A literature review has indicated that physical activity, ranging from mild to moderate intensity, can decrease the risk of dementia and AD. This is in line with our findings since the studied population was healthy. Furthermore, potential benefits were observed for patients already diagnosed with AD (Cass, 2017), and physical activities may provide benefits for the treatment or prevention of AD.

The capacity of exercise to induce alterations in genes and proteins through epigenomic manifestations that regulate synaptic and cognitive plasticity is fundamental in controlling neurological disturbances (Fernandes et al., 2017). Shireby et al. (2022) conducted a DNA methylation profiling in cortical regions associated with AD neuropathology. They identified differentially methylated positions associated with AD, utilizing measures of neuropathology to identify epigenetic signatures of AD, underscoring the importance of characterizing the disease (Shireby et al., 2022). Despite several studies demonstrating the relationship between exercise and AD, our results showcased epigenetic modulation in crucial genes to prevent AD in healthy patients.

Epigenetic biomarkers could potentially serve as a helpful tool in patient screening in the future, as changes in methylation in specific genes have already been identified as biomarkers in AD using peripheral blood leukocytes (Hou et al., 2013). Additionally, an association has been observed between diet, exercise, and improved health in AD patients related to epigenetic modifications (Athanasopoulos et al., 2016).

After 14 weeks of exercise, modulation of gene methylation correlated with AD (*ABCA7, ACE, ADAM10, BDNF, CLU, INPP5D, IQCK, PTK2B, SMARCA4, SORL1, SPI1, WWOX*) was evident. Studies have demonstrated that methylation in ABCA7 is linked to AD, as decreased *ABCA7* levels are associated with the condition. Hence, increasing functional *ABCA7* expression levels could be a valuable therapeutic approach (De Roeck et al., 2019). Our results indicated hypomethylation in *ABCA7* after exercise, suggesting a potential preventive role against AD. The *WWOX* gene's loss of function has been associated with various central nervous system pathologies. Dysfunctional *WWOX* induces tau and Aβ aggregation, leading to apoptosis. Additionally, *WWOX*'s role in brain development and pathology, such as AD, is well-documented (Stevenson-Hoare et al., 2023). This gene plays a critical role in inflammation and is associated with AD progression. Therefore, dysfunctional *WWOX*, such as loss or excessive *WWOX*, contributes to neurodegenerative diseases (AD, Parkinson's disease, among others) (Hsu et al., 2021). Hence, in our results, this gene predominantly appeared hypomethylated at most sites after exercise, although some sites exhibited hypermethylation.

The primary strength of the current study was to analyze methylation patterns using a chip capable of examining 850 thousand CpG sites, providing a comprehensive assessment of the alterations that combined physical training may induce in the methylation patterns of genes related to AD. No methods were employed to assess dietary patterns, and it is known that diet constitutes important environmental factors that can modify methylation patterns. However, participants were instructed not to make lifestyle changes during their involvement in the study, in addition to completing a 24-h dietary recall at the pre-collection stage and adhering to the same record during the post-intervention collection.

Finally, it is plausible that alterations in the methylation profile might not solely underlie the mechanisms inducing adaptations in physical performance but could represent an additional adaptation to the exercise program in a more established trained condition. In future studies, determining the gene expression associated with hypomethylated and hypermethylated sites will be crucial.

5. Conclusion

The combined physical training conducted over a 14-week period induced alterations in the methylation patterns of gene sites related to the development of AD in middle-aged and older women unaffected by neurodegenerative diseases. This epigenetic evidence highlights that physical exercise for 14 weeks was adequate to cause varying levels of methylation in genes responsible for the development of AD in women aged 50 to 70 years, acting as a protective factor for the development of the disease. Moreover, it facilitated improvements in functional capacity, physical performance, and blood pressure among these women.

Funders

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CRediT authorship contribution statement

Guilherme da Silva Rodrigues: Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Natália Yumi Noronha:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **Isabella Harumi Yonehara Noma:** Writing – review & editing, Writing – original draft, Methodology,

Table 2
Differentially Methylated Sites Across the 14 Weeks of Training.

Genes	CpG	CHR	Gene Region	Type	rs	β1 I x III	p-value
ABCA7	cg02817925	19	Body	island	rs150971192	0.009	0.020
ABCA7	cg17993394	19	Body	shelf	rs59237564	−0.025	0.008
ABCA7	cg26970077	19	Body	shore	rs542467691	−0.038	0.015
ABCA7	cg12064401	19	Body	island	rs576278275	−0.024	0.017
ABCA7	cg15681366	19	Body	shelf	rs561581862	−0.017	0.027
ABCA7	cg18846541	19	ExonBnd	shore	rs149365975	−0.017	0.030
ABCA7	cg26263477	19	TSS1500	island	rs184510773	−0.008	0.042
ACE	cg24189328	17	TSS1500	shore		−0.029	0.006
ADAM10	cg16123305	15	Body	opensea	rs113109705	−0.016	0.035
BDNF	cg12021170	11	TSS1500	opensea	rs184293959	−0.029	0.039
BDNF	cg08760147	11	Body	opensea	rs184548784	−0.022	0.042
BDNF	cg11241206	11	TSS1500	shore	rs56195957	0.01	0.046
CLU	cg08594681	8	1stExon	shelf		0.018	0.030
INPP5D	cg04975586	2	ExonBnd	opensea	rs201252871	−0.021	0.016
INPP5D	cg18097144	2	TSS1500	shore	rs188579739	−0.028	0.015
INPP5D	cg16151789	2	Body	opensea		−0.027	0.033
INPP5D	cg06831850	2	Body	opensea	rs574989226	−0.017	0.038
IQCK	cg17440572	16	Body	opensea	rs114245109	−0.024	0.014
IQCK	cg17595464	16	5'UTR	island	rs530998132	−0.007	0.017
IQCK	cg10032375	16	Body	opensea	rs540939870	−0.019	0.040
PTK2B	cg12242935	8	5'UTR	opensea	rs191315642	−0.014	0.045
SMARCA4	cg03921155	19	Body	opensea	rs568094122	−0.03	0.044
SORL1	cg25236143	11	Body	opensea	rs544790047	−0.027	0.011
SORL1	cg04707711	11	Body	opensea	rs569947455	0.021	0.038
SPI1	cg24234141	11	3'UTR	shore	rs531943594	0.024	0.046
SPI1	cg01539849	11	3'UTR	island		0.011	0.045
WWOX	cg05475027	16	Body	opensea	rs550214854	−0.022	0.018
WWOX	cg03648054	16	Body	opensea	rs527659014	−0.021	0.019
WWOX	cg03295251	16	Body	opensea	rs567996965	0.012	0.020
WWOX	cg02236679	16	Body	opensea	rs563057433	−0.026	0.022
WWOX	cg02546607	16	Body	opensea	rs373052053	0.024	0.022
WWOX	cg23054284	16	Body	opensea	rs148661660	−0.025	0.036
WWOX	cg14789524	16	Body	opensea	rs56073608	−0.027	0.037
WWOX	cg26229208	16	TSS1500	shore	rs141439937	−0.018	0.042
WWOX	cg02317358	16	Body	opensea	rs202052438	−0.019	0.045
WWOX	cg26581964	16	Body	opensea	rs145917497	−0.019	0.049

Note – CpG: Cytosine-phosphate-Guanine; CHR: Chromosome; TSS1500: Transcription Start Site 1500; TS200: Transcription Start Site 200; 5'UTR: 5' Untranslated Region; 3'UTR: 3' Unstranslated Region.

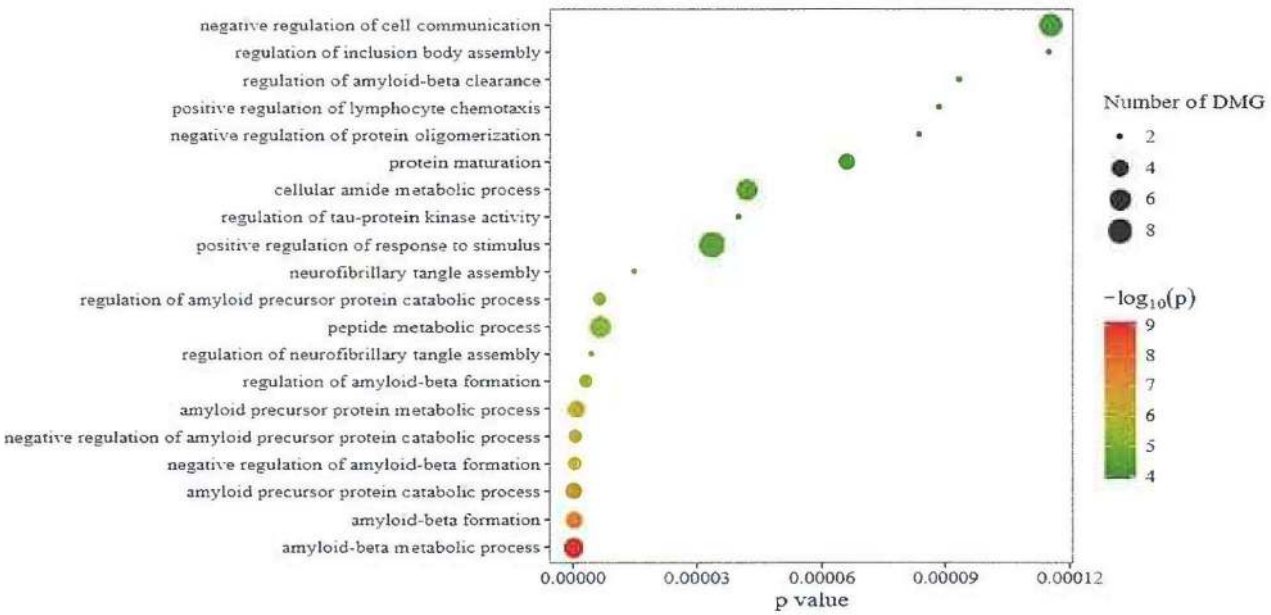


Fig. 5. Gene Ontology enrichment of probes that are differentially methylated after the 14 weeks of combined training.
Note - DMG: Number of Genes Differentially Methylated in the respective pathways.

Investigation, Formal analysis, Conceptualization. **João Gabriel Ribeiro de Lima:** Writing – review & editing, Writing – original draft, Validation, Conceptualization. **Andressa Crystine da Silva Sobrinho:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Marcela Augusta de Souza Pinhel:** Writing – review & editing, Writing – original draft, Validation, Conceptualization. **Mariana Luciano de Almeida:** Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Lígia Moriguchi Watanabe:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Conceptualization. **Carla Barbosa Nonino:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Carlos Roberto Bueno Júnior:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Data availability

The DNA methylation datasets presented in this study can be found online: https://doi.org/10.6084/m9.figshare.21763535. Other data are available upon request to the corresponding author.

References

Amati, L., Marzulli, G., Martulli, M., Chiloiro, M., Jirillo, E., 2010. Effects of a hypocaloric diet on obesity biomarkers: prevention of low-grade inflammation since childhood. Curr. Pharm. Des. 16 (7), 893–897.

Athanasopoulos, D., Karagiannis, G., Tsolaki, M., 2016 Sep. Recent findings in Alzheimer disease and nutrition focusing on epigenetics. Adv. Nutr. 7 (5), 917–927.

Barrón-Cabrera, E., Ramos-Lopez, O., González-Becerra, K., Riezu-Boj, J.I., Milagro, F.I., Martínez-López, E., et al., 2019. Epigenetic modifications as outcomes of exercise interventions related to specific metabolic alterations: a systematic review. Lifestyle Genom. 12 (1–6), 25–44.

Borg, Gunnar A.V., Noble, Bruce J., January 1974. Perceived exertion. Exerc. Sport Sci. Rev. 2 (1), 131–154.

Boyne, D.J., King, W.D., Brenner, D.R., McIntyre, J.B., Courneya, K.S., Friedenreich, C. M., 2018 Jun 28. Aerobic exercise and DNA methylation in postmenopausal women: an ancillary analysis of the Alberta Physical Activity and Breast Cancer Prevention (ALPHA) trial. PloS One 13 (6), e0198641.

Brandao, C.F.C., Nonino, C.B., de Carvalho, F.G., Nicoletti, C.F., Noronha, N.Y., San Martin, R., et al., 2020 Jan 16. The effects of short-term combined exercise training on telomere length in obese women: a prospective, interventional study. Sports Med - Open. 6 (1), 5.

Buchman, A.S., Boyle, P.A., Yu, L., Shah, R.C., Wilson, R.S., Bennett, D.A., 2012 Apr 24. Total daily physical activity and the risk of AD and cognitive decline in older adults. Neurology 78 (17), 1323–1329.

Bulla, A., De Witt, B., Ammerlaan, W., Betsou, F., Lescuyer, P., 2016 Feb. Blood DNA yield but not integrity or methylation is impacted after long-term storage. Biopreservation Biobanking. 14 (1), 29–38.

Cass, S.P., 2017 Jan. Alzheimer's disease and exercise: a literature review. Curr. Sports Med. Rep. 16 (1), 19–22.

Correia, I.M., da Silva, Rodrigues G., Noronha, N.Y., Watanabe, L.M., Luciano de Almeida, M., Sobrinho, A.C. da S., et al., 2023 Apr 10. Older postmenopausal women with lower lean mass have hypermethylated sites in the PI3K-Akt pathway. Front Physiol. 14, 1150821.

Crous-Bou, M., Minguillón, C., Gramunt, N., Molinuevo, J.L., 2017 Sep 12. Alzheimer's disease prevention: from risk factors to early intervention. Alzheimers Res. Ther. 9 (1), 71.

De Roeck, A., Van Broeckhoven, C., Sleegers, K., 2019 Aug. The role of ABCA7 in Alzheimer's disease: evidence from genomics, transcriptomics and methylomics. Acta Neuropathol (Berl). 138 (2), 201–220.

Duthey, Béatrice, 2013. Background paper 6.11: Alzheimer disease and other dementias. A public health approach to innovation, 6, pp. 1–74.

El-Hayek, Y.H., Wiley, R.E., Khoury, C.P., Daya, R.P., Ballard, C., Evans, A.R., et al., 2019 Jul 23. Tip of the iceberg: assessing the global socioeconomic costs of Alzheimer's disease and related dementias and strategic implications for stakeholders. J. Alzheimers Dis. 70 (2), 323–341.

Fernandes, J., Arida, R.M., Gomez-Pinilla, F., 2017 Sep. Physical exercise as an epigenetic modulator of brain plasticity and cognition. Neurosci. Biobehav. Rev. 80, 443–456.

Florindo, A.A., Latorre, M., do RD de O., 2003 Jun. Validation and reliability of the Baecke questionnaire for the evaluation of habitual physical activity in adult men. Rev. Bras. Med. Esporte 9 (3), 129–135.

Foster, P.P., Rosenblatt, K.P., Kuljiš, R.O., 2011 May 6. Exercise-induced cognitive plasticity, implications for mild cognitive impairment and Alzheimer's disease. Front. Neurol. 2, 28.

Fuso, A., 2013 Mar 1. The 'golden age' of DNA methylation in neurodegenerative diseases. Clin. Chem. Lab. Med. 51 (3), 523–534.

Hamer, M., Chida, Y., 2009 Jan. Physical activity and risk of neurodegenerative disease: a systematic review of prospective evidence. Psychol. Med. 39 (1), 3–11.

Higgins-Chen, A.T., Thrush, K.L., Levine, M.E., 2021 Aug. Aging biomarkers and the brain. Semin. Cell Dev. Biol. 116, 180–193.

Hou, Y., Chen, H., He, Q., Jiang, W., Luo, T., Duan, J., et al., 2013 Apr. Changes in methylation patterns of multiple genes from peripheral blood leucocytes of Alzheimer's disease patients. Acta Neuropsychiatr. 25 (2), 66–76.

Hsu, C.Y., Lee, K.T., Sun, T.Y., Sze, C.I., Huang, S.S., Hsu, L.J., et al., 2021 Jul 14. WWOX and its binding proteins in neurodegeneration. Cells 10 (7), 1781.

Izquierdo, M., Häkkinen, K., Gonzalez-Badillo, J., Ibáñez, J., Gorostiaga, E., 2002 Jul 1. Effects of long-term training specificity on maximal strength and power of the upper and lower extremities in athletes from different sports. Eur. J. Appl. Physiol. 87 (3), 264–271.

Jakovljevic, M., Westerman, R., Sharma, T., Lamnisos, D., 2021. Aging and Global Health. In: Kickbusch, I., Ganten, D., Moeti, M. (Eds.), Handbook of Global Health. Springer International Publishing, Cham.

Jia, R. Xia, Liang, J. Hong, Xu, Y., Wang, Y. Quan, 2019 Dec. Effects of physical activity and exercise on the cognitive function of patients with Alzheimer disease: a meta-analysis. BMC Geriatr. 19 (1), 181.

Kaliman, P., Párrizas, M., Lalanza, J.F., Camins, A., Escorihuela, R.M., Pallàs, M., 2011 Sep. Neurophysiological and epigenetic effects of physical exercise on the aging process. Ageing Res. Rev. 10 (4), 475–486.

Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y., Morishima, K., 2017 Jan 4. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res. 45 (D1), D353–D361.

Leandro MPG, Moura JLS de, Barros GWP, Silva Filho AP da, Farias AC de O, Carvalho PRC. Effect of the aerobic component of combined training on the blood pressure of hypertensive elderly women. Rev. Bras. Med. Esporte 2019 Dec;25(6):469–73.

Li, A., Barber, R.F., 2019 Feb 1. Multiple testing with the structure-adaptive Benjamini–Hochberg algorithm. J. R. Stat. Soc. Ser. B Stat Methodol. 81 (1), 45–74.

Li, M., Zou, D., Li, Z., Gao, R., Sang, J., Zhang, Y., et al., 2019 Jan 8. EWAS atlas: a curated knowledgebase of epigenome-wide association studies. Nucleic Acids Res. 47 (D1), D983–8.

Liang, J. Hong, Lu, L., Li, J. Yu, Qu, X. Yuan, Li, J., Qian, S., et al., 2020 Nov. Contributions of modifiable risk factors to dementia incidence: a Bayesian network analysis. J. Am. Med. Dir. Assoc. 21 (11), 1592–1599.e13.

Livingston, G., Huntley, J., Sommerlad, A., Ames, D., Ballard, C., Banerjee, S., et al., 2020 Aug. Dementia prevention, intervention, and care: 2020 report of the lancet commission. Lancet 396 (10248), 413–446.

Nicolia, V., Lucarelli, M., Fuso, A., 2015 Aug. Environment, epigenetics and neurodegeneration: focus on nutrition in Alzheimer's disease. Exp. Gerontol. 68, 8–12.

Noronha, N.Y., da Silva, Rodrigues G., de Souza Pinhel, M.A., Cazier, J.B., Watanabe, L. M., Menezes, A.N., et al., 2022 May 6. Sample preparation to bioinformatics analysis of DNA methylation: association strategy for obesity and related trait studies. J. Vis. Exp. 183, 62598.

Northey, J.M., Cherbuin, N., Pumpa, K.L., Smee, D.J., Rattray, B., 2018 Feb. Exercise interventions for cognitive function in adults older than 50: a systematic review with meta-analysis. Br. J. Sports Med. 52 (3), 154–160.

Rikli, R.E., Jones, C.J., 1999 Apr. Development and validation of a functional fitness test for community-residing older adults. J. Aging Phys. Act. 7 (2), 129–161.

Rodrigues, J.A.L., Santos, B.C., Medeiros, L.H., Gonçalves, T.C.P., Júnior, C.R.B., 2021 Jul. Effects of different periodization strategies of combined aerobic and strength training on heart rate variability in older women. J. Strength Cond. Res. 35 (7), 2033–2039.

Rodrigues, G. da S., Rodrigues, K.P., de Almeida, M.L., Sobrinho, A.C. da S., Noronha, N. Y., CJR, Benjamim, et al., 2023 Feb 2. Comparing fourteen weeks of multicomponent training versus combined training in physically inactive older women: a randomized trial. Int. J. Environ. Res. Public Health 20 (3), 2699.

Ruangthai, R., Phoemsapthawee, J., 2019 May. Combined exercise training improves blood pressure and antioxidant capacity in elderly individuals with hypertension. J. Exerc. Sci. Fit. 17 (2), 67–76.

Shireby, G., Dempster, E.L., Policicchio, S., Smith, R.G., Pishva, E., Chioza, B., et al., 2022 Sep 24. DNA methylation signatures of Alzheimer's disease neuropathology in the cortex are primarily driven by variation in non-neuronal cell-types. Nat. Commun. 13 (1), 5620.

Shokrzadeh, M., Mohammadpour, A., 2018. Evaluation of a modified salt-out method for DNA extraction from whole blood lymphocytes: a simple and economical method for gene polymorphism. Pharm Biomed Res 4 (2), 28–32.

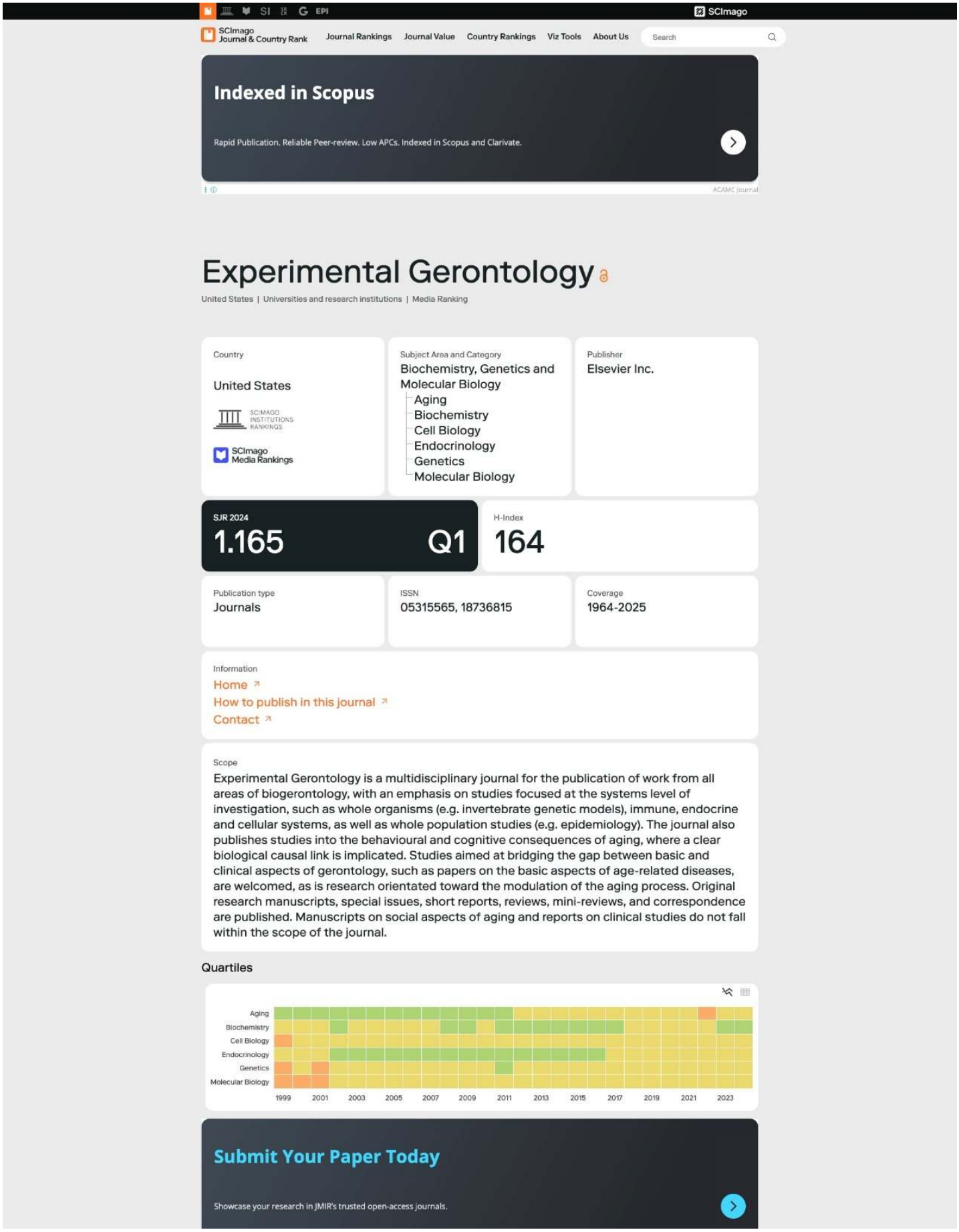
da Silva, Rodrigues G., Noma, I.H.Y., Noronha, N.Y., Watanabe, L.M., da Silva Sobrinho, A.C., de Lima, J.G.R., et al., 2023 Jul 19. Eight weeks of physical training decreases 2 years of DNA methylation age of sedentary women. Res. Q. Exerc. Sport 1–11.

da Silva, Rodrigues G., Noronha, N.Y., Almeida, M.L., Sobrinho, A.C. da S., Watanabe, L. M., Pinhel, M.A. de S., et al., 2023 Mar 1. Exercise training modifies the whole blood DNA methylation profile in middle-aged and older women. J. Appl. Physiol. 134 (3), 610–621.

Stevenson-Hoare, J., Heslegrave, A., Leonenko, G., Fathalla, D., Bellou, E., Luckcuck, L., et al., 2023 Feb 13. Plasma biomarkers and genetics in the diagnosis and prediction of Alzheimer's disease. Brain 146 (2), 690–699.

Tian, Y., Morris, T.J., Webster, A.P., Yang, Z., Beck, S., Feber, A., et al., 2017 Dec 15. ChAMP: updated methylation analysis pipeline for Illumina BeadChips. Valencia A, editor. Bioinformatics 33 (24), 3982–3984.

Verheijen, J., Sleegers, K., 2018 Jun. Understanding Alzheimer disease at the Interface between genetics and transcriptomics. Trends Genet. 34 (6), 434–447.
Wang, X., Li, F., Zhu, H., Jiang, Z., Niu, G., Gao, Q., 2022. A hierarchical Bayesian latent class model for the diagnostic performance of mini-mental state examination and Montreal cognitive assessment in screening mild cognitive impairment due to Alzheimer's disease. J. Prev Alzheimers Dis. 9 (4), 589–600.
Wu, Y.L., Lin, Z.J., Li, C.C., Lin, X., Shan, S.K., Guo, B., et al., 2023 Mar 2. Epigenetic regulation in metabolic diseases: mechanisms and advances in clinical study. Signal Transduct. Target. Ther. 8 (1), 98.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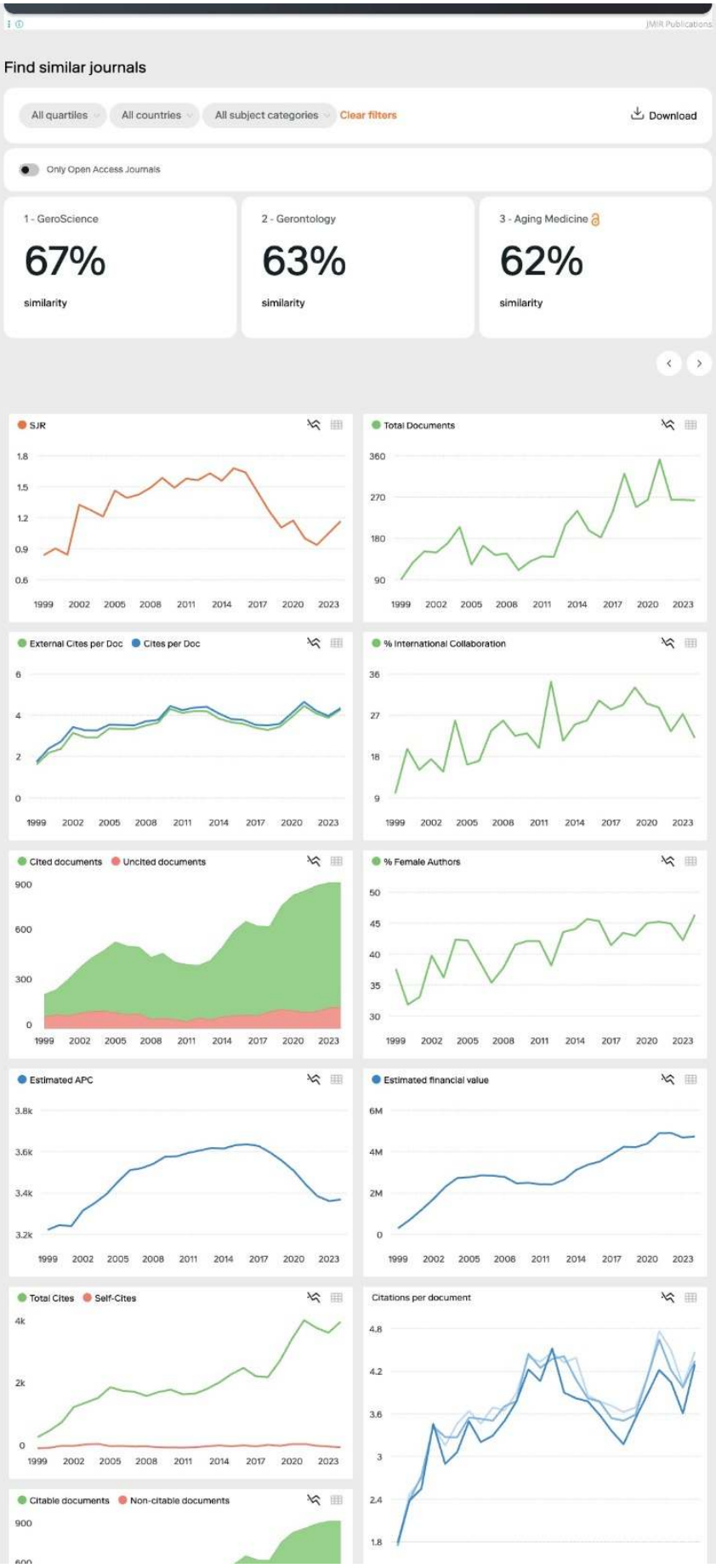
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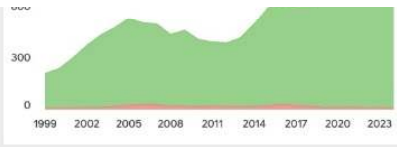

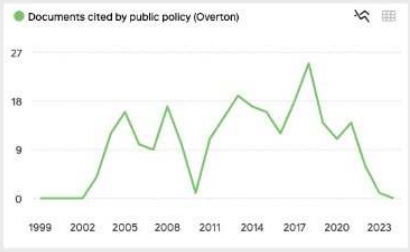

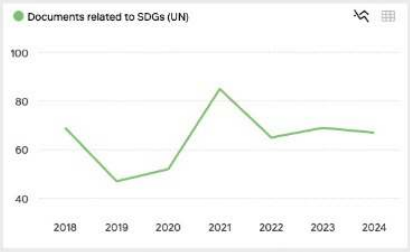

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Eight Weeks of Physical Training Decreases 2 Years of DNA Methylation Age of Sedentary Women

Guilherme da Silva Rodrigues, Isabella Harumi Yonehara Noma, Natália Yumi Noronha, Lígia Moriguchi Watanabe, Andressa Crystine da Silva Sobrinho, João Gabriel Ribeiro de Lima, ...show all

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ABSTRACT

Purpose: The acceleration of epigenetic age is a predictor of mortality and contributes to the increase in chronic diseases. Adherence to a healthy lifestyle is a strategy to reduce epigenetic age. The present study aimed to determine whether eight weeks of

combined (aerobic and strength) training (CT) can influence the epigenetic age of women between 50 and 70 years old and the differences in sites and methylated regions. **Methods**: Eighteen women (AAR_{Low}: lower age acceleration residual, $n = 10$; AAR_{High}: higher age acceleration residual, $n = 8$) participated in a combined exercise training program (60 minutes, 3× a week) for eight weeks. DNA was extracted from whole blood using the salting out technique. DNA methylation was performed using the array technique (Illumina's Infinium Methylation BeadChip 850k). We used the DNA Methylation Age Calculator platform to calculate the biological epigenetic age. Two-way ANOVA followed by FISHER LSD posthoc was Applied, adopting $p < .05$. **Results**: After eight weeks of CT, there were no changes to the epigenetic age acceleration for the AAR_{Low} group (PRE: −2.3 ± 3.2 to POST: −2.3 ± 3.6). However, the AAR_{High} group significantly decreased the age acceleration (PRE: 3.6 ± 2.6 to POST: 2.2 ± 2.7) (group effect, $p = .01$; time effect, $p = .31$; group vs. time effect, $p = .005$). **Conclusion**: CT for eight weeks benefits the epigenetic clock of women with the most accelerated age.

KEYWORDS:

DNAmAge epigenetic clock exercise older women

Disclosure statement

No potential conflict of interest was reported by the author(s).

Additional information

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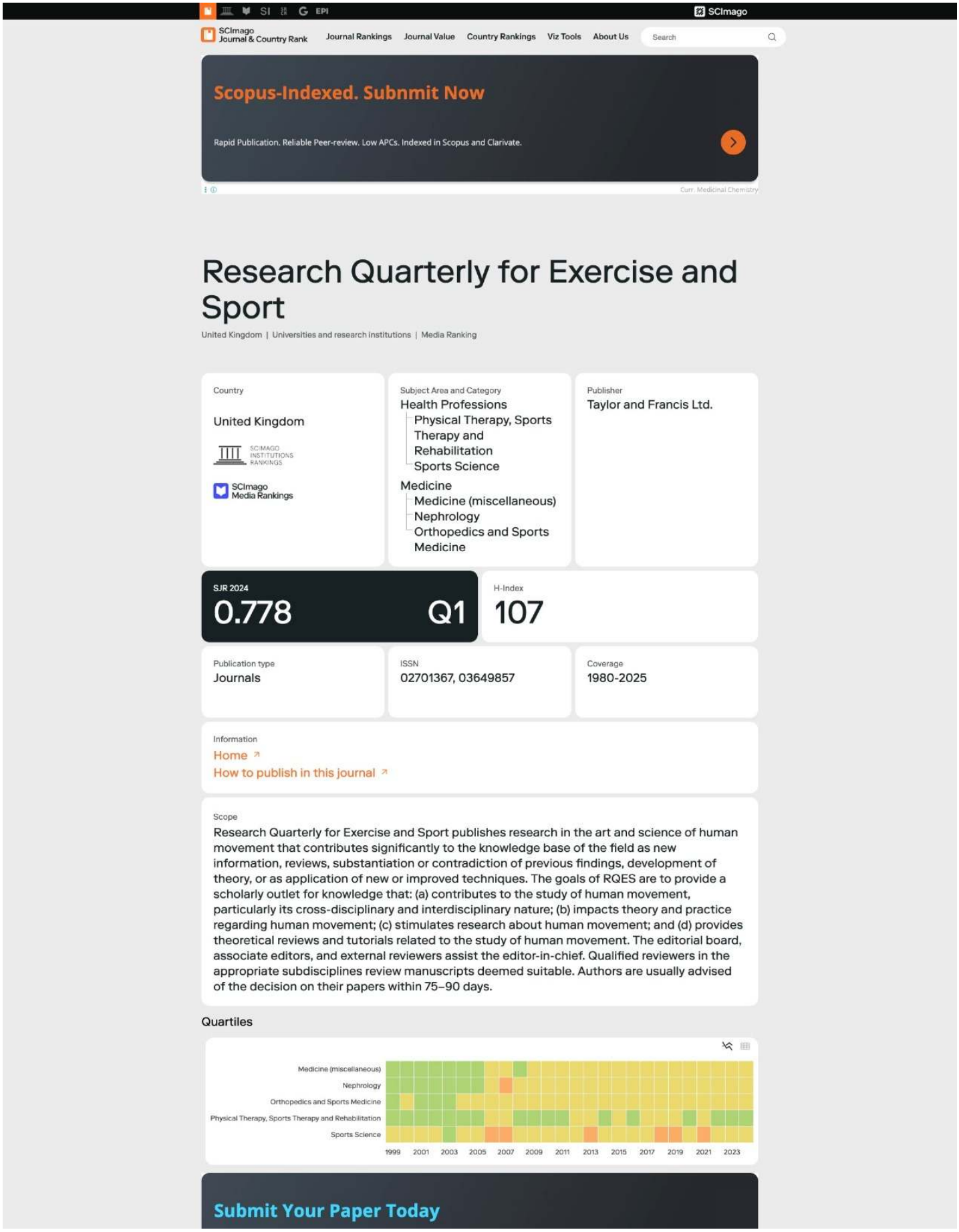
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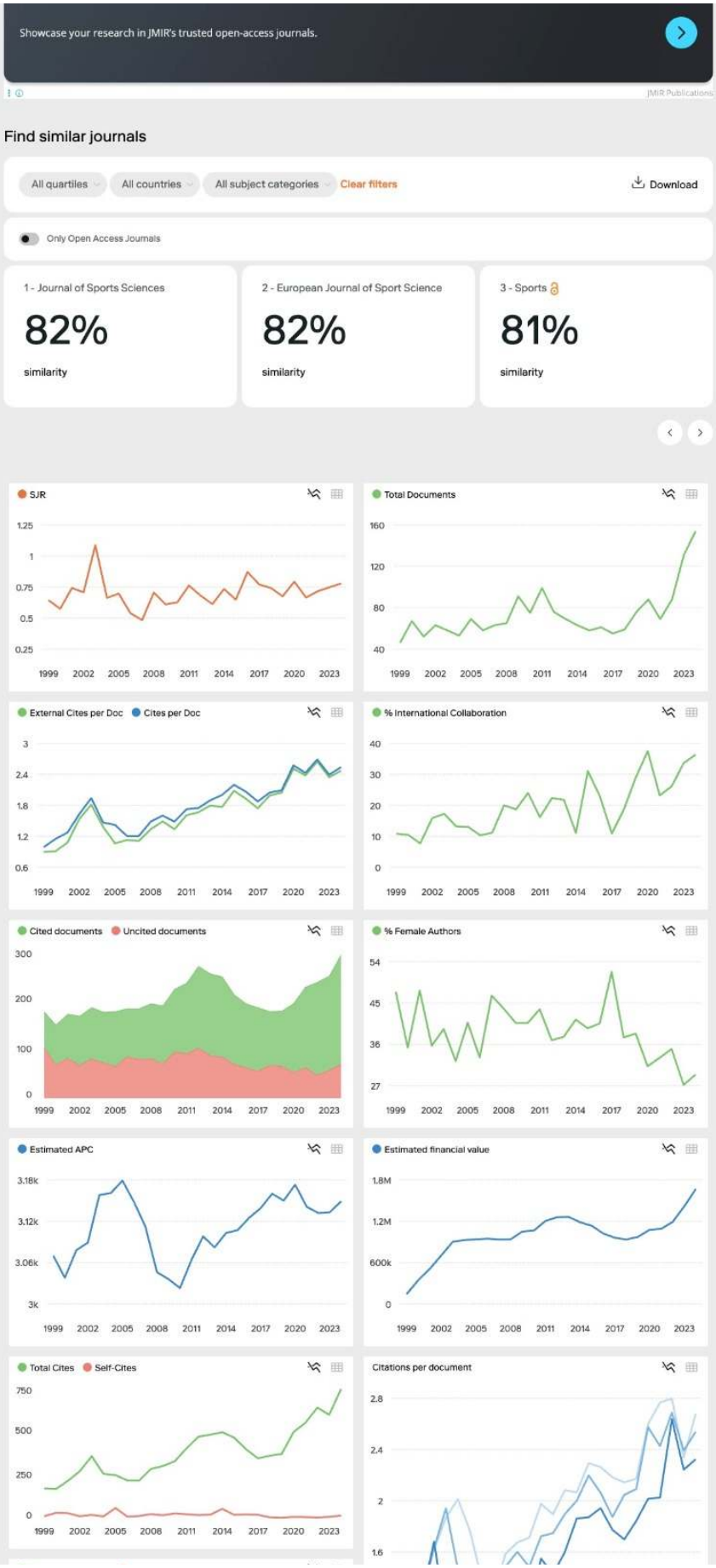
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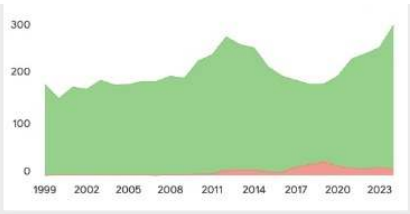
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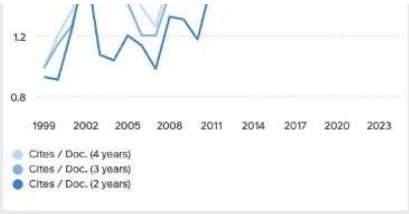
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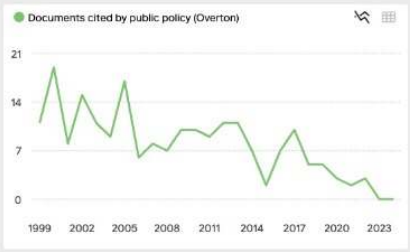
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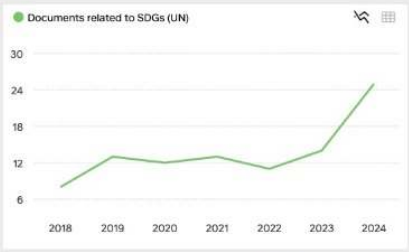
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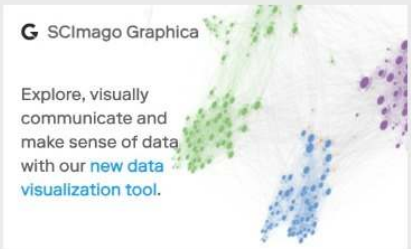
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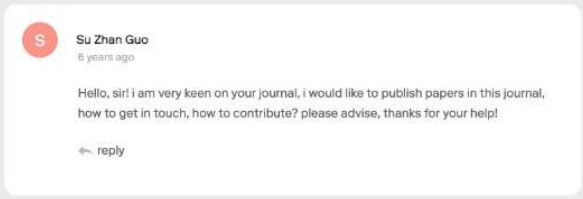
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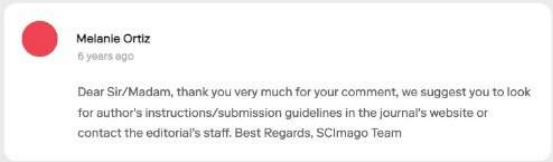
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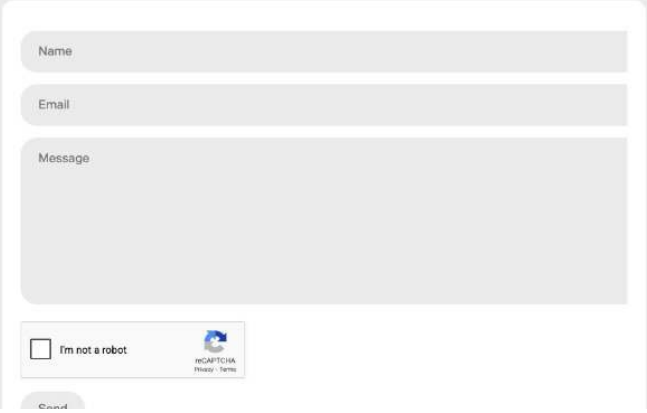
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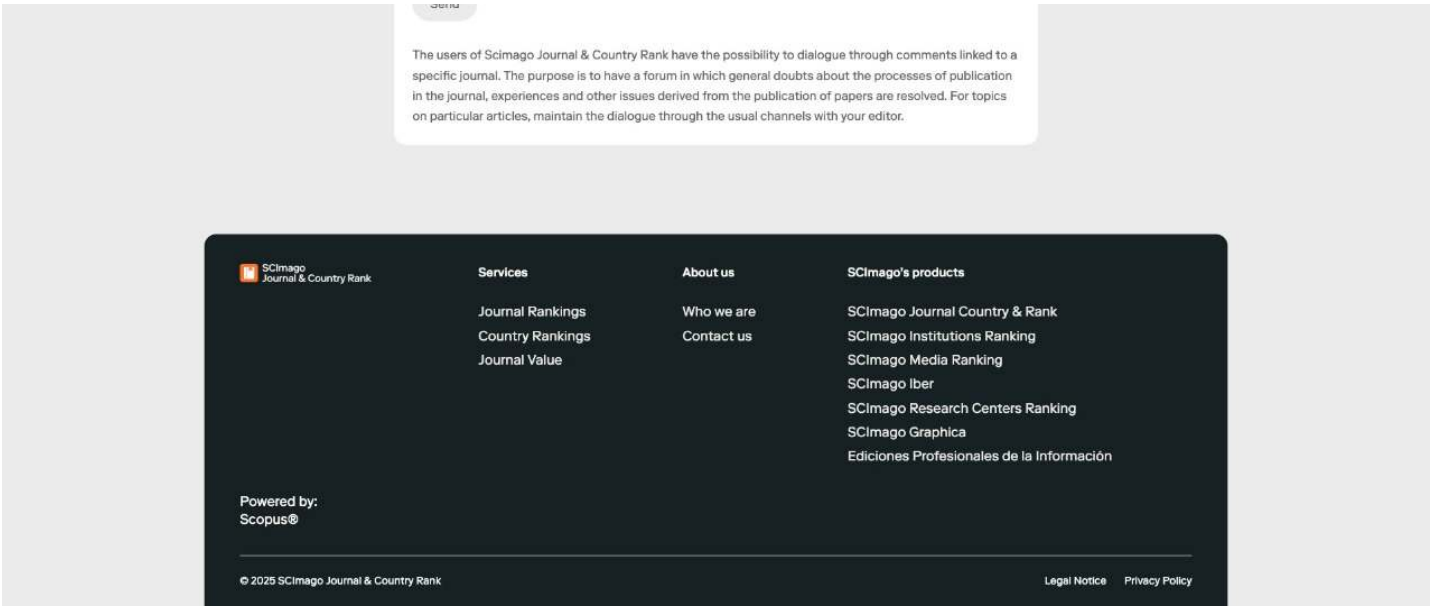
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The influence of serum selenium in differential epigenetic and transcriptional regulation of CPT1B gene in women with obesity

Lígia Moriguchi Watanabe [a], Vanessa Aparecida Batista Pereira [a], Natalia Yumi Noronha [b], Marcela Augusta de Souza Pinhel [b c], Leticia Santana Wolf [b], Cristiana Cortes de Oliveira [b], Jessica Rodrigues Plaça [d], Isabella Harumi Yonehara Noma [e], Guilherme da Silva Rodrigues [b], Vanessa Cristina Oliveira de Souza [f], Fernando Barbosa Júnior [f], Carla Barbosa Nonino [a b]

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Abstract

Introduction

The increasing prevalence of obesity has become a major health problem worldwide. The causes of obesity are multifactorial and could be influenced by dietary patterns and genetic factors. Obesity has been associated with a decrease in micronutrient intake and consequently decreased blood concentrations. Selenium is an essential micronutrient for human health, and its metabolism could be affected by obesity, especially severe obesity. This study aimed to identify differential methylation genes associated with serum selenium concentration in women with and without obesity.

Methodology

Thirty-four patients were enrolled in the study and divided into two groups: Obese (Ob) n=20 and Non-Obese (NOb) n=14, according to the Body Mass Index (BMI). Anthropometry, body composition, serum selenium, selenium intake, and biochemical parameters were evaluated. DNA

extraction and bisulfite conversion were performed to hybridize the samples on the 450k Methylation Chip Infinium Beadchip (Illumina). Bioinformatics analysis was performed using the R program and the Champ package. The differentially methylated regions (DMRs) were identified using the *Bumphunter* method. In addition, logarithmic conversion was performed for the analysis of serum selenium and methylation.

Results

In the Ob group, the body weight, BMI, fat mass, and free fat mass were higher than in the NOb group, as expected. Interestingly, the serum selenium was lower in the Ob than in the NOb group without differences in selenium intake. One DMR corresponding to the CPT1B gene, involved in lipid oxidation, was related to selenium levels. This region was hypermethylated in the Ob group, indicating that the intersection between selenium deficiency and hypermethylation could influence the expression of the CPT1B gene. The transcriptional analysis confirmed the lower expression of the CPT1B gene in the Ob group.

Conclusion

Studies connecting epigenetics to environmental factors could offer insights into the mechanisms involving the expression of genes related to obesity and its comorbidities. Here we demonstrated that the mineral selenium might play an essential role in lipid oxidation via epigenetic and transcriptional regulation of the CPT1B gene in obesity.

Introduction

Over the past 30 years, the prevalence of overweight and obesity has increased by more than 27% worldwide, affecting approximately 2.1 billion individuals and reaching epidemic proportions[1], [2]. The World Health Organization (WHO) defined obesity as an abnormal or excessive fat accumulation offering risk to health [3]. Obesity is classified primarily by body mass index (BMI), a simple metric commonly used as a screening tool to indicate overall body fatness, calculated by dividing the body weight in kilograms by the square of height in meters [3], [4], [5]. According to the BMI, obesity is categorized into different classes [1], [6], [7]; class I obesity (between 30 and 34.9 kg/m^2), class II obesity (between 35 and 39.9 kg/m^2), class III obesity (> 40 kg/m^2) [1], [6], [7]. Class III obesity could be recognized as "extreme" or "severe" obesity [5]. BMI can be used as a screening tool at an individual level but is not diagnostic of body fatness or health [5]. Excess body fat, dysfunction of adipose tissue, and body fat distribution result in metabolic disorders, such as increased oxidative stress and chronic diseases [8].

Obesity is a multifactorial disease, and its etiology comprises the interplay between genetic susceptibility, epigenetics, metagenomics, and the environment [9], [10]. Advances in epigenetics and gene-environment interaction studies have provided additional insight into obesity

development [10]. DNA methylation is one of the most studied epigenetic modifications in mammalian genomes and has a vital role in regulating gene expression without altering the DNA sequence [11]. Generally, increased DNA methylation is associated with the silencing of gene expression, while hypomethylated genes increase transcription [12]. The concept of nutrients influencing DNA stability, repair, and gene expression, became more prominent in nutritional science. Numerous dietary components can alter genetic and epigenetic events and therefore influence health [13].

Selenium, an essential nutrient to the organism, has an essential role as a methyl group donor and has been cited as an epigenetic modulator in the context of chronic and non-communicable diseases [14], [15]. As a hypothesis, selenium could actively participate in methylation processes involved with the differential gene expression between obese and non-obese individuals [16]. Also, studies have shown a correlation between obesity and lower serum selenium levels [17]. The deficiency of selenium in individuals with obesity could be directly associated with lower ingestion. Although obesity is strongly associated with an energy imbalance, characterized by excessive caloric intake and decreased energy expenditure, evidence has been shown that obesity also can be associated with micronutrient deficiencies, considering the Dietary Reference Intakes (DRI) for daily supply of vitamins and minerals [18]. Moreover, since several physiological processes besides the adipose tissue, such as gut permeability, gastric emptying, cardiac output, liver, and renal function, are influenced by obesity, the bioavailability of selenium could also be modified [19], [20].

Thus, considering the fundamental importance of selenium to metabolism, especially in health conditions such as obesity, and the promising findings of the epigenetic regulation by food and metabolites, this study aimed to identify differentially methylated genes associated with serum selenium concentration in obese and non-obese women.

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Materials and methods

This was an open, non-randomized, single-center study. Thirty-four women were enrolled in the study and divided into two groups: Obese (Ob) n=20, and Non-Obese (NOb) n=14, according to the Body Mass Index (BMI) previously obtained from medical records.

The Ethics Committee at the local institution (protocol number CAAE: 56221916.5.0000.5440) approved the study protocol. Informed consent was obtained from all individual participants. …

Anthropometric data and biochemical profile

Thirty-four participants were included and completed the study protocol. The general characteristics, biochemical assays, anthropometric data, and body composition of study participants are described in Table 1. We did not find differences between participants' ages. The variables weight, BMI, fat mass, and free fat mass were higher in the Ob group than in the NOb group, as expected for these parameters. When we analyzed the lipid profile, we did not find differences between groups, except for …

Discussion

Selenium is an essential nutrient to the organism, and adequate selenium intake is crucial for human health [27]. Although selenium intake is the primary determinant of the selenium status in the organism, several individual factors can affect its bioavailability [28], [29]. As seen in the present study, an adequate selenium intake was not determined to the serum selenium adequacy (>70 µg/L) in patients with obesity, indicating that other endogenous factors could be involved in the selenium …

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper: Carla Barbosa Nonino, Fernando Barbosa Júnior, and Lígia Moriguchi Watanabe report financial support provided by Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP). …

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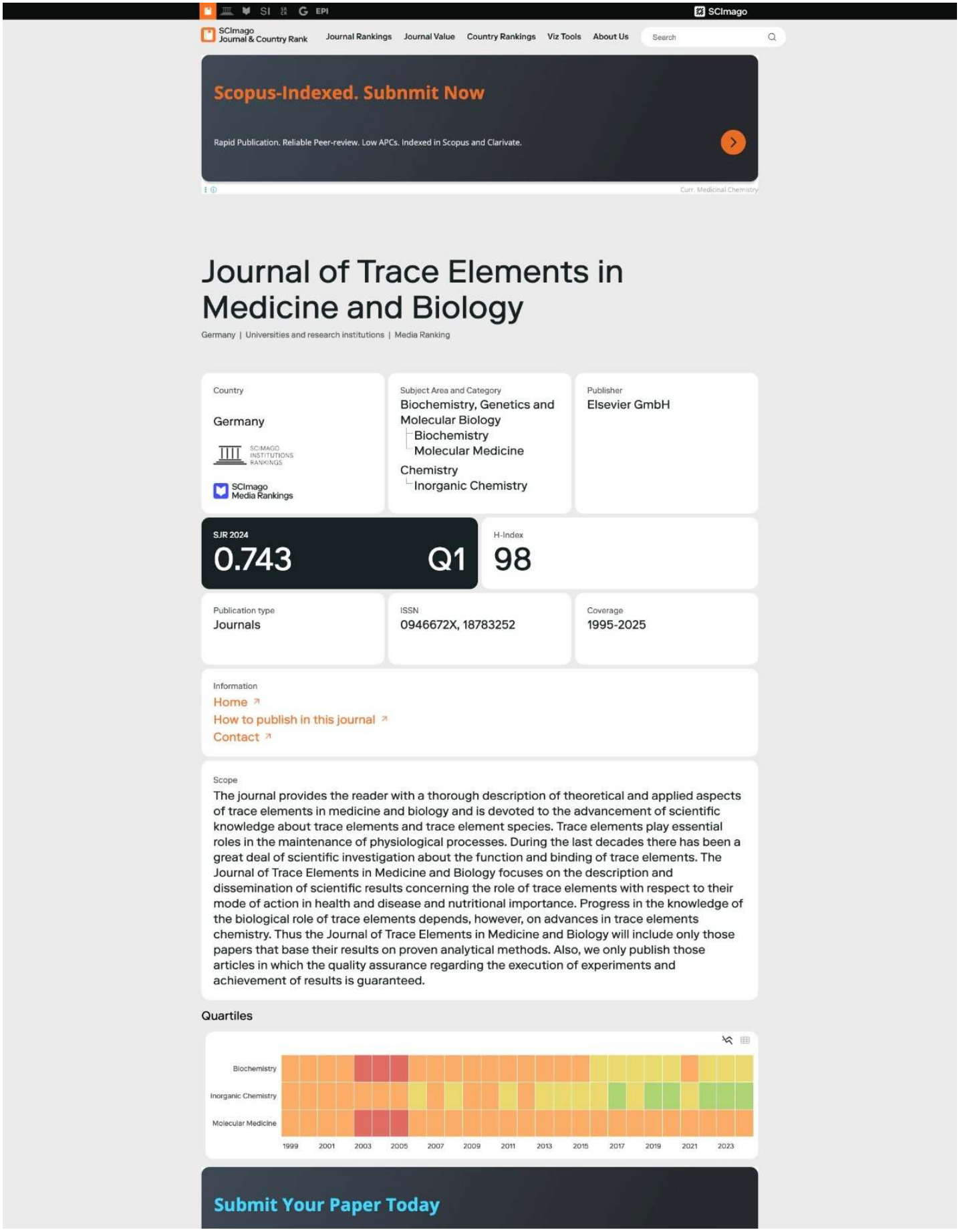
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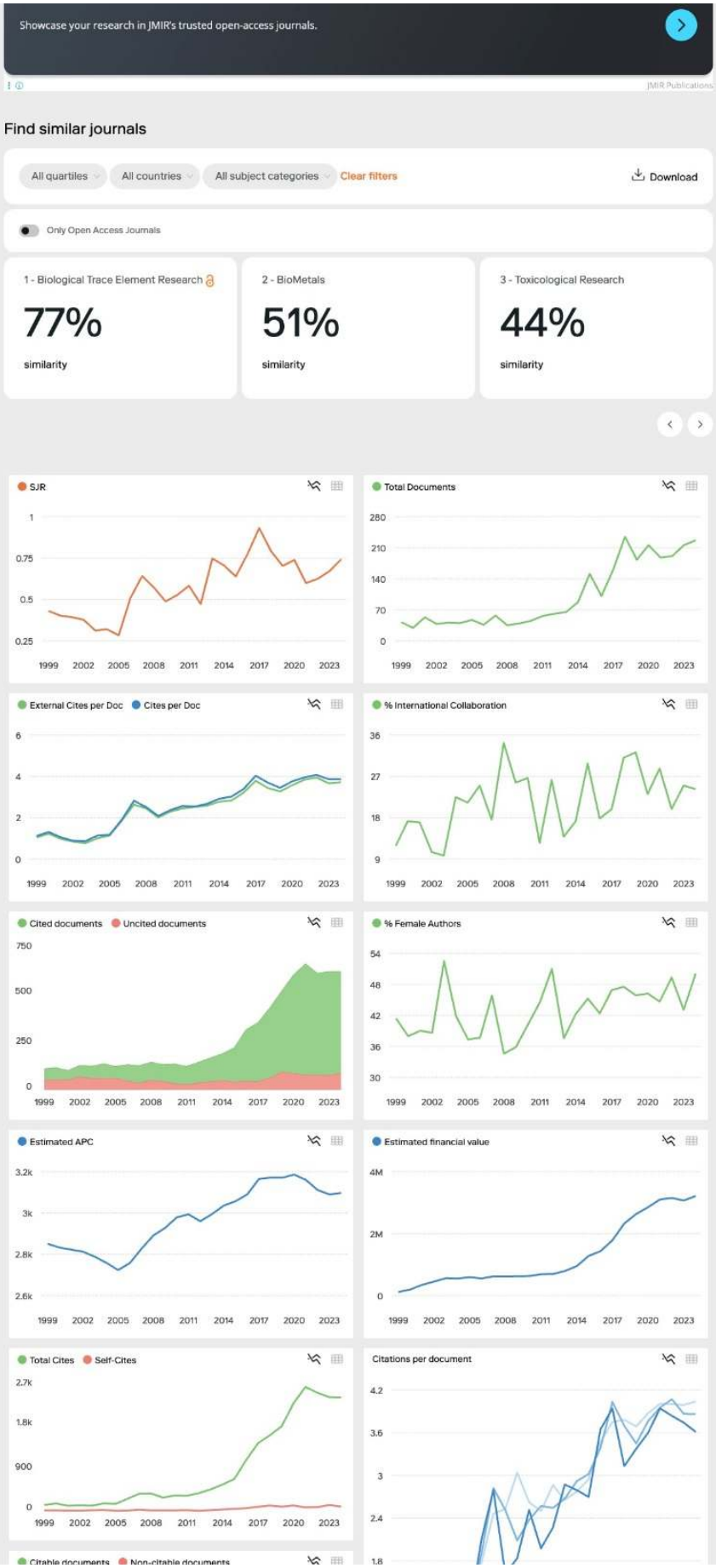
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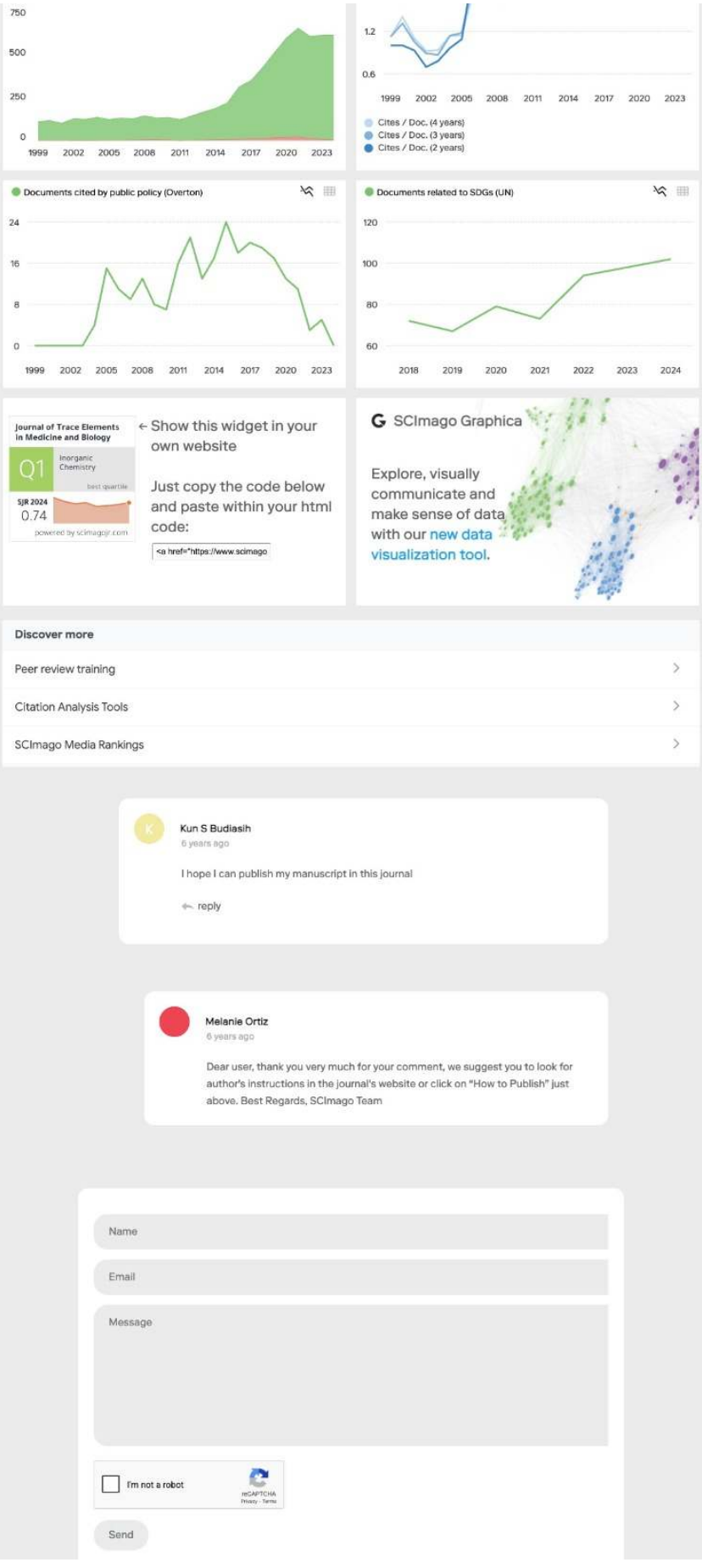
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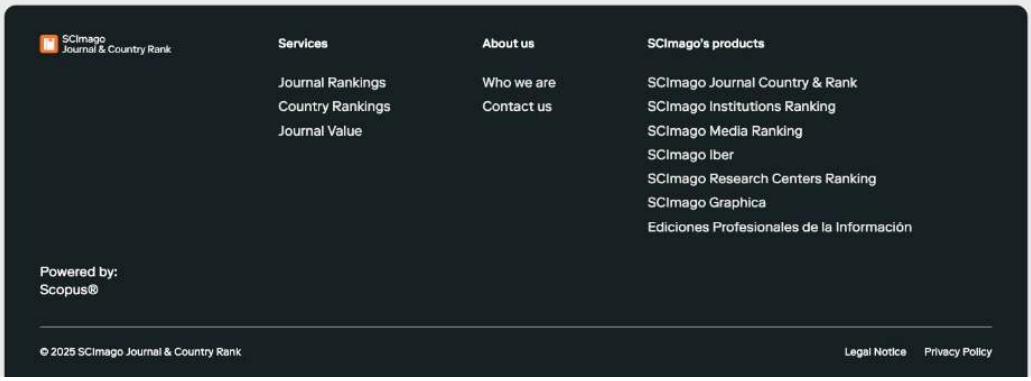

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Short Communication

Gene signatures of autopsy lungs from obese patients with COVID-19

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SUMMARY

Background & aims: Obesity is associated with low grade systemic inflammation and insulin resistance. Although metabolic and immunological changes may contribute to the increased risk for COVID-19 mortality in obese, little is known about the impact of obesity in the lungs of patients with COVID-19.
Methods: We analyzed gene expression profiles of autopsy lungs of a cohort of 14 COVID-19 patients and 4 control individuals. Patients were divided into 3 groups according to their comorbidities: hypertension, type 2 diabetes (T2D) and obesity. We then identified the molecular alterations associated with these comorbidities in COVID-19 patients.
Results: Patients with only hypertension showed higher levels of inflammatory genes and B-cell related genes when compared to those with T2D and obesity. However, the levels of *IFN-gamma*, *IL22*, and *CD274* (a ligand that binds to receptor PD1) were higher in COVID-19 patients with T2D and obesity. Several metabolic- and immune-associated genes such as *G6PD*, *LCK* and *IL10* were significantly induced in the lungs of the obese group.
Conclusion: Our findings suggest that SARS-CoV-2 infection in the lungs may exacerbate the immune response and chronic condition in obese COVID-19 patients.
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1. Introduction

Chronic inflammation and dysfunction of immune response emerge as consequences of obesity, and lead to increase in the susceptibility to pulmonary diseases [1]. Although obesity is a key comorbidity associated with severe COVID-19 [2], little is known about its impact in the lungs of COVID-19 patients.

Here, we analyzed the transcriptional profile of autopsy lungs from obese and non-obese patients with COVID-19. The expression of several immune-, metabolic- and obesity-related genes was altered in obese patients compared to control individuals. We developed an online database to display all of the results. Our work highlights the impact of nutrition on the immune response to SARS-CoV-2 in the lungs.

2. Material & methods

2.1. Targeted transcriptome analysis of post-mortem lung samples

Publicly available data from 18 individuals were downloaded using the GEOquery R package (GEO accession number: GSE151764) [3]. The primary cause of death in all 18 individuals was respiratory failure, and sometimes multi-organ failure [3].

Abbreviations: BMI, Body Mass Index; COVID-19, Coronavirus Disease 2019; DEGs, Differentially Expressed Genes; FDR, False Discovery Rate; GEO, Gene Expression Omnibus; T2D, Type II diabetes; VL, Viral Load.

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Laboratory results of COVID-19 patients showed high levels of circulating markers of inflammation: IL-6 (mean) = 5774 ng/l, C-reactive protein (mean) 216,36 mg/l [3]. Levels of circulating markers of glucose metabolism were not measured. The complete clinicopathological data of the 18 patients, including information about therapy, cause of death, thromboembolic events in the lungs, and disseminated intravascular coagulation can be found in Table S1 (which was adapted from [3]).

We summed the count expression of genes from technical replicates and performed differential expression analysis using the edgeR package. The cutoff for identifying the differentially expressed genes (DEGs) were $|\log_2$ fold-change$| > 0.5849$ and FDR < 0.05. We also correlated body mass index (BMI), SARS-CoV-2 viral load (in $\log_{10}$) and age of COVID-19 patients with gene expression. We then used the significant Pearson correlations ($P < 0.05$) to create a network using Cytoscape. The online database was created in python using streamlit, pandas and altair libraries for data manipulation and visualization.

3. Results

Recently, Nienhold et al. [3] described the immunopathological profiles in autopsy lungs of COVID-19 patients. However, they did not characterize the expression profiles according to the comorbidities associated with the severe form of the disease. We divided the COVID-19 patients into 3 groups: Hypertension (N = 8), Hypertension + T2D (N = 3), Hypertension + T2D + Obesity (N = 3) (Fig. 1A). Given the limited number of samples, we considered overweight patients as non-obese patients. We then identified the differentially expressed genes between each one of these 3 groups and the control individuals not infected with SARS-CoV-2 (N = 4). To display the results, we developed an online database which is located at https://covid19.sysbio.tools/obesity. Most of the genes up- and down-regulated in COVID-19 when compared to controls were shared by all 3 groups (Fig. 1B). However, some of the genes, including *IL1A*, *IL1B*, *IL23A*, *IFNA17*, and *IFNB1* were up-regulated in the hypertension group but down-regulated in either the Hypertension + T2D or Hypertension + T2D + Obesity groups (Fig. 1C). The expression of 15 genes was exclusively induced in patients with Hypertension + T2D + Obesity relative to controls, even including both normal weight and overweight controls (Fig. 1B). These included several genes associated with leptin (*LCK*, *IRF1*, *VCAM1*, *SNAI1*), MHC-class I and II (*HLA-A*, *HLA-C*, *HLA-DQA2*), glucose metabolism (*G6PD*), and immune regulatory functions (*IL10*, *CD40*, *NT5E*, *CORO1A*).

Using only the 14 COVID-19 patients, we then performed a correlation analysis between gene expression and age, SARS-CoV-2 viral load (VL) or BMI (Fig. 2). While genes associated with type I interferon were positively correlated with VL, genes related to Interleukin-2 signaling pathway (*CD52*, *RPS6*, *AIF1*, *LY9*, *CTSS*, *IL2*, *CD2*, *KLRK1*, *GNLY*, *IRF4*, *CXCR3*, *CD27*, *CCR5*, *CD68*, *CCR2*) were negatively correlated with VL (Fig. 2). There were 17 genes exclusively associated with BMI. Among them, genes involved with lipid metabolism, insulin signaling and cell cycle/maturation, such as *LCK*, *EGR2*, *CDKN3* and *MELK* were positively correlated with BMI (Fig. 2). Some of the genes whose expression was negatively correlated only with BMI were associated with cytokine signaling and immune responses, such as *CD40*LG, *CXCR2*, *ADORA2A*, *PIK3CD* and *CD37*, as well as receptors for the Fc region of immunoglobulins gamma (*FCGR3A* and *FCGR3B*) (Fig. 2).

4. Discussion

Obesity is a key comorbidity associated with severe COVID-19 [2]. Here, we revealed significant alterations in gene expression in the lungs of obese patients that died from COVID-19 complications [3]. Type-I interferon and interleukin-1 genes were up-regulated only in non-obese and non-T2D patients, suggesting a lower capacity in inducing proper antiviral defense in the lungs of patients with T2D/obesity. Interestingly, genes up-regulated exclusively in the lungs of obese individuals were associated with energy metabolism, antigen presentation and immune regulatory functions, which might suggest an interplay between metabolism and immunity.

Obese people have high levels of leptin. We found that the expression of lymphocyte-specific kinase (*LCK*) gene and Early Growth Response 2 (*EGR2*) genes was exclusively up-regulated in obese patients and positively correlated with BMI. In kidney, it has been demonstrated that LCK plays an important role in leptin-induced inflammation [4]. Additionally, Jaedicke et al. showed

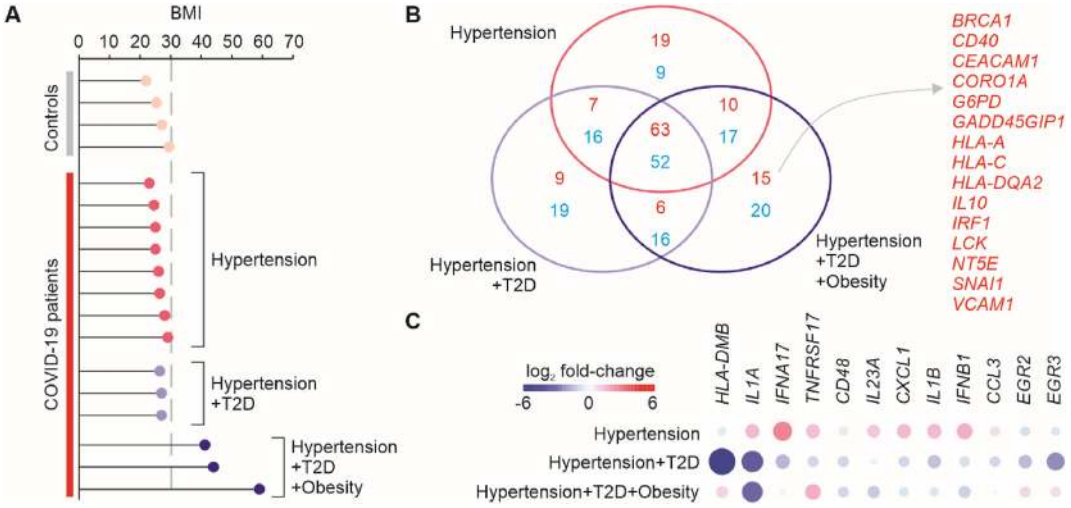

Fig. 1. Differential gene expression analysis of the lungs of COVID-19 patients compared to controls. (A) Individuals utilized in the analysis grouped according to their comorbidities. The x-axis shows the body mass index (BMI) for each individual. Individuals with a BMI above 30 (grey dashed line) were considered obese. (B) Number of differentially expressed genes (DEGs) of each COVID-19 patient group compared to control. The Venn diagram shows the number of upregulated genes (red) and down-regulated genes (blue) that were shared or unique to each group. (C) DEGs with opposite expression among the groups. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

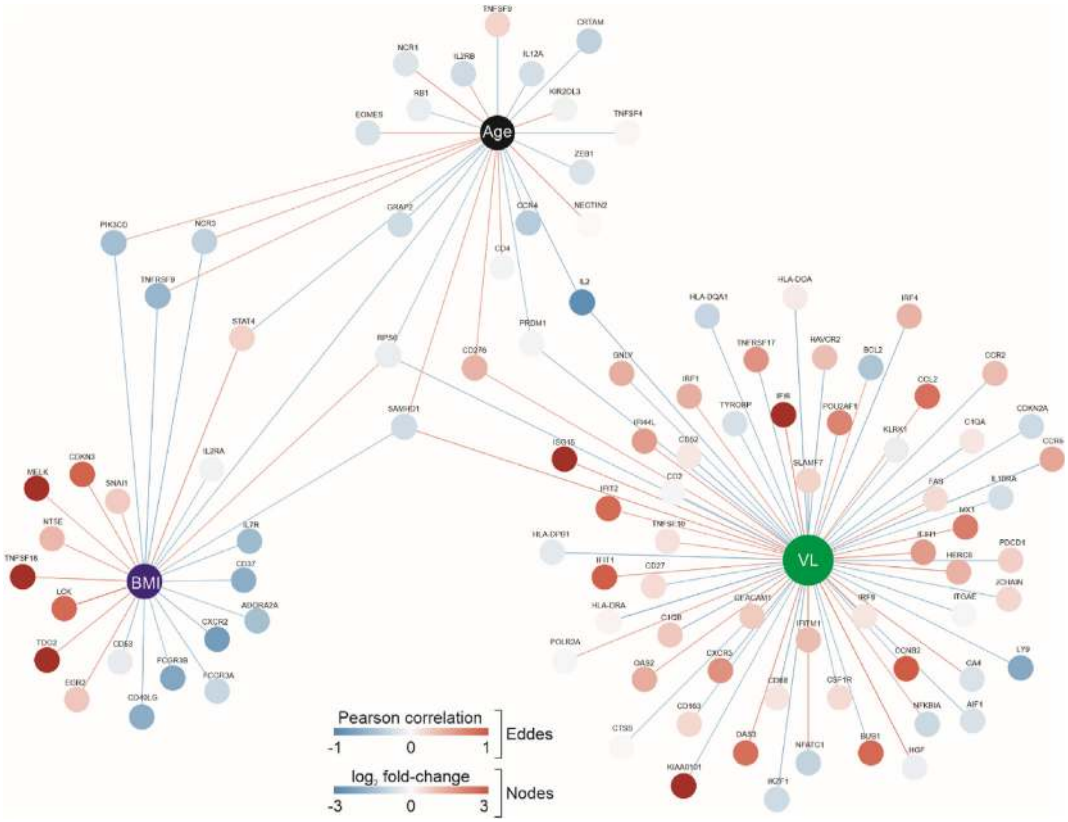

Fig. 2. Network of genes associated with BMI, age and SARS-CoV-2 viral load. The network shows the genes whose expression was correlated with BMI, age and log10 viral load (VL) ($P < 0.05$). The edges colors represent the direction of correlation (negative correlation in blue and positive correlation in red). The nodes are colored with the log2 fold-change from Hypertension + T2D + Obesity group compared to controls. The size of the nodes is proportional to their degree. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

that leptin up-regulates the expression of EGR2 in primary human monocytes [5]. Together with the fact that leptin dysregulation in the lungs is associated with abnormal ventilation, excessive remodeling, and immune dysregulation seen in pulmonary diseases [6], we think that leptin may play a key role in COVID-19 physiopathology in obese individuals.

Our findings also suggest that neutrophils may migrate less to the lungs of obese COVID-19 patients, compared to non-obese ones. This was supported by the fact that the expression of *CXCR2* (a chemokine that mediates neutrophil migration to sites of inflammation), and *FCGR3A* and *FCGR3B* genes was negatively correlated with BMI. FCGR3A and FCGR3B are the two subunits of CD16 surface molecule, which is found in neutrophils, as well as in natural killer cells, monocytes, and macrophages. There is increase evidence that neutrophils and Neutrophil Extracellular Traps play an important role in the lungs of COVID-19 patients [7]. Nienhold et al. [3] have estimated the number of neutrophils per lung tissue section by immunohistochemical stains for CD15 and MPO. We found that in 83% of lung tissue sections of obese patients, few or no neutrophils were found, whereas in non-obese patients, the number of tissue sections with few or no neutrophils was around 60%.

Since obesity is associated with an increased risk of developing T2D and hypertension, we must also consider its impact as the driver of the signatures in the present data. However, our correlation analysis was performed taking into account these comorbidities. Also, our online database provides the scientific papers which are related to the genes and to the comorbidities shown in this work.

We recognize the study limitations, which mainly consist in a reduced number of samples and genes, absence of obese people in the healthy control group, and the presence of confounding factors [3], such as smoker status and the drug therapy taken by the COVID-19 patients before death. Indeed, these limitations prevent us to determine others key factors that may interfere to the observed phenomena. However, our results present novel insights about the mechanism and severity of COVID-19 in obese individuals.

5. Conclusion

The lungs of obese patients with COVID-19 display an altered metabolic and immunological gene profile that may explain the association between obesity and the increased risk for the severe COVID-19.

Declaration of competing interest

None of the authors have conflicts of interest to disclose.

Acknowledgements

We are thankful to the participants of the Systems Biology course (02.2020) and the members of Computational Systems Biology Laboratories (CSBL) for the discussions that refined this work, including Allan Rodrigo for his help with the online database.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2021.05.004.

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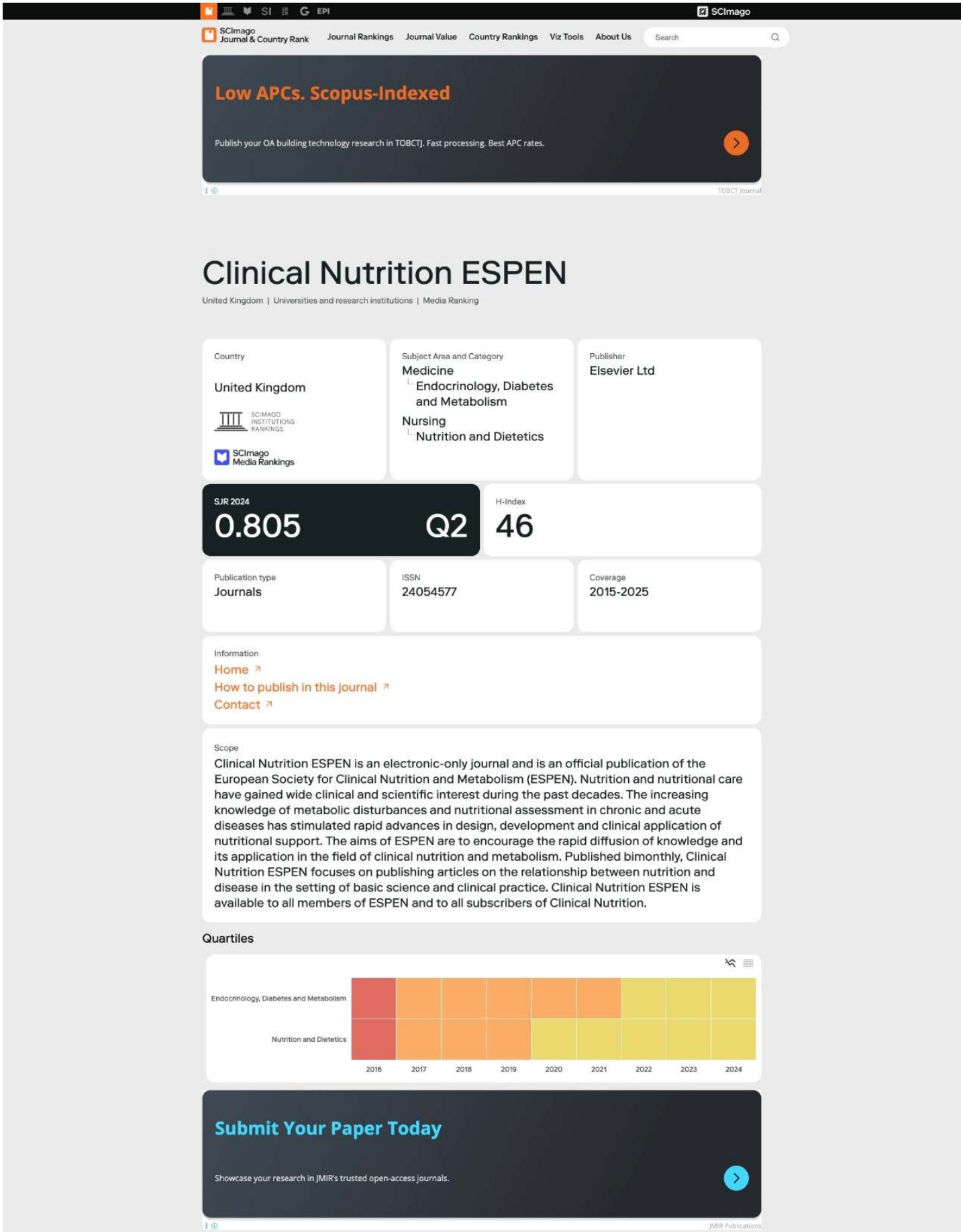
Author contribution

Conceptualization, Investigation, Data Curation and Writing: JCSS, APV, IHYN, NYN, HIN. Software Programming, Formal analysis: JCSS, APV, HIN, AGCM, VS. Database was developed by RA, JG and LD. Resources, Writing Review & Editing, Supervision, Funding acquisition: PMMV, HIN.

References

[1] Qi Y, Xie M, Wei L, Hou G. Insulin resistance exacerbates lung inflammation in obese patients via PI3K/Akt signaling pathway. Eur Respir J 2019;54.
[2] Simonnet A, Chetboun M, Poissy J, Raverdy V, Noulette J, Duhamel A, et al. High prevalence of obesity in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) requiring invasive mechanical ventilation. Obesity 2020;28(7):1195–9.
[3] Nienhold R, Ciani Y, Koelzer VH, Tzankov A, Haslbauer JD, Menter T, et al. Two distinct immunopathological profiles in autopsy lungs of COVID-19. Nat Commun 2020;11(1):5086.
[4] Kim DH, Park JW, Jeong HO, Lee B, Chung KW, Lee Y, et al. Novel role of lck in leptin-induced inflammation and implications for renal aging. Aging Dis 2019;10(6):1174–86.
[5] Jaedicke KM, Roythorne A, Padget K, Todryk S, Preshaw PM, Taylor JJ. Leptin upregulates TLR2 in human monocytes. J Leukoc Biol 2013;93(4):561–71.
[6] Jutant EM, Tu L, Humbert M, Guignabert C, Huertas A. The thousand faces of leptin in the lung. Chest 2021;159(1):239–48.
[7] Wang J, Li Q, Yin Y, Zhang Y, Cao Y, Lin X, et al. Excessive neutrophils and neutrophil extracellular Traps in COVID-19. Front Immunol 2020;11:2063.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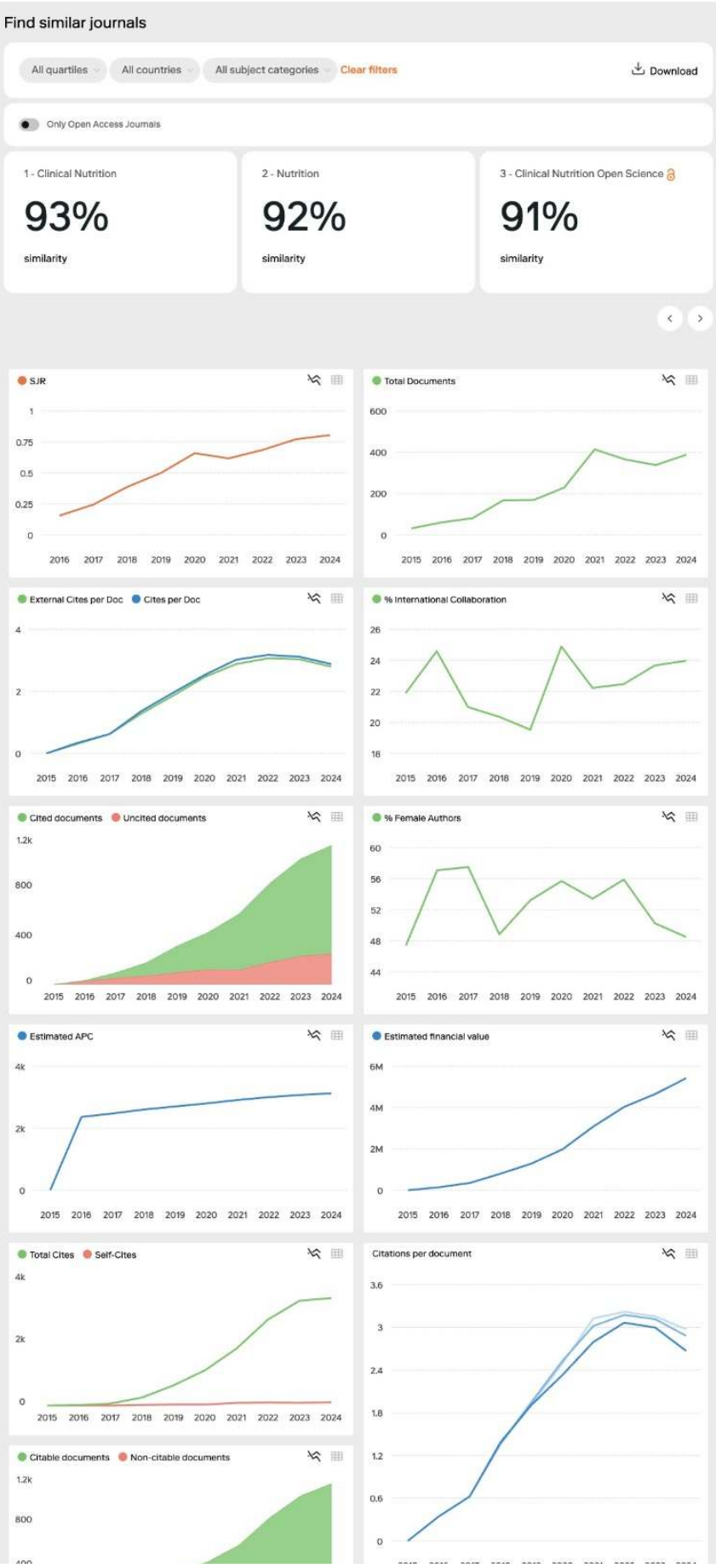
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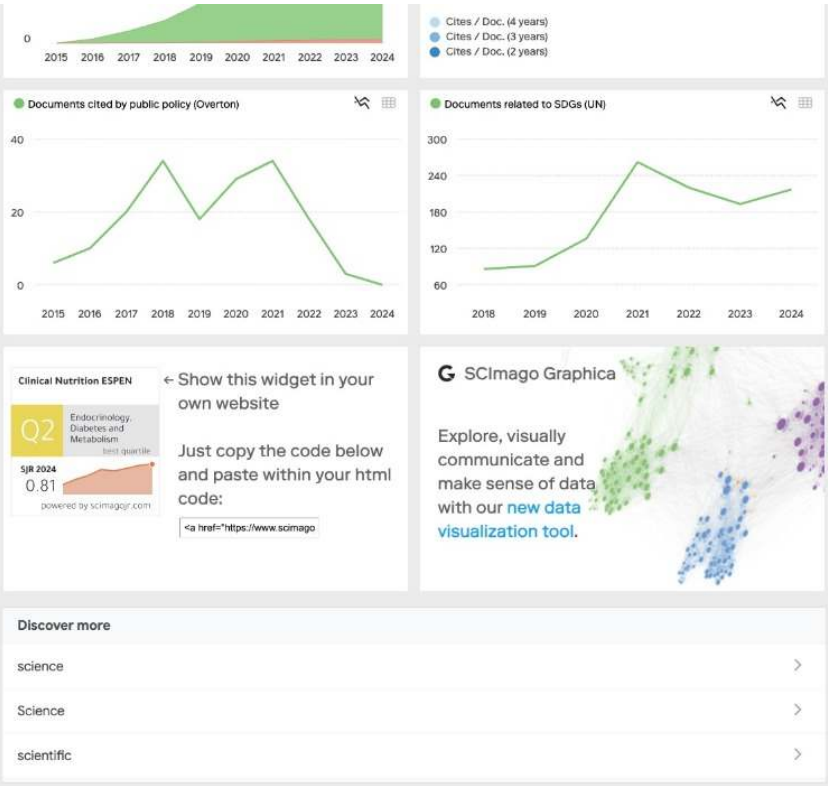

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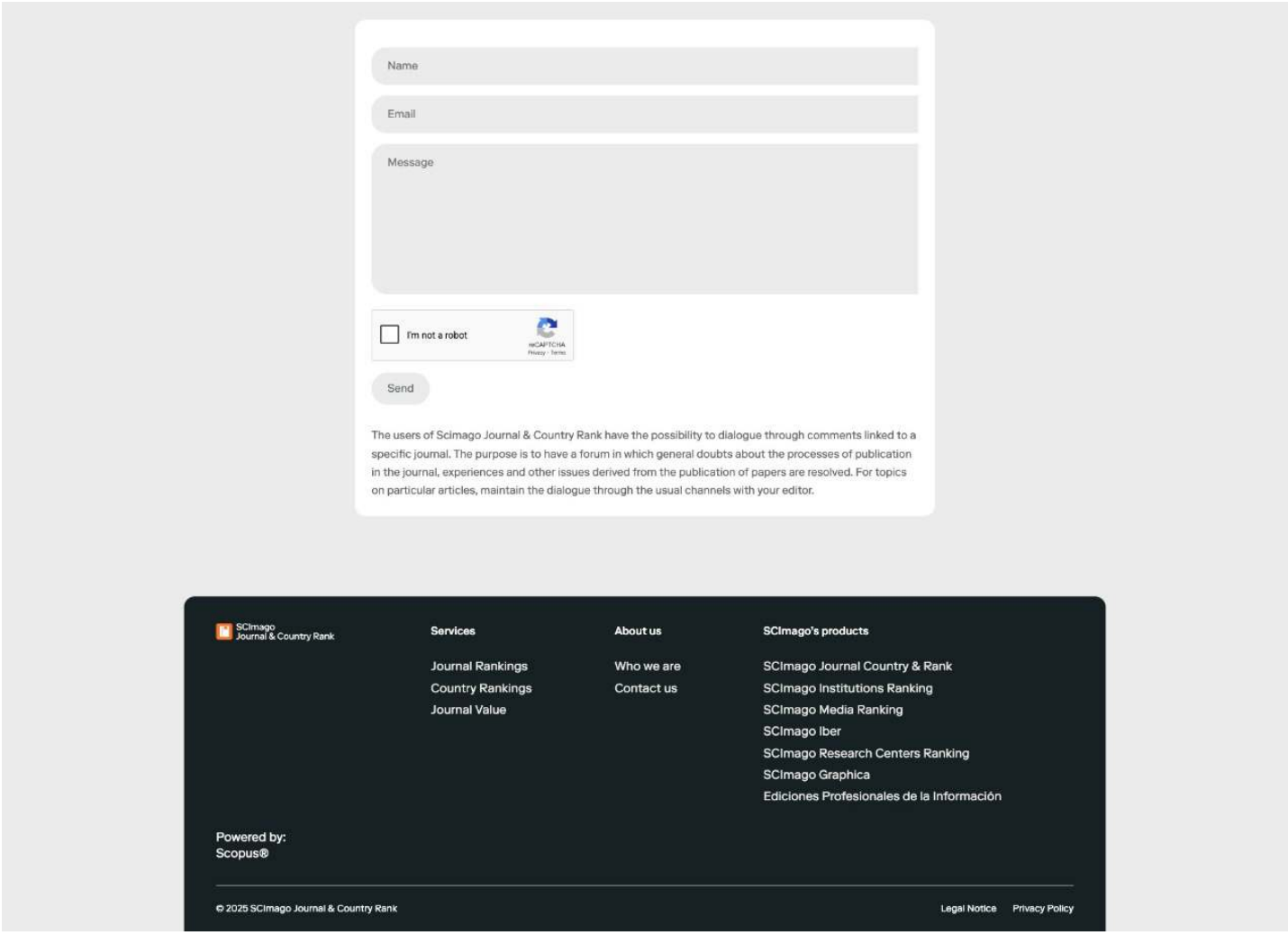
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E.12. Evidence of publication in Journal of Visualized Experiments (JoVE) (2022)

jove

Sample Preparation to Bioinformatics Analysis of DNA Methylation: Association Strategy for Obesity and Related Trait Studies

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Abstract

Obesity is directly connected to lifestyle and has been associated with DNA methylation changes that may cause alterations in the adipogenesis and lipid storage processes contributing to the development of the disease. We demonstrate a complete protocol from selection to epigenetic data analysis of patients with and without obesity. All steps from the protocol were tested and validated in a pilot study. 32 women participated in the study, in which 15 individuals were classified with obesity according to Body Mass Index (BMI) (45.1 ± 5.4 kg/m^2); and 17 individuals were classified without obesity according to BMI (22.6 ± 1.8 kg/m^2). In the group with obesity, 564 CpG sites related to fat mass were identified by linear regression analysis. The CpG sites were in the promoter regions. The differential analysis found 470 CpGs hypomethylated and 94 hypermethylated sites in individuals with obesity. The most hypomethylated enriched pathwayswere in the *RUNX*, *WNT* signaling, and response to hypoxia. The hypermethylated pathways were related to insulin secretion, glucagon signaling, and Ca^{2+}. We conclude that the protocol effectively identified DNA methylation patterns and trait-related DNA methylation. These patterns could be associated with altered gene expression, affecting adipogenesis and lipid storage. Our results confirmed that an obesogenic lifestyle could promote epigenetic changes in human DNA.

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Introduction

Large-scale omics technologies have been increasingly used in studies of chronic diseases. An interesting feature of these methods is the availability of a large amount of generated data to the scientific community. Therefore, a demand to standardize the protocols has arisen to allow technical comparison between studies. The present study suggests the standardization of a protocol to obtain and analyze DNA methylation data, using a pilot study as an applicated example.

Negative energy expenditure predominates in modern human lifestyles, leading to an excessive accumulation of adipose tissue and, consequently, the development of obesity[1]. Many factors have increased obesity rates, such as sedentarism, high-calorie diets, and stressful routines. The World Health Organization (WHO) estimated that 1.9 billion adults were obese in 2016, which means that more than 20% of the world's population has over 30 kg/m^2 BMI[2]. The most recent update of 2018 revealed that the prevalence of obesity in the United States of America (USA) was higher than 42%[3].

Epigenetics is the structural adaptation of chromosomal regions to register, signal, or perpetuate altered activity states[4]. DNA methylation is a reversible chemical alteration in the cytosine-guanosine dinucleotides sites (CpG sites), forming 5-methylcytosine-pG (5mCpG). It can modulate gene expression by regulating the access of the transcription machinery to the DNA[5,6,7,8]. In this context, it is essential to understand which CpG sites are associated with obesity-related traits[9]. Many factors can support or prevent site-specific DNA methylation. Necessary enzymes for this process, such as DNA methyltransferases[10] (DNTMs) and ten-eleven translocations (TETs), can promote DNA methylation or demethylation under environmental exposures[11].

Considering the growing interest in DNA methylation studies over the last years, choosing the most appropriate analysis strategy to precisely answer each question has been an essential concern of researchers[12,13,14]. The 450K DNA methylation array is the most popular method, used in more than 360 publications[14] for determining the DNA methylation profile. It can determine the methylation of up to 485,000 CpGs located in 99% of known genes[15]. However, this array has been discontinued and replaced with the EPIC, covering 850,000 CpG sites. The present protocol can be applied for both 450K and EPIC[16,17,18].

The protocol is presented step-by-step in **Figure 1** and comprises the following steps: population selection, sampling, experiment preparation, DNA methylation pipeline, and bioinformatics analysis. A pilot study performed in our laboratory is demonstrated here to illustrate the steps of the proposed protocol.

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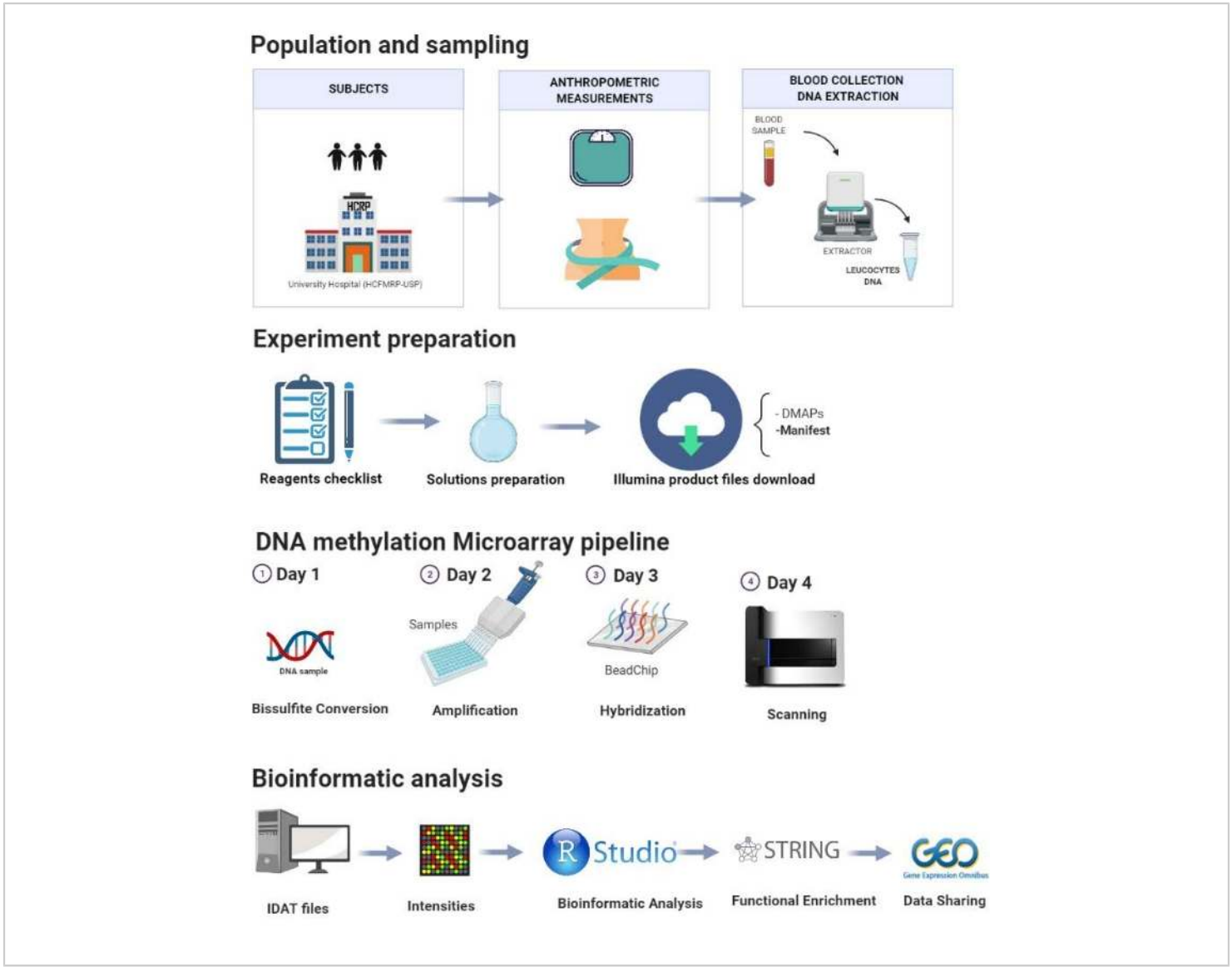

Figure 1: Schematic of the presented protocol. Please click here to view a larger version of this figure.

Protocol

The ethics committee of Ribeirão Preto Medical School University Hospital of the University of São Paulo (HCRP-USP) approved the study (CAAE:14275319.7.0000.5440). The participants signed the consent form, and all procedures were conducted following the Declaration of Helsinki.

1. Population and sampling

1. Recruit participants with BMI >30 kg/m^2 for the study. For the present study, 32 women from an admixture population[19] between 18 to 60 years old were recruited. NOTE: In the HCRP-USP, higher rates of obesity were observed mainly in women[20]. Participants with obesity, with BMI ≥30 kg/m^2 (n = 15), were recruited from the

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Metabolic Diseases outpatient clinic in HCRP-USP. The participants without obesity, with BMI between 18.5 kg/m^2 and 24.9 kg/m^2 (n = 17), were recruited from other clinics and did not have severe diseases; clinical data are shown in **Table 1**.

2. Exclude women who are pregnant, nursing mothers, smokers, and consume alcohol.

2. Anthropometry and body composition

1. Measure the weight of the participants using a 200 kg digital anthropometric scale. Ensure to remove shoes and excess clothing.
2. Assess height using a stadiometer with a 0.5 cm graduation. Participants were instructed to stand barefoot, feet together, and arms by their sides[21].
3. Collect the fat mass (FM) information using an electric bioimpedance. After 12 h of fasting, assess with an empty urinary bladder. Place the participants in the supine position, with legs apart and arms parallel without touching the body. Instruct participants to remove all metal accessories[22].
4. Obtain the waist circumference (WC) using an inextensible measuring tape with a 0.1 mm graduation. The tape was placed horizontally around the middle waist, just above the hip bones. The measurement occurred just after a breath out. According to the Centers for Diseases Control and Prevention (CDC), the WC cut-off for women is 88 cm[23].

Table 1: Population characteristics. All variables were parametric ($p > 0.05$, Shapiro Wilk), and differences were evaluated using an Independent t-Test, in which $p < 0.05$ was considered significant. *$p < 0.0001$. Please click here to download this Table.

3. Collection of biological material

1. Collect peripheral blood after 12 h of fasting, as described previously[24].
2. Collect 4 mL of the whole blood samples in collection tubes with anticoagulant (e.g., EDTA) and store them on ice for transportation.

4. DNA extraction

1. Centrifuge each tube containing the blood at 2,000 x *g* for 10 min. Collect around 300 µL of the buffy coat (white interphase).
2. Extract DNA from the peripheral blood using a commercially available instrument (see **Table of Materials**), as described previously[25]. Elute the DNA in 480 µL of ultrapure H_2O.
3. Assess DNA concentration and quality using a fluorometer. The integrity was evaluated using a 1% agarose gel analysis[25]. Store in a freezer at -80 ˚C.

5. Preparation before methylation analysis

1. Ensure that the samples have the minimum DNA concentration (500 ng is the ideal initial input to start), but dilution may vary between samples in this step.
2. Some reagents are not included in the microarray kit[26], so purchase them separately[27].
3. Check if the kit contains all reagents used during the procedure; they are irreplaceable. Check the expiration date of all the reagents. Ensure other reagents and

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solutions not provided in the kit are also available (95% formamide/1 mM EDTA, 0.1 N NaOH, absolute ethanol, 2-propanol).

CAUTION: Formamide is a volatile reagent; the quality of the results can be improved if the product is fresh. The integrity and quality of formamide and absolute ethanol are considered critical because they can affect the staining process proposed by the manufacturer. The chosen alcohols must be ultrapure products specific for molecular biology analysis.

4. Once the microarray kit is acquired, download the Chips Decode Maps (DMAPs). Those files are available on the manufacturer's website 24-48 h after the kit is shipped and are excluded on the expiration date[28]. To download the mentioned files, perform the steps below.
    1. Ensure a computer with internet access is available. Perform the download using the Software Decode File Client.
    2. Before installing the program, ensure that the computer has outbound and inbound access granted by the institution; the Decode File Client does not have security restrictions.
    3. Log in to the Decode File Client software using the Access by BeadChip option. This login is performed using MyIllumina user ID and password. Registration on this page is required[28].
    4. After logging in to the Decode File Client, enter the information listed below in their respective fields on the software home page[28]: List of barcodes; List of Box Ids; Purchase order number (PO); and Sales order number (remember not to include the letters that follow the numbers).
    5. Select the DMAPs to download according to the barcode number in the purchase box and specify the destination in the dialog box to save the DMAPs.
    6. Click the button to start the download.

       NOTE: At the end of the download, make sure that the downloaded folders are not empty and store the files safely, as they will be necessary for the final step to convert the experiments to Intensity Data Files (IDAT files). After the expiry date, Illumina may remove the DMAPs data from its online database.

6. DNA methylation pipeline

NOTE: The DNA methylation experiment is divided into 4 days (**Figure 1**). Follow the manufacturer's recommendations and specifications to obtain accurate results.

1. Day 1: Bisulfite treatment
    1. Start by denaturing 500 ng of genomic DNA, then add the conversion reagents. To perform this step, follow the recommendations of the bisulfite and microarray kits[29].
    2. Treat the denatured DNA with 130 µL of the bisulfite conversion reagent at the standard concentration provided by the manufacturer.
    3. Incubate the tube in a thermocycler for 16 cycles at 95 °C for 30 s and 50 °C for 1 h. Ramp down the thermocycler to 4 °C for 10 min.
    4. Perform the washing step by adding 100 µL of the washing buffer and then centrifuging at full speed for 30 s. The concentration is not provided in the user guide, and the manufacturer standardizes the volumes.

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NOTE: This reagent comes with the kit and does not need to be diluted or prepared.

2. Day 2: Amplification - Methylation Assay, Manual Protocol

1. Preheat the hybridization oven to 37 °C and allow the temperature to equilibrate.
2. Apply a barcode label to a 0.8 mL microplate.
3. Thaw the amplification reagents (Multi-Sample Amplification Mix 1, Random Primer Mix, and Multi-Sample Amp Master Mix) at room temperature (those reagents come with the kit and do not need to be diluted or prepared). Then, perform a gentle inversion at least 10x until the tubes' entire contents are mixed.
4. Dispense 20 µL of the Multi-Sample Amplification Mix 1 (MA1) into the wells of the plate.
5. Transfer 4 µL of the DNA sample from the bisulfite converted plate to the corresponding wells on the 0.8 mL plate. At this point, record the original DNA sample's ID on the laboratory tracking form corresponding to the plate wells.
6. Dispense 4 µL of 0.1 N NaOH into each well of the plate containing the Multi-Sample Amplification Mix 1 (MA1) and the DNA sample.
7. Seal the plate with a plastic seal.
8. Vortex the plate to mix the reagents with the samples at 1,600 rpm for 1 min. Incubate for 10 min at room temperature and carefully remove the seal.
9. Pipette 68 µL of Random Primer Mix (RPM) into each well of the plate. Pipette 75 µL of Multi-Sample Amp Master Mix (MSM) into each well of the plate (without removing the previous solution). Use a new seal to cover the plate.

   NOTE: Take care to combine the wells with the plate and orient it correctly.
10. Vortex the sealed plate at 1,600 rpm for 1 min. Incubate in the hybridization oven for 20-24 h at 37 °C.

3. Day 3: Hybridization - Methylation Assay, Manual Protocol

1. Preheat the block to 37 °C.
2. Thaw the contents of the tube at room temperature. Carefully and gently invert the Fragmentation Solution (FMS) tubes at least 10x to thoroughly mix the contents.
3. Carefully remove the plate from the hybridization oven. Pulse-centrifuge the plate at 280 x *g*.
4. Now remove the seal from the plate. In each well of the plate containing a sample, add 50 µL of Fragmentation Solution (FMS) and seal the plate again.Vortex the plate at 1,600 rpm for 1 min.
5. Centrifuge the plate at 280 x *g* for a few seconds (perform pulse centrifugation). Then, place the sealed plate on the heating block at 37 °C for 1 h.
6. If it was decided to pause the experiment and freeze the plate, thaw it at room temperature, and centrifuge it at 280 x *g*. If it is not frozen, skip this step and move to Step 6.3.7.
7. Leave the heating block to preheat to 37 °C.
8. Leave the Wash Buffer to thaw at room temperature. When defrosted, invert it at least 10x to mix the contents.
9. Remove the seal from the plate.

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10. Add 100 µL of Precipitation Solution (PM1) to each well of the plate containing samples.
11. Seal the plate again with the seal.
12. Vortex the plate at 1,600 rpm for 1 min.
13. Incubate at 37 °C for 5 min.
14. Then, place in the pulse centrifuge at 280 x *g* for 1 min.
    NOTE: Set the centrifuge at 4 °C for the next step.
15. Carefully remove the seal from the plate and discard it.
16. Add 300 µL of 2-propanol (100%) to each well containing the sample.
17. Seal the plate with extreme care using a new seal. Take care not to shake the plate.
18. Invert the plate at least 10x, mixing the entire contents.
19. Incubate at 4 °C for 30 min.
20. Centrifuge at 3,000 x *g* at 4 °C for 20 min.
    NOTE: At the end of Step 6.3.20, immediately remove the centrifuge plate.
21. Remove the seal from the plate and discard it.
22. Quickly invert the plate and drain the liquid on a pad to discard the supernatant. Then, hit the plate in a dry area on a paper towel to drain the liquid.
23. Tap firmly several times for 1 min or until all the wells are free of any liquid.
24. Leave the plate inverted and uncovered for 1 h at room temperature.
    NOTE: It is expected to see blue pellets at the bottom of the wells.
25. Preheat the hybridization oven to 48 °C.
26. Turn on the heat block to preheat. Allow 20 min for this process.
27. Thaw Resuspension, Hybridization, and Wash Solution at room temperature. Invert at least 10x. Check that there is no salt precipitated in the solution.
28. Add 46 µL of Resuspension, Hybridization, and Wash Solution (RA1) to each well of the plate.
    NOTE: Any remaining reagents must be reserved for the staining and hybridization steps.
29. Apply the seal to the plate. Use an aluminum seal to cover the plate. Hold tight to perform the sealing evenly.
30. Check if all wells are safely closed to avoid evaporation.
31. Carefully place the newly sealed plate in the hybridization oven and incubate at 48 °C for 1 h.
32. Vortex the plate at 1,800 rpm for 1 min.
33. Pulse centrifuge at 280 x *g*.
34. Transfer the plate to the heat block at 95 °C for 1 h.
35. Prepare the Hybridization (Hyb) Chambers by placing them together. Dispense 400 µL of Humidifying Buffer (PB2) into the reservoirs of the Hyb Chambers. Put the lid on immediately.
36. Load each sample from the plate into the sample entry opening of the microarray.
37. Load the Hyb Chamber inserts containing the microarrays into the Hyb Chamber. Close the Hyb Chamber crosswise to avoid possible shifting of the Hyb Chamber.

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NOTE: Hyb Chambers need to be incubated at 48 °C for at least 16 h, but not more than 24 h.

4. Day 4: Staining - Methylation Assay, Manual Protocol

    1. Resuspend the Staining Solution 4 with 330 mL of 100% EtOH, obtaining a final volume of 350 mL. Shake the Staining Solution 4 bottle vigorously to ensure complete resuspension. Keep at room temperature.

        NOTE: Staining Solution 4 can be stored for up to 2 weeks at a temperature between 2 °C and 8 °C.

    2. Remove the chambers from the hybridization oven and allow them to cool on the bench for 30 min before opening.

    3. While the Hyb Chamber is cooling, fill two washing containers with Wash Buffer (200 mL per washing container). Remember to label each container as "Wash Buffer" (PB1 and PB2).

    4. Fill the microarray alignment accessories with 150 mL of Wash Buffer.

    5. Separate the plastic spacers and, if necessary, clean the glass.

    6. To wash the microarray slides, attach the wire loop to the grid and immerse the dripping equipment in the containers with 200 mL of Wash Buffer for a total of 10x.

    7. Remove all inserts from the Hybridization Chamber and remove each array from the inserts. Remove the seal.

        NOTE: During removal, do not touch the exposed matrices.

    8. While removing the microarray slides from the Hybridization Chamber, be careful not to spill anything.

    9. Wash the microarrays in the wash buffer (PB1) solution slowly and lightly, moving up and down 10x. Move to a fresh PB1 solution and perform the wash by slowly moving the microarrays 10x up and down in the solution.

    10. Remove the microarray from the container and confirm the absence of residue.

    11. Use a transparent spacer at the top of each microarray and guide the spacers to the corresponding positions.

        NOTE: Be careful to use only the transparent spacers, not the white ones.

    12. Position the alignment bar on the alignment accessory.

    13. Place a glass backplate on the top of the clear spacer; be careful to cover the entire microarray. Then, attach metal clamps to the flow chambers. If necessary, use scissors to trim the ends of the flow chamber spacers.

    14. Immediately wash the Hybridization Chamber reservoirs using ddH_2O and scrub with a small cleaning brush. Do not allow any Humidifying Buffer to remain in the reservoir.

    15. Move to the next step that is responsible for the Extending and Staining Microarray.

        NOTE: Leave all the flow chambers horizontally mounted on the laboratory bench. Only re-handle them when the entire staining step is ready. Heat the Resuspension, Hybridization, and Wash Solution to

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a temperature between 20 °C and 25 °C. Mix gently until no crystal is seen in the solution.

16. Place the reagents in a rack in sequence. If any are frozen, leave them at room temperature and then invert them at least 10x to mix the solutions.
17. Fill the water circulator to the appropriate level.
18. Turn on the pump and set the temperature to 44 °C.
19. Remove any bubbles that are attached to the chamber rack.
20. Perform tests on the chamber rack using a temperature probe. All tested locations must be between 44 °C ± 0.5 °C.
    NOTE: The following steps must be performed without interruption.
21. When the rack reaches a temperature of 44 °C, quickly place each assembly in the flow chamber.
    NOTE: For the following steps, pipette all reagents into the back glass plate.
22. Pipette 150 µL of Resuspension, Hybridization, and Wash Solution (RA1). Then, incubate for 30 min. Repeat this step a total of 5x.
23. Pipette 450 µL of Staining Solution 1 (XC1) and incubate for 10 min.
24. Pipette 450 µL of Staining Solution 2 (XC2) and incubate for 10 min.
25. Pipette 200 µL of Two-Color Extension Master Mix (TEM) and incubate for 15 min.
26. Pipette 450 µL of 95% formamide/1 mM EDTA and incubate for 1 min. Repeat twice.
27. Incubate for 5 min.
28. Decrease the temperature of the chamber rack to 32 °C (indicated on Superior Two-Color Master Mix).
29. Pipette 450 µL of Stain Solution 3 (XC3) and incubate for 1 min. Repeat 1x.
30. Wait for the rack in the chamber to reach the correct temperature.
    NOTE: To read the microarray image immediately after the coloring process, turn on the scanner at this stage.
31. Now dispense 250 µL of Superior Two-Color Master Mix (STM), followed by a 10 min incubation.
32. Then, dispense 450 µL of Stain Solution 3 (XC3), followed by 1 min incubation. Repeat 1x.
33. Wait for 5 min and then add 250 µL of Anti-Stain 2-Color Master Mix (ATM) and incubate for 10 min.
34. Now dispense 250 µL of Superior Two-Color Master Mix (STM), followed by a 10 min incubation.
35. Then, dispense 450 µL of Stain Solution 3 (XC3), followed by 1 min incubation. Repeat 1x.
36. Wait for 5 min.
37. Add 250 µL of Anti-Stain 2-Color Master Mix (ATM) and incubate for 10 min.
38. Add 450 µL of Stain Microarray Solution 3 (XC3) and incubate for 1 min, then repeat again.
39. Wait for 5 min.
    NOTE: Do the following repetition scheme: Step 6.4.34., Step 6.4.35. and Step 6.4.36.; Step 6.4.37., 6.4.38., and Step 6.4.39.; Step 6.4.34., Step 6.4.35., and Step 6.4.36.
40. Now, immediately remove the flow chambers from the rack and carefully place them horizontally on

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the laboratory bench. Make sure the temperature is ambient.

41. 6.4.41.Place 310 mL of Wash Solution (PB1) for each microarray in a wash container.
42. Remove the metal clamps and the glass, and finally, the microarray.
43. Place the microarrays on a staining rack and place them in the wash container containing 10x PB1 solution. Ensure that the barcodes are facing away from whoever is handling them and that all the microarrays are submerged.
44. Move slowly and lightly with up and down movements 10x. The microarray should be removed entirely from the solution before being submerged again. Then, let it remain submerged for 5 min.
45. Vigorously shake the Stain Solution 4 (XC4) reagent to ensure total resuspension. Place 310 mL of XC4 into a wash container.
    NOTE: Do not let the solution sit in the wash container for more than 10 min.
46. Lightly move the staining stand to the other container with XC4.
47. Move slowly and lightly with up and down movements 10x. Ensure that the microarray is removed entirely from the solution before being submerged again.
48. Then, let the microarray remain submerged for 5 min.
49. Move the staining stand out of the solution and place it in a tube holder with the barcodes facing up.
50. Carefully remove the microarray using locking forceps and place them on a shelf to dry.
51. Dry them using the vacuum desiccator for 50-55 min at 675 mm Hg (0.9 bar).
52. Clean the back of each microarray using EtOH.
    CAUTION: Avoid touching the stripes, and do not allow EtOH to drip on the stripes in any way.
53. Proceed to image the microarray.
54. Carefully store the microarray in a storage box at room temperature.
    NOTE: Microarray images must be taken within 72 h.

7. Bioinformatics analysis

NOTE: It is necessary to change some attributes of the kind of microarray (e.g., arraytype = "450k" should be replaced by arraytype = "EPIC"). The author of the ChAMP pipeline describes in detail all fields that should be modified to analyze arrays on the Bioconductor page of the package[30].

1. Sample sheet
    1. Transfer the IDAT files to the computer to proceed with bioinformatics analysis[31]. Create a sample sheet to carry out the data analysis[31].
       NOTE: Three columns are essential for this spreadsheet file. The first is Sample_name. This column must include the names or codes used to identify the samples. The second is the Sentrix_position. This column places the corresponding R01C01 of the samples, with R01 being responsible for the chip row and the column being referred to as C01. The third essential column is the Sentrix_ID, and it is responsible for receiving the number that identifies the microarray. Make sure to put the correct numbers. It is possible to add other information on the sample sheet as variable

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values. In the present experiment, data from the metal analysis were included.

2. ChAMP pipeline

1. Install RStudio (V.4.0.2) on the computer to perform the analysis[30].

NOTE: Ensure that the computer has a minimum of 8 G of RAM. Data analysis will struggle or crash with a lower amount of RAM.

2. After installation, open RStudio and install the ChAMP Bioconductor package[30] using the respective command line (see **Supplementary Material 1**).

NOTE: If there is any error at the end of the installation, install the required ChAMP libraries manually, one by one[26].

3. Now, import the raw data and perform the analysis. See **Supplementary Material 1**.

champ.load() imports the raw data and does the filtering process, excluding probes with low detection pval, multihit sites, non-CG probes, CpG sites near to SNPs, and CpGs located in sexual chromosomes.

champ.impute() performs KNN imputation as default.

champ.QC retrieves data quality graphs (e.g., density plot graph).

champ.SVD evaluates batch effects based on provided metadata in the sample sheet.

champ.refbase() performs cell type estimation and correction.

champ.DMP( ) retrieves differentially methylated positions and trait associations such as fat mass to check the DNA methylation between groups and change the parameters, as shown in **Supplementary Material 1**.

3. **Enrichment (String DB)**

NOTE: Functional enrichment is used to infer the biological functions of a set of genes.

1. To enrich data in a string, select the list of targets to enrich and use as input from: https://string-db.org/cgi/input?sessionId=bywt5GI8Bawy&input_page_active_form=multiple_identifiers

2. For organism, select homo sapiens and press the Search button.

3. To navigate through the enrichment, click on the Analysis button.

4. **GEO submission**

1. Deposit the data to a public repository like NCBI's GEO. For this, register for the submitter account.

NOTE: Complete the GEO submission (https://submit.ncbi.nlm.nih.gov/geo/submission/) by downloading the metadata sheet with descriptive information and protocols for the overall experiment and individual samples. GEO archive submissions can be created in any spreadsheet software. Second, add the raw data file generated from the cell-ranger count script for all libraries into the directory. IDAT and associated files or a matrix worksheet contains non-normalized data. The third folder is the processed data files. The matrix table is a spreadsheet containing the final, normalized values comparable across rows and samples and preferably processed as described in any accompanying manuscript.

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2. Place processed data files generated from the cell-ranger count script for all libraries into the directory. Use the GEO submitter's FTP server credentials to transfer the directory containing all three components.

Representative Results

After using the ChAMP pipeline, 409,887 probes were considered in the analysis after all filters (unqualified CpGs, non-CG probes, CpG near SNPs, multihit sites, and CpGs related to XY); a scheme is represented in **Figure 2**.

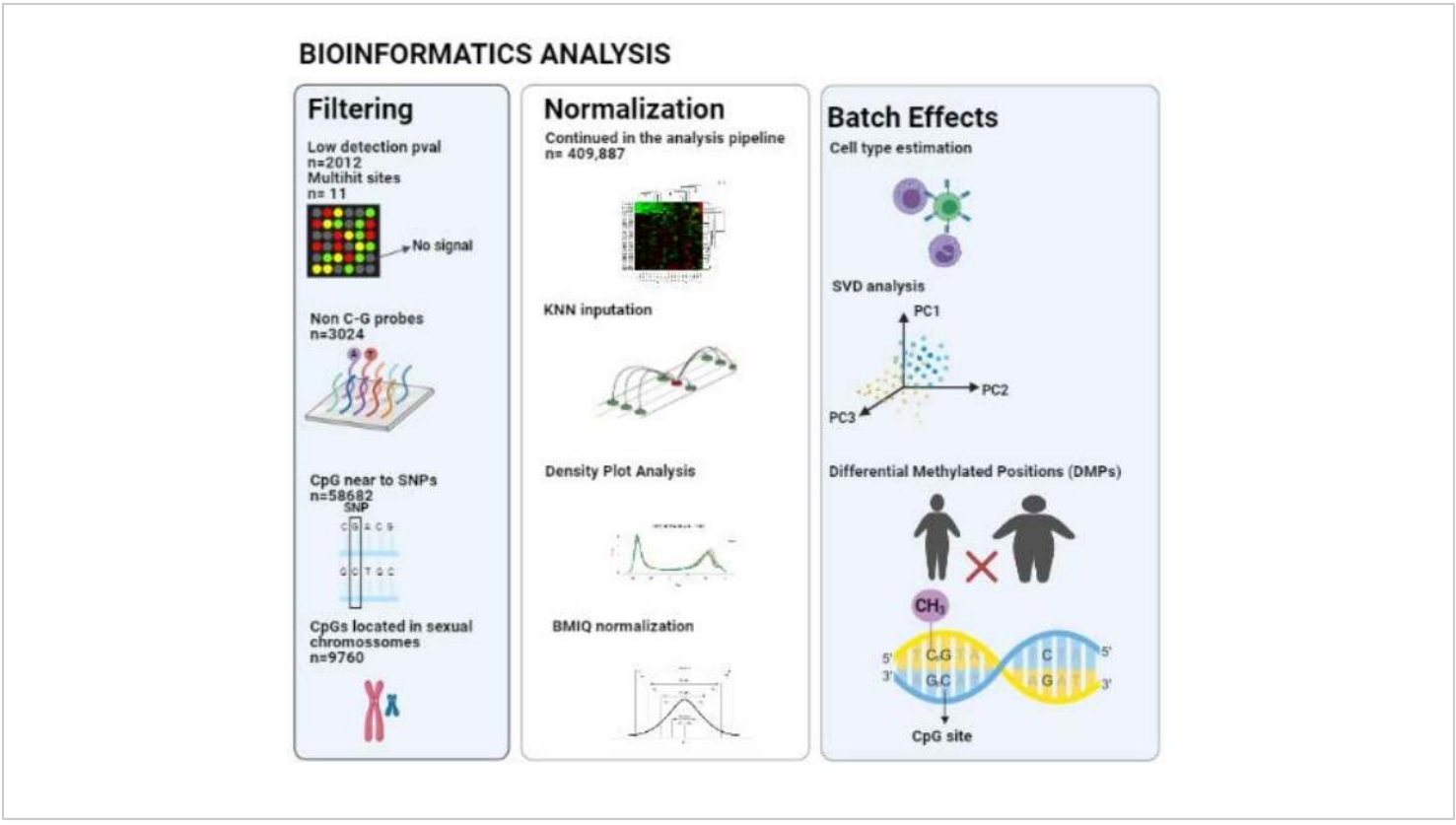

Figure 2: Pipeline of the bioinformatics analysis. Please click here to view a larger version of this figure.

Furthermore, the density plot revealed that all samples had similar densities of the beta distribution related to the quality control steps. This analysis evaluates the distribution of beta values and points out if there are any samples that need to be excluded. In this batch, no samples were excluded based on this analysis.

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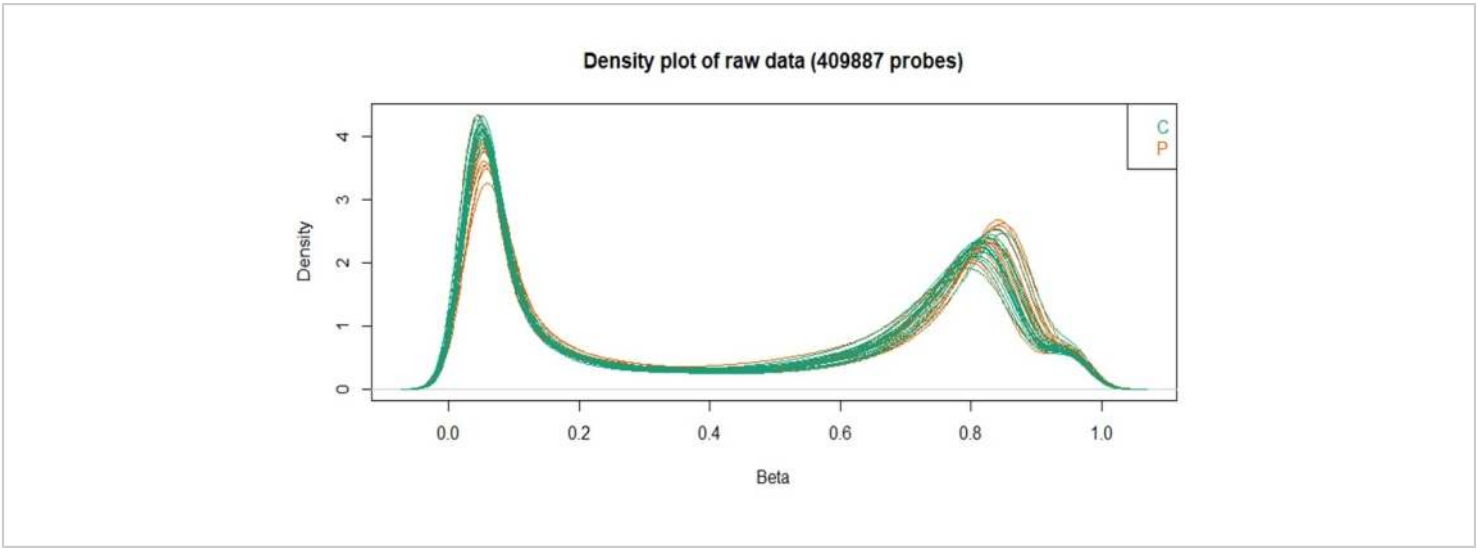

Figure 3: Beta values density plot obtained with the ChAMP package. Please click here to view a larger version of this figure.

The singular value decomposition (SVD) analysis was used to verify the principal components that significantly influenced DNA methylation data variability. The data revealed that BMI, WC ($p < 1e10\text{-}5$), and FM ($p < 0.05$) had a significant effect on the data variability (**Figure 4**).

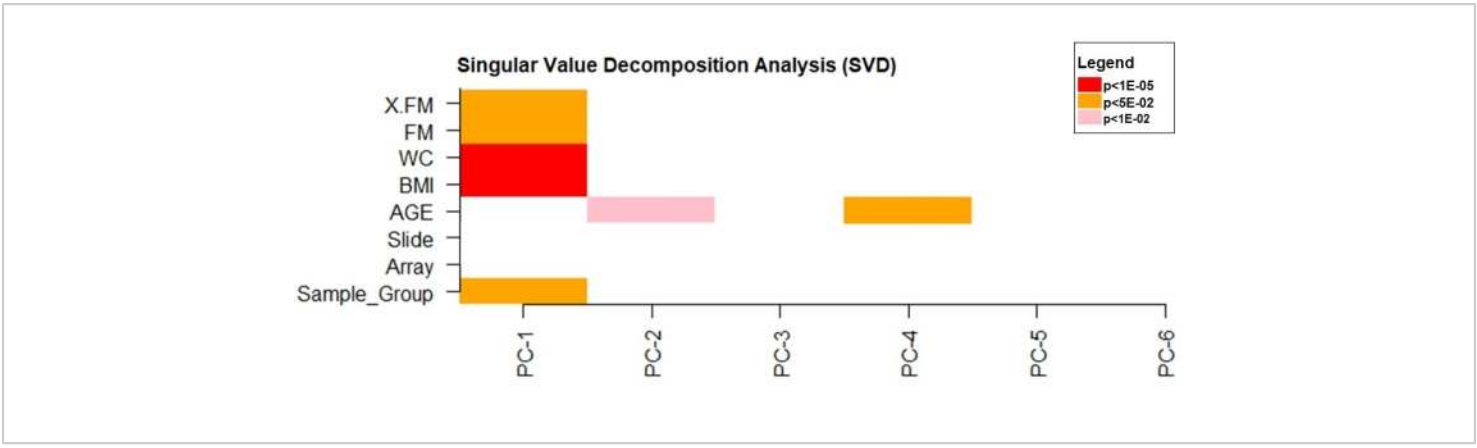

Figure 4: Singular value decomposition analysis. Please click here to view a larger version of this figure.

Cell type estimation revealed that both natural killer cells (NK) and B cells were higher in obese women (**Figure 5**).

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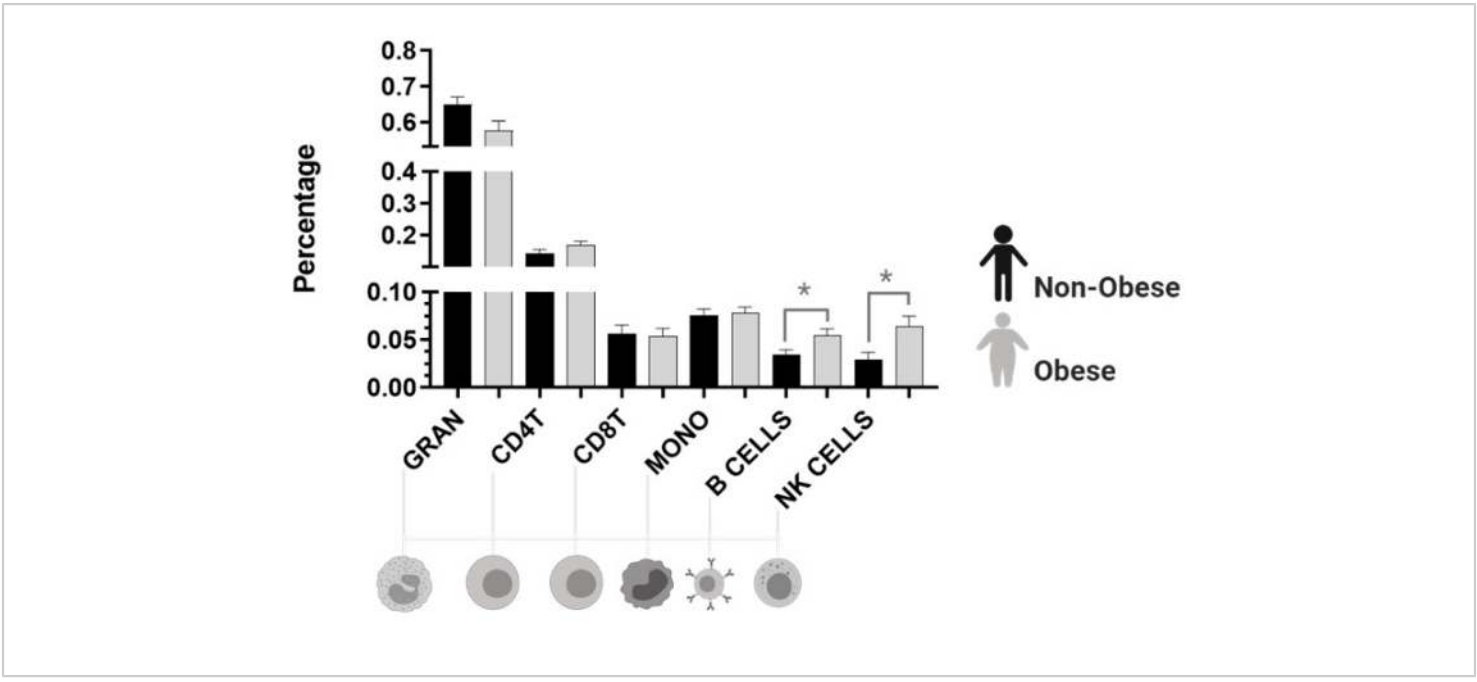

Figure 5: Cell fractions estimated by Houseman's method. Gran: granulocyte. CD4T: helper T cell, lymphocyte. CD8T: cytotoxic T cell, lymphocyte. Mono: monocyte. B Cell: B lymphocyte. NK: natural killer cells, lymphocytes. *p < 0.05. Please click here to view a larger version of this figure.

DNA methylation levels between obese and non-obese women differed before and after cell-type correction. Before DNA methylation data correction for cell types, 43,463 differentially methylated positions (DMPs) were observed, and 3,329 CpGs remained significant after. 445 CpG sites were in intergenic regions (IGR), and 2,884 were in genic regions, with most in the promoter region (n = 1,438). The distribution along all regions were TSS1500 (n = 612), TSS200 (n = 826), 5'UTR (n = 390), first exon (n = 273), body (n = 724), and 3'UTR (n = 59). Considering Δβ values <-0.05 and >0.05, 162 CpGs were hypomethylated and 576 were hypermethylated in obese compared to non-obese individuals (**Figure 6**). The data are available in the GEO database under the register code GSE166611.

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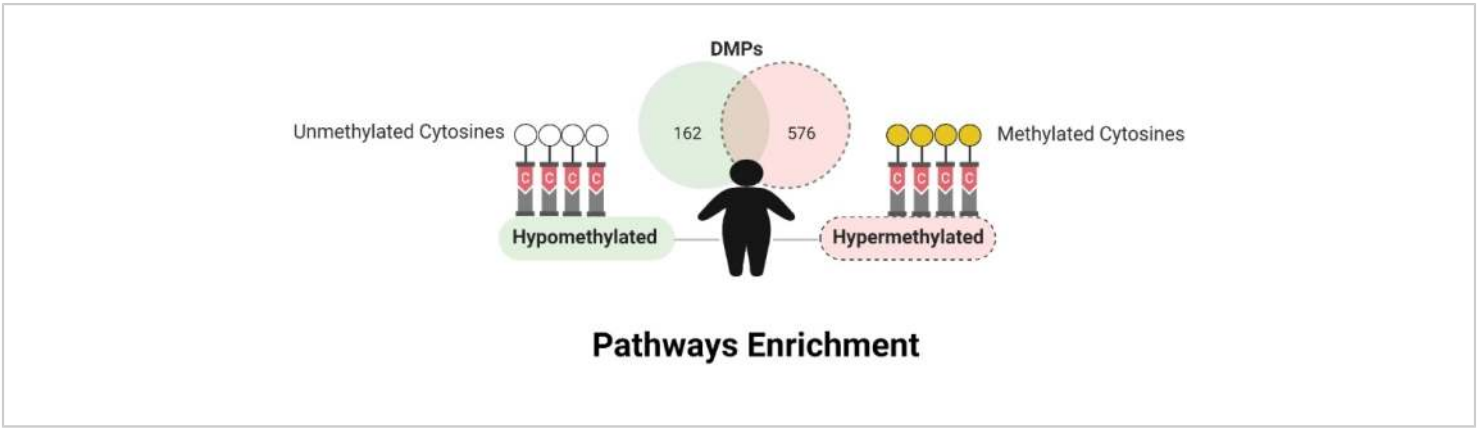

Figure 6: Differentially methylated positions. Please click here to view a larger version of this figure.

It is possible to evaluate the different methylation between groups and find CpG sites associated with specific traits in methylation studies. For fat mass studies, 13,222 CpG sites were found. 6,159 CpGs in the promoter region were related to fat mass, with 470 hypermethylated and 94 hypomethylated (**Figure 7**), and the respective genes were enriched (**Table 2**).

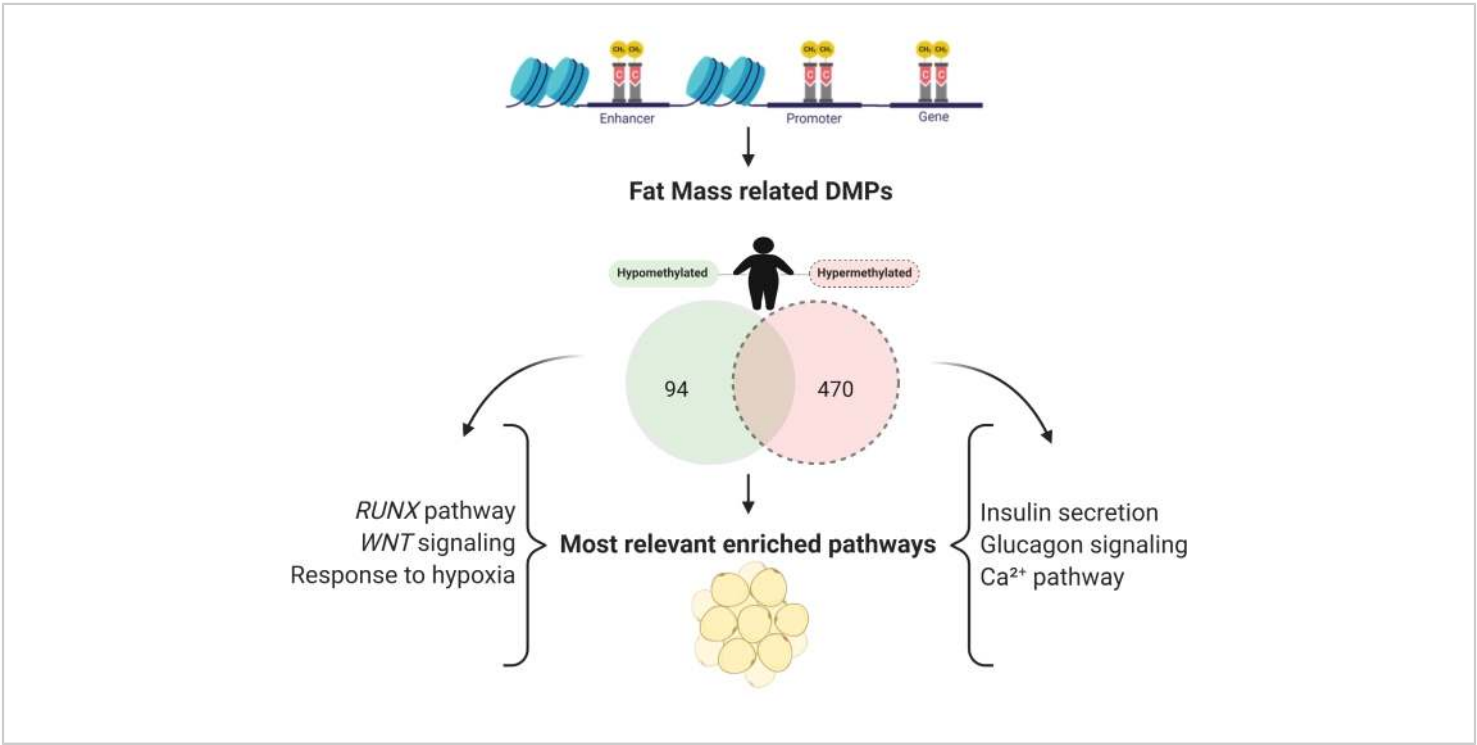

Figure 7: Promoter regions of hypomethylated and hypermethylated genes, independently related to fat mass.
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Table 2: Functional enrichment of the genes from the differentially methylated CpG sites. Please click here to download this Table.

Supplemental Material 1: Please click here to download this File.

Discussion

DNA methylation arrays are the most used methods to access DNA methylation due to their cost-benefit ratio[14]. The present study described a detailed protocol using a commercially available microarray platform to evaluate DNA methylation in a pilot study performed in a Brazilian cohort. The obtained results from the pilot study confirmed the effectiveness of the protocol. **Figure 3** shows the sample comparability and the complete bisulfite conversion[32].

As a quality control step, the ChAMP algorithm recommended the exclusion of CpGs sites during the filtering process. The aim of excluding probes is to improve data analysis and eliminate bias. The low-quality CpGs (p-values lower than 0.05) were removed to eliminate experimental noise in the dataset. The targets remained passed in the density plot analysis. Zhou[33] described the importance of filtering CpGs near SNPs to avoid mismatches, misinterpretation of polymorphic cytosines' methylation, and causing switch color of type I probe design[34]. Also, as XY chromosomes are differentially impacted by imprinting, Heiss and Just[35] reinforced the importance of filtering those probes because, in females, the problems with hybridization may be confounding factors[35].

The DMAPs expiration date, the formamide opening date, the analytical quality of the absolute ethanol, and total leucocyte counts are considered critical steps in the protocol.

Furthermore, according to our observations, the cell-type estimation is essential in performing the bioinformatics analysis. The Houseman method performs the cell type estimation as described in Tian's study[30]. This method is based on 473 specific CpG sites that can predict the percentages of the most important cell types, such as granulocytes, monocytes, B cells, and T cells[36]. We used the recommended function "myRefbase" from the ChAMP package. After the estimation, the ChAMP algorithm adjusts the beta values and eliminates this bias from the dataset. This step is crucial in studies focused on obesity because this population has a considerable difference in white blood cells due to their chronic inflammatory state.

We only changed the original cap map for the common PCR seal regarding method modification and troubleshooting. After each centrifugation process, the seal was changed for a new one. We could not use the standard heat sealing and adapted it using aluminum foil around the plate.

Although commercial assays have been considered a gold standard for epigenetic studies, one limitation of the protocol could be the specificity of the reagents and equipment from a unique brand[37,38,39,40]. Another limitation is the lack of indicators that allow identification of the correct progress of the experiment[41].

The standardization of the present protocol represents a great guide for epigenetic research, reducing human errors during the process and allowing successful data analysis and comparability between different studies.

According to our results, DNA methylation experiments are suitable for studies comparing individuals with and without obesity[43]. Also, the proposed bioinformatics analysis

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provided high-quality data and could be considered in large-scale studies.

Using the SVD analysis, we identified that the obesity-related traits (BMI, WC, and FM) influenced the variability in DNA methylation data. As a significant result, the cell-type estimation indicates that both natural killer cells (NK) and B cells were higher in women with obesity than in women without obesity (**Figure 5**). The higher counts of those cells could be explained by the low-grade inflammatory state of these individuals[44]. We observed that patients with obesity have hypo- and hypermethylated CpGs in promoter regions of genes associated with fat mass. Most of the sites were hypomethylated, which could be related to the natural increase in reactive oxygen species (ROS) levels in these individuals. This oxidative stress condition may promote guanine perturbance at the dinucleotide site, forming 8-hydroxy-2'-deoxyguanosine (8-OHdG), resulting in a 5mCp-8-OHdG dinucleotide site, and causing TET enzymes recruitment. All these events could be responsible for promoting DNA hypomethylation and hypermethylation by different mechanisms of action[45].

In addition, the rate of adipogenesis seems to increase in individuals with obesity, with approximately 10% of new cells to old cells[46,47]. Epigenetic contributions, emphasizing the obesogenic environment, can alter the cells' proliferation and differentiation rates, favoring the development of fat mass[48]. Epigenetic changes can also affect adipogenic programs, facilitating or restricting their development. Primary transcription factors (PPARγ or C / EBPα) or the assembly of multiprotein complexes, positioned in downstream promoter regions operated by including or excluding epigenetic modifying enzymes, regulate gene expression through hyper- or hypomethylation[45]. The PPARγ pathway has been previously described to alter the WNT pathway, which had genes enriched in this study. Although it is still unknown how WNT signaling occurs during adipogenesis, recent studies have reported that it might have essential roles in adipocyte metabolism, particularly under obesogenic conditions[49].

Disclosures

The authors of this article have no conflicts of interest and have no financial disclosure to declare.

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References

1. Manna, P., Jain, S. Obesity, oxidative stress, adipose tissue dysfunction, and the associated health risks: causes and therapeutic strategies. *Metabolic Syndrome and Related Disorders.* **13** (10), 423-444 (2015).

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2. *World Health Organization.* Obesity (2017). Available at: [https://www.who.int/health-topics/obesity#tab=tab_1]. Accessed on February 16, (2021).
3. *Center for Disease Control and Prevention.* Adult Obesity Facts. Available at https://www.cdc.gov/obesity/data/adult.html. (2021).
4. Bird, A. Perceptions of epigenetics. *Nature.* **396** (2007).
5. Kouzarides, T. Chromatin modifications, and their function. *Cell.* **128** (4), 693-705 (2007).
6. Berger, F. The strictest usage of the term epigenetic. *Seminars in Cell & Developmental Biology.* **19** (6), 525-526 (2008).
7. Henikoff, S., Greally, J. M. Epigenetics, cellular memory, and gene regulation. *Current Biology.* **26** (14), 644-648 (2016).
8. Laker, R. C. et al. Transcriptomic and epigenetic responses to short-term nutrient-exercise stress in humans. *Scientific Reports.* **7**(1), 1-12 (2017).
9. Wahl, S. et al. Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity. *Nature.* **541,** 81-86 (2017).
10. Jin, B., Robertson, K. D. DNA methyltransferases, DNA damage repair, and cancer. *Epigenetic Alterations in Oncogenesis.* **754**, 3-29 (2013).
11. Jang, H. S., Shin, W. J., Lee, J. E., Do, J. T. CpG and non-CpG methylation in epigenetic gene regulation and brain function. *Genes.* **8** (6), 148 (2017).
12. Shen, L., Waterland, R. A. Methods of DNA methylation analysis. *Current Opinion in Clinical Nutrition & Metabolic Care.* **10** (5), 576-581(2007).
13. Olkhov-Mitsel, E., Bapat, B. Strategies for discovery and validation of methylated and hydroxymethylated DNA biomarkers. *Cancer Medicine.* **1**, 237-60 (2012).
14. Kurdyukov, S., Bullock, M. DNA methylation analysis: choosing the right method. *Biology.* **5** (1), 3 (2016).
15. Yong, W.-S., Hsu, F.-M., Chen, P.-Y. Profiling genome-wide DNA methylation. *Epigenetics & Chromatin.* **9** (1),1-16 (2016).
16. Dugué, P. A. et al. Alcohol consumption is associated with widespread changes in blood DNA methylation: Analysis of cross-sectional and longitudinal data. *Addiction Biology.* **1**, e12855 (2021).
17. Colicino, E. et al. Blood DNA methylation sites predict death risk in a longitudinal study of 12, 300 individuals. *Aging.* **14**, 14092-14124 (2020).
18. Karlsson, L., Barbaro, M., Ewing, E., Gomez-Cabrero, D., Lajic, S. Genome-wide investigation of DNA methylation in congenital adrenal hyperplasia. *The Journal of Steroid Biochemistry and Molecular Biology.* **201**, 105699 (2020).
19. Ribeiro, R. R., Guerra-Junior, G., de Azevedo Barros-Filho, A. Bone mass in schoolchildren in Brazil: the effect of racial miscegenation, pubertal stage, and socioeconomic differences. *Journal of Bone and Mineral Metabolism.* **27** (4), 494-501 (2009).
20. Filozof, C. et al. Obesity prevalence and trends in Latin-American countries. *Obesity Reviews.* **2** (2), 99-106 (2001).
21. Chadid, S., Kreger, B. E., Singer, M. R., Bradlee, M. L., Moore, L. L. Anthropometric measures of body fat and obesity-related cancer risk: sex-specific differences

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in Framingham Offspring Study adults. *International Journal of Obesity.* **44** (3), 601-608 (2020).

22. Nicoletti, C. F. et al. DNA methylation pattern changes following a short-term hypocaloric diet in women with obesity. *European Journal of Clinical Nutrition.* **74** (9), 1345-1353 (2020).
23. *Centers for Disease Control and Prevention.* Assessing Your Weight (2020). Available at: [https://www.cdc.gov/healthyweight/assessing/index.html]. Accessed on February 14, (2021).
24. Giavarina, D., Lippi, G. Blood venous sample collection: Recommendations overview and a checklist to improve quality. *Clinical Biochemistry.* **50** (10-11), 568-573 (2017).
25. Duijs, F. E., Sijen, T. A rapid and efficient method for DNA extraction from bone powder. *Forensic Science International: Reports.* **2**, 100099 (2020).
26. *Infinium HD Methylation Assay, Manual Protocol.* (2015). Available at: [https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/infinium_assays/infinium_hd_methylation/infinium-hd-methylation-guide-15019519-01.pdf]. Accessed on February 14, (2021).
27. Serrano, J., Snuderl, M. Whole genome DNA methylation analysis of human glioblastoma using Illumina BeadArrays. In *Glioblastoma.* 31-51. Humana Press. New York, NY (2018).
28. Leti, F., Llaci, L., Malenica, I., DiStefano, J. K. Methods for CpG methylation array profiling via bisulfite conversion. *Disease Gene Identification.* **1706**, 233-254 (2018).
29. Noble, A. J. et al. A validation of Illumina EPIC array system with bisulfite-based amplicon sequencing. *PeerJ.* **9**, e10762 (2021).
30. Tian, Y. et al. ChAMP: updated methylation analysis pipeline for Illumina BeadChips. *Bioinformatics.* **33**, 3982-3984 (2017).
31. Turinsky, A. L., et al. EpigenCentral: Portal for DNA methylation data analysis and classification in rare diseases. *Human Mutation.* **41** (10), 1722-1733 (2020).
32. Tian, Y. et al. *The Chip Analysis Methylation Pipeline (2020).* Available at: [https://www.bioconductor.org/packages/release/bioc/vignettes/ChAMP/inst/doc/ChAMP.html]. Accessed on February 14, (2021).
33. Zhou, W., Laird, P. W., Shen, H. Comprehensive characterization, annotation and innovative use of Infinium DNA methylation BeadChip probes. *Nucleic Acids Research.* **45** (4), e22 (2017).
34. Wanding, Z., Triche Jr, T. J. Laird, P. W., Hui, S. SeSAMe: reducing artifactual detection of DNA methylation by Infinium BeadChips in genomic deletions. *Nucleic Acids Research.* **46** (20), e123 (2018).
35. Heiss, J. A., Just, A. C. Improved filtering of DNA methylation microarray data by detection p values and its impact on downstream analyses. *Clinical Epigenetics.* **11**, 15 (2019).
36. Houseman, E. A. et al. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics.* **13**, 86 (2012).
37. Valavanis, I., Sifakis, E. G., Georgiadis, P., Kyrtopoulos, S., Chatziioannou, A. A. A composite framework for the statistical analysis of epidemiological DNA

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methylation data with the Infinium human Methylation 450K BeadChip. I*EEE Journal of Biomedical and Health Informatics.* **18** (3), 817-23 (2014).

38. Sun, N., Zhang, J., Zhang, C., Shi, Y., Zhao, B., Jiao, A., Chen, B. Using Illumina Infinium HumanMethylation 450K BeadChip to explore genomewide DNA methylation profiles in a human hepatocellular carcinoma cell line. *Molecular Medicine Reports.* **18** (5), 4446-56 (2018).
39. Moran, S., Arribas, C., Esteller, M. Validation of a DNA methylation microarray for 850,000 CpG sites of the human genome enriched in enhancer sequences. *Epigenomics.* **8** (3), 389-399 (2016).
40. Bibikova, M. et al. High density DNA methylation array with single CpG site resolution. *Genomics.* **98** (4), 288-295 (2011).
41. Lehne, B., et al. A coherent approach for analysis of the Illumina HumanMethylation450 BeadChip improves data quality and performance in epigenome-wide association studies. *Genome Biology.* **16** (1), 1-12 (2015).
42. Wang, J., Zhang, H., Rezwan, F. I., Relton, C., Arshad, S. H., Holloway, J. W. Pre-adolescence DNA methylation is associated with BMI status change from pre- to post-adolescence. *Clinical Epigenetics.* **25**, 64 (2021).
43. Maugeri, A. The effects of dietary interventions on DNA methylation: implications for obesity management. *International Journal of Molecular Sciences.* **21**, 8670 (2020).
44. DeFuria, J. et al. B cells promote inflammation in obesity and type 2 diabetes through regulation of T-cell function and an inflammatory cytokine profile. *Proceedings of the National Academy of Sciences.* **110** (13), 5133-5138 (2013).
45. Kietzmann, T., Petry, A., Shvetsova, A., Gerhold, J. M., Görlach, A. The epigenetic landscape related to reactive oxygen species formation in the cardiovascular system. *British Journal of Pharmacology.* **174**, 1533-1554 (2017).
46. Chase, K., Sharma, R. P. Epigenetic developmental programs and adipogenesis: implications for psychotropic induced obesity. *Epigenetics.* **8** (11), 1133-1140 (2013).
47. Spalding, K. L. et al. Dynamics of fat cell turnover in humans. *Nature.* **453**, 783-787 (2008).
48. Ross, S. E. et al. Inhibition of adipogenesis by Wnt signaling. *Science.* **289**, 950-953 (2000).
49. Bagchi, D. P. et al. Wnt/β-catenin signaling regulates adipose tissue lipogenesis and adipocyte-specific loss is rigorously defended by neighboring stromal-vascular cells. *Molecular Metabolism.* **42**, 101078 (2020).

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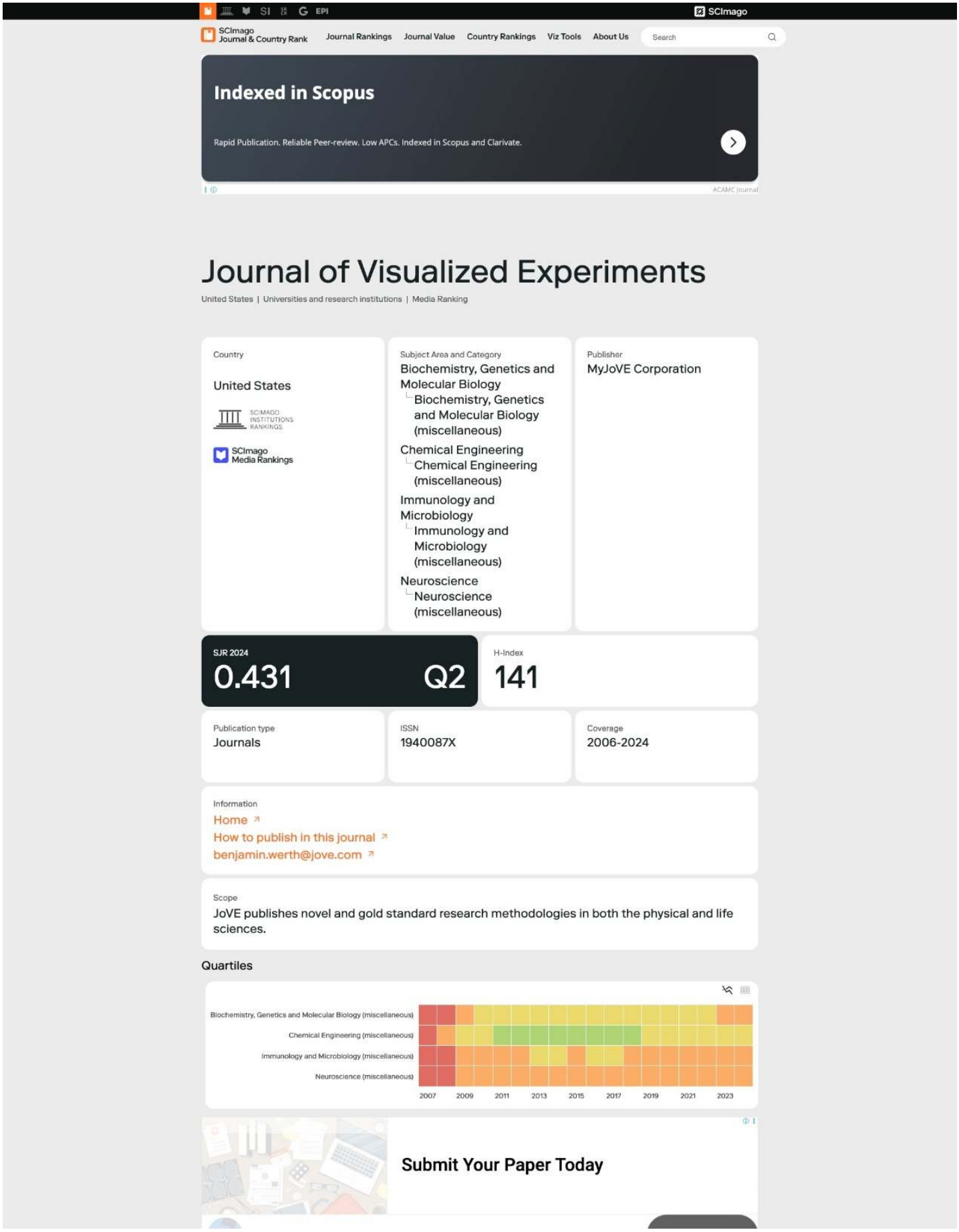
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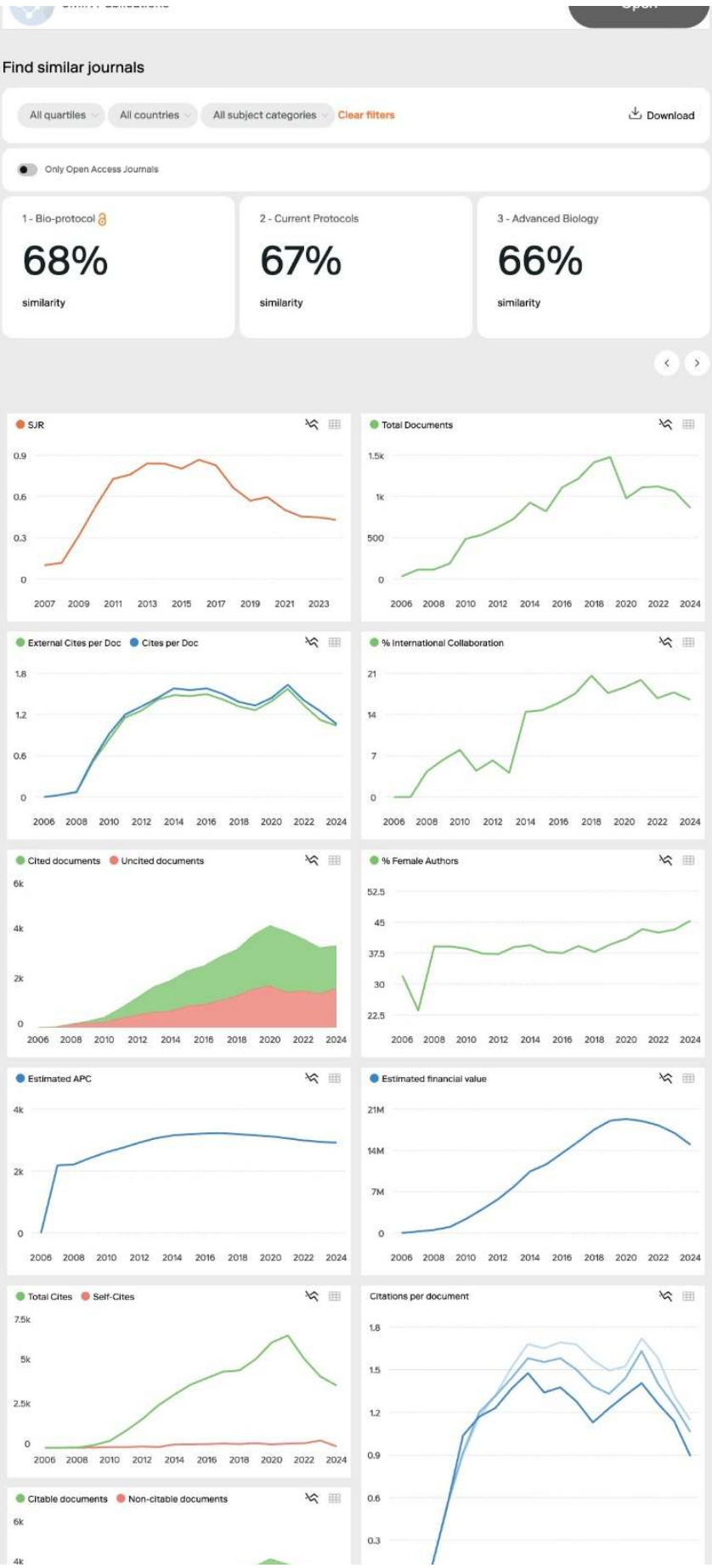
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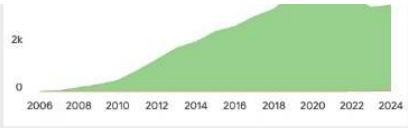
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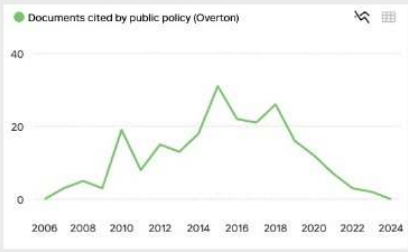
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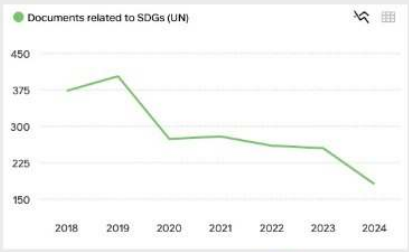
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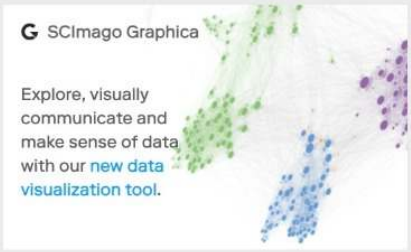
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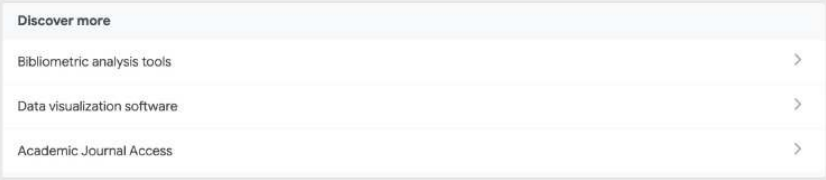
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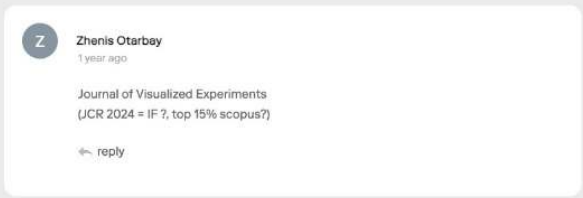
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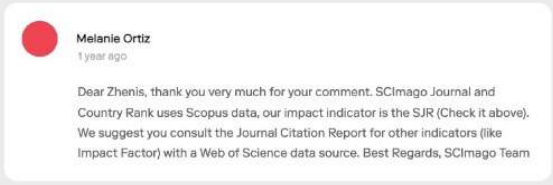
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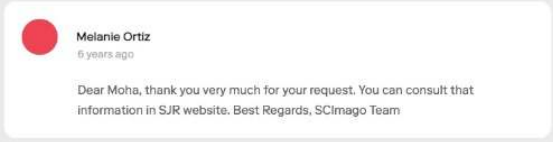
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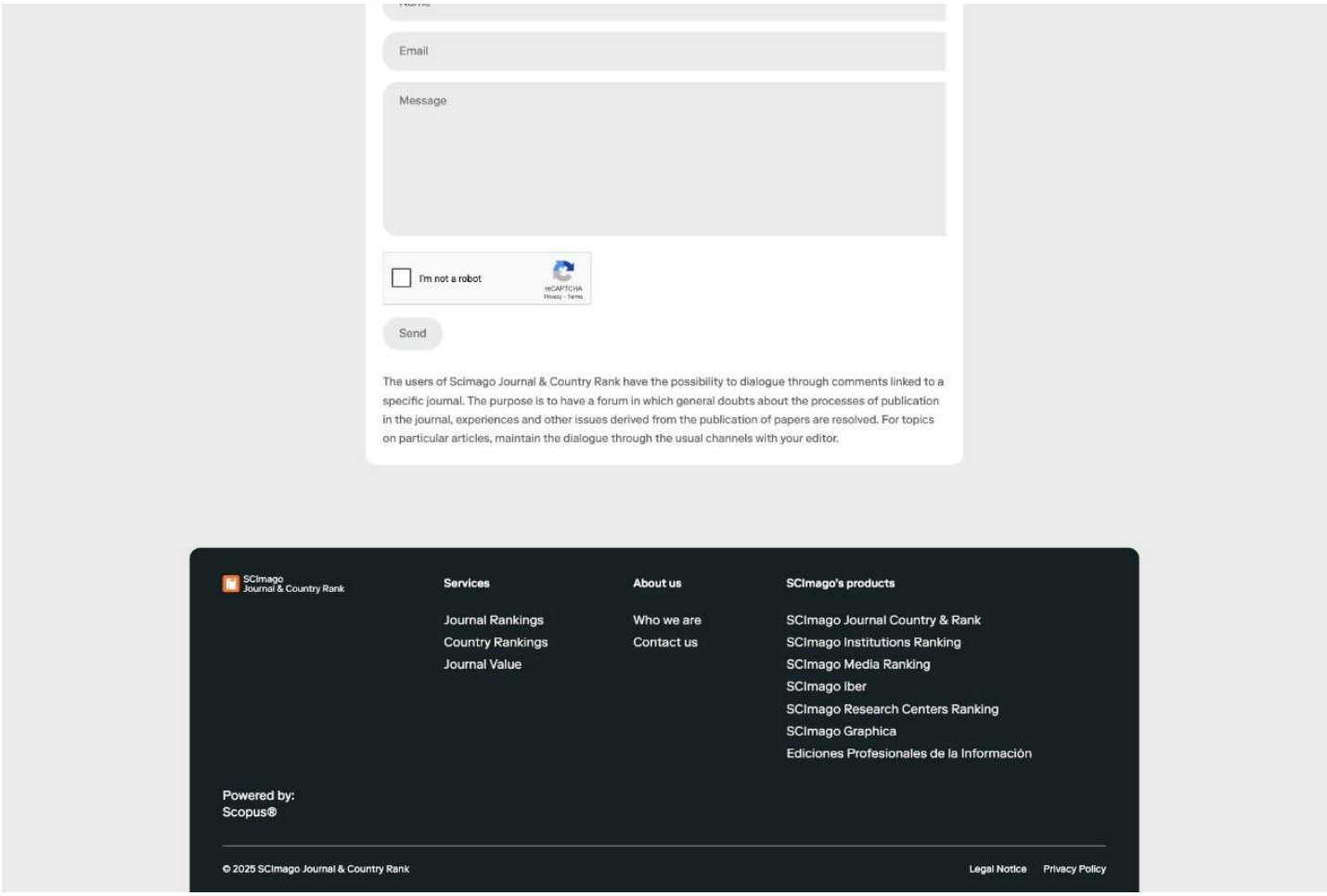
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E.13. Evidence of publication in Asian Pacific Journal of Cancer Prevention (APJCP) (2021):

RESEARCH ARTICLE

Editorial Process: Submission:01/14/2021 Acceptance:04/16/2021

Association of Righ-Risk Human Papillomavirus and *Ureaplasma parvum* Co-Infections with Increased Risk of Low-Grade Squamous Intraepithelial Cervical Lesions

Isabella Harumi Yonehara Noma, Cristiane Suemi Shinobu-Mesquita, Tamy Taianne Suehiro, Fabricio Morelli, Maria Vitória Felipe de Souza, Edilson Damke, Vânia Ramos Sela da Silva, Marcia Edilaine Lopes Consolaro*

Abstract

Objective: The present report investigated the rates of coinfections between high-rik human papillomavirus (hrHPV) and the most important human mycoplasmas including *Mycoplasma hominis, M. genitalium, Ureaplasma urealyticum* and *U. parvum* in cervical samples of asymptomatic brazilian population. **Methods:** Were included a total of 283 women aged 25–64 years screened by Papanicolaou smears for determining cervical abnormalities, single-target polymerase chain reaction (PCR) and real-time PCR (rt-PCR) for hrHPV and mycoplasmas, respectively. **Results:** A total of 273 (94.5%) women were negative for intraepithelial lesions or malignancy cytology (NILM) and 10 (3.5%) presented abnormal cytology, all low-grade intraepithelial lesions (LSIL). The prevalence of hrHPV was 12.7% and 53.7% for mycoplasmas. *U. parvum* was the most frequently bacteria detected, followed by *Mycoplasma hominis* and *U. urealyticum. M. genitalium* was not detected. Women positive for *U. parvum* presented a 5-fold increased risk of LSIL (OR = 5.33; 95% CI = 1.09-26.04, P = 0.02) and co-infections between *U. parvum* and hrHPV increased the risk for LSIL (OR = 3.88; 95% CI = 1.75-8.58, P = 0.0003). However, these associations were not dependent on the concentration of the bacteria. **Conclusion:** Our results reinforced the hypothesis that some mycoplasmas may play a role as cofactors in HPV-mediated cervical carcinogenesis, at least in some populations.

Keywords: Cervical cancer- high-risk HPV- Co-factors- mycoplasmas- risk

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Introduction

Cervical cancer is the fourth leading cause of cancer in women worldwide, despite the existence of highly effective prevention and screening methods (Bray et al., 2018). Persistent high-risk human Papillomavirus (hrHPV) infection is the central factor in the development of squamous cell cervical carcinoma and hrHPV is a prerequisite for progression to high-grade squamous intraepithelial lesions (HSIL) (Kjaer et al., 2010). hrHPV infections are most eventually cleared, but few cases progress to HSIL (10% of HPV infections) and squamous cervical cancer (< 1% of HPV infections) (Ho et al., 1998; Tota et al., 2011; Monsego et al., 2015). The reasons for this variable natural history are poorly understood, but it is generally assumed that other causes or cofactors are important for the development of neoplasia in HPV-infected women (Bodily et al., 2011; Tota et al., 2011). The identification of these cofactors will improve the understanding of cervical carcinogenesis and will help to identify new opportunities for cervical cancer prevention and treatment (Adebamowo et al., 2017).

Several possible risk factors were associated with persistent hrHPV and contributed to cervical carcinogenesis (Moreno et al., 2002; Castle et al., 2003, Appleby et al., 2006; Carrillo-Gárcia et al., 2014). Among them, coinfection with sexually transmitted infections (STI) (de Abreu et al., 2016) as genital mycoplasmas, are gradually becoming more important (Ye et al., 2018). Genital mycoplasmas are groups of small free-living pathogenic bacterium belonging to class Mollicutes that lives on the ciliated epithelial cells of the urinary and genital tracts in humans (Tully et al., 1981), generally transmitted through direct interaction between hosts—venereally through genito-genital or oro-genital contact and vertically from mother to child (either in utero or at birth) (Taylor-Robinson, 2017). Genital mycoplasmas often refer to six species that colonize the genital tract, involving *Ureaplasma urealyticum, U. parvum, Mycoplasma hominis, M. genitalium, M. primatum*, and

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M. spermatophilum. M. primatum and M. spermatophilum are considered non-pathogenic for humans (Ljubin-Sternak and Mestrovic, 2014). Mycoplasmas have been associated with some asymptomatic and symptomatic genital tract conditions, including cervicitis, bacterial vaginosis and endometritis. Cervicitis, an acute or chronic inflammation of the uterine cervix, may promote the infection by human immunodeficiency virus and other STIs, including HPV, by the disruption of epithelial barrier integrity, and subsequent recruitment of inflammatory cells (Patel and Nyirjesky, 2010; Herfs et al., 2011; Silva et al., 2014). However, the results of previous studies on the association between prevalent mycoplasmas and hrHPV infections have been contradictory (biernat-Sudolska et al., 2011; Magaña-Contreraas et al., 2015; de Abreu et al., 2016; Kim et al., 2016; Klein et al., 2020). Therefore, it is still under discussion whether genital mycoplasmas may be a cofactor associated with hrHPV in cervical carcinogenesis.

The real prevalence of co-infections between hrHPV and mycoplasmas in cervical samples is not clear. The overall prevalence of genital mycoplasmas is an under-recognized condition in several populations despite its clinical importance, which is likely because of difficulties in the diagnoses that depend on traditional methods, such as culture (Daley et al., 2014). The recent development and introduction of cultivation-independent molecular-based techniques, such as polymerase chain reaction (PCR), revolutionized the diagnosis of genital tract infections (DÍnzeo et al., 2017) including mycoplasmas. Although studies have investigated cervical infections in Brazilian women, to date, few studies evaluated the coinfections between hrHPV and mycoplasmas in the Brazilian female population. Therefore, the present report investigated the rates of coinfections between hrHPV and the most important human mycoplasmas including *M. hominis, M. genitalium, U. urealyticum* and *U. parvum* in cervical samples of asymptomatic brazilian population. We found that *U. parvum* was the primary pathogen associated with hrHPV for increasing the risk of low-grade squamous intraepithelial lesions in this population, suggesting a possible synergistic action in cervical lesion development.

Materials and Methods

Study population

This cross-sectional study enrolled asymptomatic women for STI and without a history of abnormal cytology residing in Maringá city/Brazil attending at the Public Health System to cervical cancer screening consult between March to October 2016. Women aged 25-64 years old were recruited because this is the recommended screening cohort by the Brazilian Cancer Institute. Women with any of the following factors were excluded: pregnancy, post-partum, previous hysterectomy, vaginal bleeding, previous history of cancer, sexual inactivity, recent treatment for any pathological condition of the urogenital tract, ablative or excisional therapy for the cervix within the previous 12 months, receiving treatment with antimicrobials (oral or topical) within the previous 4 weeks, and those using intrauterine devices or contraceptives delivered directly to the vaginal mucosa. Prior to undergoing gynecological examination, all women signed a written informed consent. Each woman voluntarily agreed to provide a sample of uterine cervix for Pap screening, hrHPV test and molecular mycoplasmas detection. This study was conducted with the supervision and approval of the Committee for Ethics in Research Involving Humans at the State University of Maringá (UEM)/Paraná/Brazil (CAAE: 37091114.2.0000.0104).

Study procedures

Demographic and baseline characteristics were obtained from analysis of the data from the standard registration form of each woman. Cytological samples were obtained from asymptomatic women for cervical cancer screening consult. Ecto/endocervical samples were collected with an Ayre's spatula and cytobrush and were used for conventional Pap smear preparation. For hrHPV and mycoplasmas DNA analyses, samples were immediately suspended in liquid medium (HC2 DNA Collection Device; Digene, Gaithersburg, MD, USA) and stored at −10°C until analysis.

Cytological diagnosis

Cytological examination was conducted without the knowledge of hrHPV status at an accredited clinical laboratory (Clinical Cytology Laboratory of UEM) and reported according to the Bethesda 2001 nomenclature (Solomon and Nayar, 2015). The threshold of abnormal cytology was used to define all abnormal cytological findings including low-grade squamous intraepithelial lesions (LSIL), HSIL and cervical cancer. Negative for intraepithelial lesion or malignancy (NILM) was used to define normal cytological findings.

HPV detection and typing by Cobas HPV test

Cervical specimens (2–5 mL of the liquid medium with sample) were tested for hrHPV DNA using the cobas HPV test (Roche Molecular Systems, USA) run on the fully automated cobas 4800 testing platform. The Cobas HPV test is a DNA test for hrHPV, and the results are reported in three separate channels: HPV16 individually, HPV18 individually, and a pool of 12 other hrHPV genotypes [11 definite high-risk, cancer-associated, HPV types (HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) plus one possibly hrHPV genotype (HPV66)]. A fourth channel measures β-globin for specimen adequacy. Inadequate specimens were retested and if still inadequate, results were treated as positive for patient safety purposes.

Single-target polymerase chain reaction (sPCR) for mycoplasmas detection

sPCR was conducted for qualitative detection of *Ureaplasma* spp., *M. hominis* and *M. genitalium*. DNA was extracted using the AxyPrep Body Fluid Miniprep Kit (Axygen, California, USA) according to the manufacturer's instructions. The quality and quantity of the purified DNA were determined by spectrophotometry on a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Massachusetts, USA).

For the design of primers (supplementary Table 1),

specificity was checked against all sequences in GenBank and primers were aligned by using the Pairwise Sequence Alignment. Subsequently, the primers were evaluated by the OligoAnalyzer Tool (Integrated DNA Technologies) and selected according to melting temperatures, amplification temperature and amplicon size. All primers were evaluated performing a Basic Local Alignment Search Tool (BLAST) analysis against the sequences in the National Center for Biotechnology Information (NCBI) database. To assess the specificity of the primers, all primers were tested in sPCR reactions with different samples and positive control.

The optimized protocol for each assay was a mixture of 25 µL containing 2.5 mM of each deoxynucleotide triphosphates (dNTP), 1U of Taq DNA polymerase (Invitrogen, Carlsbad, CA), 0.6 mM of $MgCl_2$, 25 mM of each primer and 50 ng of the extracted DNA for a final volume of 15 mL. The PCR conditions were comprised of thirty-five amplification cycles; denaturation for 10 min at 94°C, annealing for 1 min at 55°C, extension for 1 min at 72°C, and final extension for 10 min at 72°C (Thermal cycler, Biosystem,CA, USA). PCR products were electrophoresed on 1.0% agarose gel, stained with 1 mg/mL UniSafe Dye (Uniscience, USA), and photodocumented under UV light. Amplification of the human β-globin gene was performed as an internal control using primers GH20 and PC04. Positive controls for all studied bacteria were derived from positive clinical detected samples using reference methods (culture). Two types of controls were also included in each reaction, a "no-DNA" (negative control) and "positive DNA" for each bacteria (positive control). Samples positive for Ureaplasma spp. by sPCR were differentiated in U. urealyticum or *U. parvum* by real-time PCR as follows.

Real-time PCR (rt-PCR) assay for Ureaplasma spp. identification and mycoplasmas quantification

rt-PCR was conducted for the identification of Ureaplasma spp. in *U. urealyticum* or *U. parvum* and for all mycoplasmas quantification using the standardized kits: Bio Gene Research Max *Mycoplasma* spp., Bio Gene Research Max *Ureaplasma parvum*, and Bio Gene Research Max *Ureaplasma urealyticum* (Bioclin, Brazil), following the manufacturer's instructions. This assay has a detection range of 2 to $2X10^5$ copies/µL of each bacteria. For the analysis, we inferred a low concentration of bacteria between 2 and $2x10^2$ copies/µL and as high those between $2X10^3$ and $2X10^5$ copies/µL.

Statistical analysis

Different variables were evaluated for comparisons between hrHPV positive and negative findings for analytical calculations, and between NILM and LSIL women. A two-sided Fisher's exact test for 2×2 contingency table was performed to evaluate the statistical significance between different groups. Crude odds ratios (OR, relative risk) with 95% confidence intervals (CI) were calculated to estimate the association of HPV and mycoplasmas positive findings with socio-demographic data. The age was expressed as a mean and standard deviation (SD). Statistical significance was defined as P values <0.05. All statistical analyses were performed using GraphPad Prism 6.0 (San Diego, California, USA).

Results

Baseline features

Table 1 outlines the baseline features of the study cohort. A total of 360 women were attended during the study period for cervical cancer screening. Of these, 283 women fit the inclusion criteria of the study and were included with a mean (SD) age of 45.84 ± 11.04 years old (range 25-64). Most women exhibited the following characteristics: between 31-50 years old (51.2%); were married (68.2%); had education level ≥8 years (61.1%); were white (55.1%); and had 1-2 children (50.5%).

A total of 273 (96.5%) women presented normal cytology (NILM) and 10 (3.5%) presented LSIL (Table

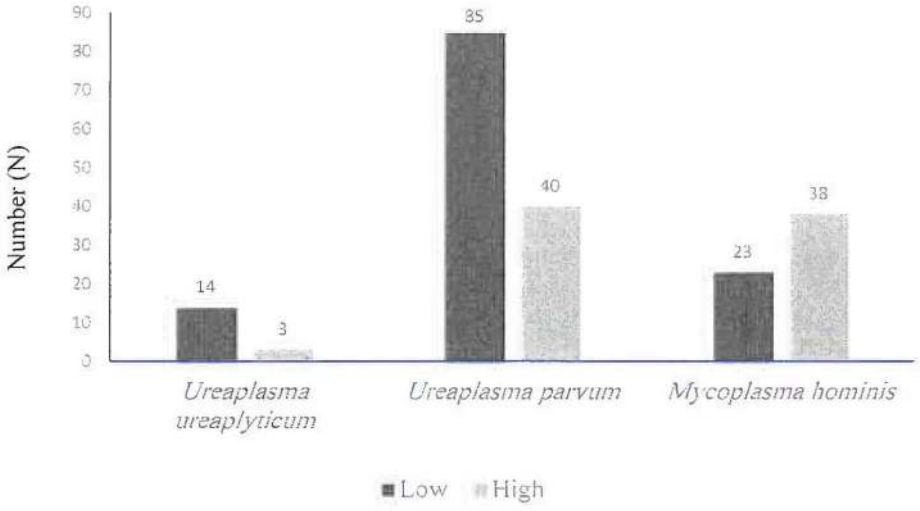

Figure 1. Quantification of Mycoplasmas in Copies/µL by rt-PCR as Low (between 2 and $2x10^2$ copies/µL) and high (between $2X10^3$ and $2X10^5$ copies/µL) Concentration.

Table 1. Overall Baseline Features of the Study Cohort

Characteristics	Overall (n=283) n (%)	hrHPV+ (n=36) n (%)	hrHPV- (N=247) n (%)	OR (95%CI)	P value	*Ureaplasma* spp. + (n=142) n(%)	*Ureaplasma* spp. - (n=141) n (%)	OR (95%CI)	P value	*Mycoplasma hominis*+ (n=61) n (%)	*Mycoplasma hominis*- (n=222) n (%)	OR (95%CI)	P value
Age groups													
≤30 years	33 (11.7)	5 (13.9)	28 (11.34)	2.47 (0.71-8.52)	0.13	16 (11.3)	17 (12.1)	1.09 (0.49-2.41)	0.81	8 (13.1)	25 (11.3)	1.19 (0.47-3.01)	0.70
31-40 years	60 (21.2)	12 (33.3)	48 (19.43)	3.46 (1.25-9.58)	0.01	37 (26.0)	23 (16.3)	1.87 (0.97-3.62)	0.06	16 (26.2)	44 (19.8)	1.35 (0.64-2.85)	0.42
41-50 years	85 (30.0)	12 (33.3)	73 (29.55)	2.28 (0.84-6.13)	0.09	40 (28.2)	45 (31.9)	1.03 (0.58-1.84)	0.90	15 (24.6)	70 (31.5)	0.79 (0.38-1.66)	0.54
>50 years	104 (36.8)	7 (19.4)	97 (39.27)	1	-	48 (33.8)	56 (39.7)	1	-	22 (36.1)	82 (36.9)	1	-
No related	1 (0.3)	0 (0.0)	1 (0.40)	-	-	1 (0.7)	0 (0.0)	-	-	0 (0.0)	1 (0.5)	-	-
Marital status													
Married	193 (68.2)	20 (55.6)	173 (70.0)	1	-	93 (65.5)	100 (70.9)	1	-	36 (59.0)	157 (70.7)	1	
Unmarried	87 (30.7)	15 (41.7)	72 (29.2)	1.80 (0.87-3.73)	0.11	47 (33.1)	40 (28.4)	1.26 (0.76-2.10)	0.37	25 (41.0)	62 (27.9)	1.75 (0.97-3.18)	0.06
No related	3 (1.1)	1 (2.7)	2 (0.8)	-	-	2 (1.4)	1 (0.7)	-	-	0 (0.0)	3 (1.4)	-	
Education level													
<8 years	101 (35.7)	11 (30.6)	90 (36.4)	1.19 (0.55-2.57)	0.65	55 (38.7)	46 (32.6)	1.29 (0.79-2.12)	0.30	21 (34.4)	80 (36.0)	1.03 (0.56-1.89)	0.90
≥8 years	173 (61.1)	22 (61.1)	151 (61.1)	1	-	83 (58.5)	90 (63.8)	1	-	37 (60.7)	136 (61.3)	1	-
No related	9 (3.2)	3 (8.3)	6 (2.5)	-	-	4 (2.8)	5 (3.6)	-	-	3 (4.9)	6 (2.7)	-	-
Ethnic group													
White	156 (55.1)	18 (50.0)	138 (55.9)	1	-	78 (54.9)	78 (55.3)	1		32 (52.5)	124 (55.9)	1	-
No white	118 (41.7)	15 (41.7)	103 (41.7)	1.11 (0.53-2.32)	0.77	59 (41.6)	59 (41.9)	1		28 (45.9)	90 (40.5)	1.20 (0.67-2.14)	0.52
No related	9 (3.2)	3 (8.3)	6 (2.4)	-	-	5 (3.5)	4 (2.8)	-	-	1 (1,6)	8 (3.6)	-	-
Number of children													
0	25 (8.8)	4 (11.1)	21 (8.5)	1.32 (0.40-4.31)	0.64	12 (8.5)	13 (9.2)	1.11 (0.47-2.60)	0.81	8 (13.1)	17 (7.7)	2.59 (0.98-6.82)	0.04
1 or 2	143 (50.5)	18 (50.0)	125 (50.6)	1	-	65 (45.8)	78 (55.3)	1	-	22 (36.1)	121 (54.5)	1	-
≥3	93 (32.9)	10 (27.8)	83 (33.6)	0.83 (0.37-1.90)	0.67	52 (36.6)	41 (29.1)	1.52 (0.89-2.58)	0.11	24 (39.3)	69 (31.1)	1.91 (0.99-3.68)	0.04
No related	22 (7.8)	4 (11.1)	18 (7.3)	-	-	13 (9.1)	9 (6.4)	-	-	7 (11.5)	15 (6.7)	-	-

hrHPV, high-risk human papilomavirus

Table 2. Association between hrHPV and Mycoplasmas with Cytological Findings

	Overall (n = 283) n (%)	Normal (n = 273) n (%)	LSIL (n = 10) n (%)	OR (95%CI)	P -value
hrHPV					
Positive	3 (12.7)	26 (9.5)	10 (100.0)	-	< 0.0001
Negative	247 (87.3)	247 (90.5)	0 (0.0)	1	
Ureaplasma parvum					
Positive	125 (44.2)	117 (42.9)	8 (80.0)	5.33 (1.09-26.04)	0.02
Negative	158 (55.8)	156 (57.1)	2 (20.0)	1	
Ureaplasma urealyticum					
Positive	17 (6.0)	17 (6.3)	0 (0.0)	-	> 0.99
Negative	266 (94.0)	256 (93.7)	10 (100.0)	1	
Mycoplasma hominis					
Positive	61 (21.5)	59 (21.6)	2 (20.0)	1.10 (0.26-5.27)	> 0.99
Negative	222 (78.5)	214 (78.4)	8 (80.0)	1	

hrHPV, high-risk human papillomavirus; LSIL, low-grade squamous intraepithelial lesions

Table 3. Overall Rates of hrHPV and Mycoplasmas co-Infections in Women Included in the Study

Mycoplasmas	Overall (n=283) n (%)	hrHPV + (n=36) n (%)	hrHPV – (n=247) n (%)	OR (95%CI)	P- value
Ureaplasma parvum					
Positive	125 (44.2)	26 (72.2)	99 (40.1)	3.88 (1.75-8.58)	0.0003
Negative	158 (55.8)	10 (27.8)	148 (59.9)	1	
Ureaplasma urealyticum					
Positive	17 (6.0)	2 (5.6)	15 (6.1)	0.91 (0.19-4.16)	0.90
Negative	266 (94.0)	34 (94.4)	232 (93.9)	1	
Mycoplasma hominis					
Positive	61 (21.6)	8 (22.2)	53 (21.5)	1.04 (0.45-2.43)	0.92
Negative	222 (78.4)	28 (77.8)	194 (78.5)	1	

hrHPV, high-risk human papillomavirus

2). Women with NILM and LSIL had similar ages (45.90 ± 10.99 and 44.10 ± 12.84 years old, respectively; P = 0.30).

hrHPV positivity

Thirty-six women (12.7%) were positive for hrHPV (Tables 2 and 3). HPV16 was detected in 4 (11.1%) and HPV18 in 2 (5.6%) women. In the remaining 30 (83.3%) women, other types of hrHPV were detected. Twenty nine (10.6%) women with NILM and all (100%) women with LSIL were hrHPV positive. Thus, hrHPV was associated with LSIL (P < 0.0001) (Table 2).

hrHPV positive women were younger than hrHPV negative women (41.42 ±10.52 vs. 46.49 ±10.99, respectively, p-value: 0.004) and most (51.2%) had between 31-40 years (P = 0.01) (Table 1).

Table 4. Rates of hrHPV and *U. parvum* co-Infections in Relation to Bacteria Concentrations

U. parvum concentration	hrHPV+ (n=26) n (%)	hrHPV- (n=99) n (%)	OR (95%CI)	P value
Low	23 (88.5)	92 (92.9)	1	-
High	3 (11.5)	7 (7.1)	1.71 (0.45-7.07)	0.43

hrHPV, high-risk human papillomavirus; Low, between 2 and $2x10^2$ copies/µL of *U. parvum*; High, between $2X10^3$ and $2X10^5$ copies/µL of U. parvum.

Cervical mycoplasmas positivity and quantification

The PCR results for cervical mycoplasmas presented high rates of these bacteria (n = 152/283, 53.7%), with 142/283 (50.2%) positive women for *Ureaplasma* spp. and 61 (21.6%) for *Mycoplasma* spp. (Tables 2 and 3). In 51/152 of the women positive for mycoplasmas (33.6%). *Ureaplasma* spp. and *Mycoplasma* spp. were detected simultaneously (OR = 7.34, 95%CI = 3.36-16.00, P < 0.0001). For *Mycoplasma* spp. (n = 61), all samples (100%) were positive for *M. hominis* and none positive for M. genitalium.

Women positive for *Ureaplasma* spp. had similar mean age to negative women (44.84 ± 10.65 vs. 46.85 ± 11.37 respectively, P = 0.07). Women positive for *M. hominis* also had similar mean age to negative women (45.05 ± 11.09 vs. 46.06 ± 11.04 respectively, P = 0.26). Women positive for *M. hominis* were associated with no children and with ≥3 children (P = 0.04 for both).

By rt-PCR, the samples of *Ureaplasma* spp. (n = 142) were identified as *U. parvum* (n = 125, 88%)

and *U. urealyticum* (n = 17, 12%). None of the women was positive to both bacteria. Thus, *U. parvum* (n = 125/152, 82.2%) was the most frequently bacteria detected, followed by M. hominis (n = 61/152, 40.1%) and U. urealyticum (n = 17/152, 11.2%).

Regarding cytological findings, women positive for *U. parvum* have a 5-fold increased risk of LSIL (OR = 5.33; 95% CI = 1.09-26.04, P = 0.02) (Table 2).

Figure 1 represents the quantification of mycoplasmas in copies/μL by rt-PCR as low and high concentration. It was possible to observe that despite *U. parvum* being the most detected mycoplasma it was quantified mainly in low concentrations (n = 85/125, 68%). On the other hand, the second most prevalent bacteria (*M. hominis*) was found mainly in higher concentrations (n = 38/61, 62.3%). *U. urealyticum* was quantified mainly in low concentrations (n = 14/17, 82.4%).

hrHPV and mycoplasmas co-infections

Women positive for *U. parvum* had a 4-fold increased risk of hrHPV positivity (OR = 3.88; 95% CI = 1.75-8.58, P = 0.0003) (Table 3). Although we have evidenced the association between *U. parvum* with hrHPV, these associations were not dependent on the concentration of the bacteria, as shown in Table 4.

Discussion

This study investigated the rates of co-infections between hrHPV and important human mycoplasmas in cervical samples of asymptomatic Brazilian population. We found that *U. parvum* was the primary mycoplasma associated with hrHPV for increasing the risk of LSIL in this population. Therefore, *U. parvum* might play a synergistic role in the initial stage of cervical lesions caused by hrHPV infection.

Previous reports presented high rates of ureaplasmas and *M. hominis* detection in asymptomatic sexually mature females (Taylor-Robinson et al., 2017). Epidemiological data showed that *Ureaplasma* spp. can be detected in the cervix or vagina of 40-80% of sexually mature asymptomatic females, whereas *M. hominis* and M. genitalium only in 20-50% and 0-5%, respectively (Morse et al., 2010). Our results are consistent with these evidences since *Ureaplasma* spp. was detected in 142 (50.2%) women, *M. hominis* in 61 (21.6%), and *M. genitalium* was not detected. Additionally, we found that *U. parvum* was the most frequent bacterial agent detected (n = 125/283, 44.2%). Our results are similar to obtained in a recent study, which *U. parvum* was the most prevalent mycoplasma in the cervix (Carneiro et al., 2020). However, for these authors, *U. parvum*, *M. hominis* and *U. urealyticum* were detected at much lower rates (14.9%, 8.5% and 4.2%, respectively) than in our study, and *M. genitalium* was also not detected. Foschi et al., (2018) also found that *U. parvum* was the most prevalent mycoplasma (67.3%), followed by *M. hominis* (14.3%), U. urealyticum (9.2%) and *M. genitalium* (3.1%) in the group of asymptomatic healthy women.

HPV is necessary, but not sufficient, to cause cervical cancer, which supports the influence of additional factors in carcinogenesis associated with hrHPV (Ho et al., 1998; Kjaer et al., 2010; Tota et al., 2011; Ko et al., 2015; Monsonego et al., 2015). Mycoplasmas have been suggested as a possible risk factor for the development of cervical lesions (Ekiel et al., 2009) and cervical cancer (Biernat-Sudolska et al., 2011), and with HPV infections in samples with abnormal cytology (Biernat-Sudolska et al., 2011; Kawaguchi et al., 2012). Our results are in agreement with these findings once women positive for *U. parvum* showed a 5-fold increased risk of LSIL. Additionally, we observed significant association between *U. parvum* and hrHPV infections, indicating that women with both infections might be more likely to develop cervical lesions. Our results are consistent with the hypothesis that mycoplasmas can exert a synergistic action with hrHPV and possibly act as cofactors in the development of cervical lesions (Ho et al., 1998; Bodily et al., 2011; Tota et al., 2011). Therefore, detecting and eradicating *U. parvum* infection might contribute to reduce HPV-related carcinogenesis (Drago et al., 2016).

However, our results also suggest that there exist differences between different mycoplasmas regarding the potential to act as a cofactor for hrHPV since only *U. parvum* was associated with LSIL and hrHPV. These results are in agreement with Drago et al., (2016) which found a strong correlation between *U. parvum*-HPV co-infection and CIN1. According to these authors, this data indicates that *U. parvum* may be involved in HPV persistence and in promoting the development of cytological abnormalities. Additionally, in a recent meta-analysis, *U. parvum* was also associated with a significantly increased risk of abnormal cervical cytopathology (Ye et al., 2018). Finally, Camporiondo et al., (2016) showed that the probability of HPV-positive women being infected by *U. parvum* was 2.5 fold higher than in HPV-negative women.

The association between *U. urealyticum* and *M. hominis* with abnormal cervical cytopathology and with hrHPV was not observed in our results, in contrary to other authors (Magaña-Contreras et al., 2015; de Abreu et al., 2016; Kim et al., 2016; Kim et al., 2018; Klein et al., 2020). This can be explained, at least in part, by the differences between the populations evaluated in the different studies as well as in the techniques used to detect and identify mycoplasmas. Thus, it is very important that future studies should be carried out in different populations with similar clinical conditions to elucidate the participation of different mycoplasmas as cofactors of hrHPV in cervical carcinogenesis.

Although we have evidenced the association between *U. parvum* with LSIL and with hrHPV, these associations were not dependent on the concentration of the bacteria. This data may indicate that possibly the presence of the bacteria regardless of their concentration in the cervical environment would be sufficient to act synergistically with hrHPV in the development of cervical lesions. In a recent study, Klein et al., found different results regarding the mycoplasmas concentration and cervical lesions. More specifically, these authors showed that *M. hominis* prevalence was similar despite the severity of cervical lesions; however the abundance of *M. hominis* increased significantly in women with cervical lesions. Therefore,

the role of *U. parvum* concentration as a risk factor for the development of cervical lesions has not been completely elucidated and requires further investigation.

The present study had no data on the history of hrHPV and vaginal infections as well as mycoplasmas agents in the women prior to enrollment in the study or during follow-up, which limits our interpretations on the influence of hrHPV persistence. As we evaluated mycoplasmas and hrHPV infections at the same time, we were not able to rule out reverse causation. It is important to note that our study did not aim to assess the carcinogenicity mechanisms of the synergistic action between hrHPV and mycoplasmas agents, but assessed the possible occurrence of this synergistic action.

In conclusion, this study provided an opportunity to determine the rates of co-infection between hrHPV and important mycoplasmas in different cytological cervical samples. We found that *U. parvum* was the primary pathogen associated with hrHPV for the increased risk of cervical abnormalities, particularly for LSIL in an asymptomatic population. Our results reinforce the hypothesis that some mycoplasmas may play a role as cofactors in hrHPV-mediated cervical carcinogenesis. Further cross-sectional studies are needed in different populations mainly in those with high rates of HSIL and cervical cancer.

Author Contribution Statement

IHY Noma, CS Shinobu-Mesquita, TT Suehiro, MEL Consolaro, and F Morelli designed and wrote the study protocol; IHYNoma, MVF de Souza, and E Damke were in charge of HPV analysis; IHY Noma, VRS da Silva, and CS Shinobu-Mesquita managed the data and performed the statistical analysis; and IHY Noma, CS Shinobu-Mesquita, TT Suehiro, F Morelli, MVF de Souza, E Damke, VRS da Silva, and MEL Consolaro wrote the manuscript. All of the authors have read and approved the final manuscript.

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Approvation by scientific Body/part of an student thesis: This study was part of an approved student thesis (Isabella Harumi Yonehara Noma).

Ethics approval and consent to participate

Prior to undergoing gynecological examination, all women signed a written informed consent. Each woman voluntarily agreed to provide a sample of uterine cervix for Pap screening, hrHPV test and molecular mycoplasmas detection. This study was conducted with the supervision and approval of the Committee for Ethics in Research Involving Humans at the State University of Maringá (UEM)/Paraná/Brazil (CAAE: 37091114.2.0000.0104).

Statement conflict of Interest

The authors declare no conflicts of interest or industry supports of the project in this study.

References

Adebamowo SN, Ma B, Zella D, et al (2017). Mycoplasma hominis and Mycoplasma genitalium in the vaginal microbiota and persistent high-risk human papillomavirus infection. *Front Public Health*, **5**, 140.

Appleby P, Beral V, et al (2006). Carcinoma of the cervix and tobacco smoking: Collaborative reanalysis of individual data on 13,541 women with carcinoma of the cervix and 23,017 women without carcinoma of the cervix from 23 epidemiological studies. *Int J Cancer*, **118**, 1481-95.

Biernat-Sudolska M, Szostek S, Rojek-Zakrzewska D, et al (2011). Concomitant infections with human papillomavirus and various mycoplasma and ureaplasma species in women with abnormal cervical cytology. *Adv Med Sci*, **56**, 299-303.

Bodily J, Laimins LA (2011). Persistence of human papillomavirus infection: keys to malignant progression. *Trends Microbiol*, **19**, 33-9.

Bray F, Ferlay J, Soerjomataram I, et al (2018). Global cancer statistics 2018. GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*, **68**, 394–424.

Camporiondo MP, Farchi F, Ciccozzi M, et al (2016). Detection of HPV and co-infecting pathogens in healthy Italian women by multiplex real-time PCR. *Infez Med*, **24**, 12-7.

Carneiro FP, Darós AC, Darós ACM, et al (2020). Cervical cytology of samples with Ureaplasma urealyticum, Ureaplasma parvum, Chlamydia trachomatis, Trichomonas vaginalis, Mycoplasma hominis, and Neisseria gonorrhoeae detected by multiplex PCR. *Biomed Res Int*, **2020**, 7045217.

Carrillo-Garcia A, Ponce-de-León-Rosales S, Cantú-de-León D, et al (2014). Impact of human papillomavirus coinfections on the risk of high-grade squamous intraepithelial lesion and cervical cancer. *Gynecol Oncol*, **134**, 534-9.

Castle PE, Giuliano AR (2003). Chapter 4: Genital tract infections, cervical inflammation, and antioxidant nutrients-assessing their roles as human papillomavirus cofactors. *J Natl Cancer Inst Monogr*, **31**, 29-34.

D'Inzeo T, De Angelis G, Fiori B, et al (2017). Comparison of Mycoplasma IES, Mycofast Revolution and Mycoplasma IST2 to detect genital mycoplasmas in clinical samples. *J Infect Dev Ctries*, **11**, 98-101.

Daley GM, Russell DB, Tabrizi SN, et al (2014). Mycoplasma genitalium: a review. *Int J STD AIDS*, **25**, 475-87.

de Abreu ALP, Malaguti N, Souza RP, et al (2016). Association of human papillomavirus, Neisseria gonorrhoeae and Chlamydia trachomatis co-infections on the risk of high-grade squamous intraepithelial cervical lesion. *Am J Cancer Res*, **6**, 1371–83.

Discacciati MG, Gimenes F, Pennacchi PC, et al (2015). MMP-9/RECK imbalance: a mechanism associated with high-grade cervical lesions and genital infection by human papillomavirus and chlamydia trachomatis. *Cancer Epidemiol Biomarkers Prev*, **24**, 1539-47.

Drago F, Herzum A, Ciccarese G, et al (2016). Ureaplasma parvum as a possible enhancer agent of HPV-induced cervical intraepithelial neoplasia: preliminary results. *J Med*

Virol, **88**, 2023-24.
Ekiel AM, Friedek DA, Romanik MK, et al (2009). Occurrence of Ureaplasma parvum and Ureaplasma urealyticum in women with cervical dysplasia in Katowice, Poland. *J Korean Med Sci*, **24**, 1177-81.
Foschi C, Salvo M, D'Antuono A, et al (2018). Distribution of genital Mollicutes in the vaginal ecosystem of women with different clinical conditions. *New Microbiol*, **41**, 225-9.
Herfs M, Hubert P, Moutschen M, et al (2011). Mucosal junctions: open doors to HPV and HIV infections?. *Trends Microbiol*, **19**, 114-20.
Ho GYF, Bierman R, Beardsley L, et al (1998). Natural history of cervicovaginal papillomavirus infection in young women. *N Engl J Med*, **338**, 423-8.
Kawaguchi S, Shigehara K, Sasagawa T, et al (2012). Liquid-based urine cytology as a tool for detection of human papillomavirus, Mycoplasma spp., and Ureaplasma spp. in men. *J Clin Microbiol*, **50**, 401-6.
Kim HS, Kim TJ, Lee IH, et al (2016). Associations between sexually transmitted infections, high-risk human papillomavirus infection, and abnormal cervical Pap smear results in OB/GYN outpatients. *J Gynecol Oncol*, **27**, e49.
Kim S II, Yoon JH, Park DC, et al (2018). Co-infection of Ureaplasma urealyticum and human papilloma virus in asymptomatic sexually active individuals. *Int J Med Sci*, **15**, 915-20.
Kjær SK, Frederiksen K, Munk C, et al (2010). Long-term absolute risk of cervical intraepithelial neoplasia grade 3 or worse following human papillomavirus infection: role of persistence. *J Natl Cancer Inst*, **102**, 1478-88.
Klein C, Samwel K, Kahesa C, et al (2020). Mycoplasma co-infection is associated with cervical cancer risk. *Cancers (Basel)*, **12**, 1093.2020.
Koh W-J, Greer BE, Abu-Rustum NR, et al (2015) Cervical Cancer, Version 2.2015. *J Natl Compr Cancer Netw*, **13**, 395-404.
Ljubin-Sternak S, Meštrović T (2014). Chlamydia trachomatis and genital mycoplasmas: pathogens with an impact on human reproductive health. *J Pathog*, **2014**, 183167.
Magaña-Contreras M, Contreras-Paredes A, Chavez-Blanco A, et al (2015). Prevalence of sexually transmitted pathogens associated with HPV infection in cervical samples in a Mexican population. *J Med Virol*, **87**, 2098-2105.
Monsonego J, Cox JT, Behrens C, et al (2015). Prevalence of high-risk human papilloma virus genotypes and associated risk of cervical precancerous lesions in a large U.S. screening population: data from the ATHENA trial. *Gynecol Oncol*, **137**, 47-54.
Moreno V, Bosch FX, Muñoz N, et al (2002). Effect of oral contraceptives on risk of cervical cancer in women with human papillomavirus infection: the IARC multicentric case-control study. *Lancet*, **359**, 1085-92.
Morse SA, Holmes K, King K, Ballard R, Ronald C (2010). Atlas of sexually transmitted diseases and AIDS: Expert Consult. 4th Edition, Saunders 2010 [Published: 17 Sep 2010].
Patel MA, Nyirjesy P (2010). Role of Mycoplasma and Ureaplasma species in female lower genital tract infections. *Curr Infect Dis Rep*, **12**, 417-22.
Silva J, Cerqueira F, Medeiros R (2014). Chlamydia trachomatis infection: implications for HPV status and cervical cancer. *Arch Gynecol Obstet*, **289**, 715-23.
Solomon D, Nayar R (2015). The Bethesda system for cervicovaginal cytology. definitions, criteria, and explanatory notes. *Anticancer Res*, **35**, 5708.
Taylor-Robinson D (2017). Mollicutes in vaginal microbiology: Mycoplasma hominis, Ureaplasma urealyticum, Ureaplasma parvum and Mycoplasma genitalium. *Res Microbiol*, **168**, 875-81.
Tota JE, Chevarie-Davis M, Richardson LA, et al (2011). Epidemiology and burden of HPV infection and related diseases: implications for prevention strategies. *Prev Med*, **53**, S12-21.
Tully JG, Taylor-Robinson D, Cole RM, Rose DL (1981). A newly discovered mycoplasma in the human urogenital tract. *Lancet*, **1**, 1288-91.
Ye H, Song T, Zeng X, et al (2018). Association between genital mycoplasmas infection and human papillomavirus infection, abnormal cervical cytopathology, and cervical cancer: a systematic review and meta-analysis. *Arch Gynecol Obstet*, **297**, 1377-87.

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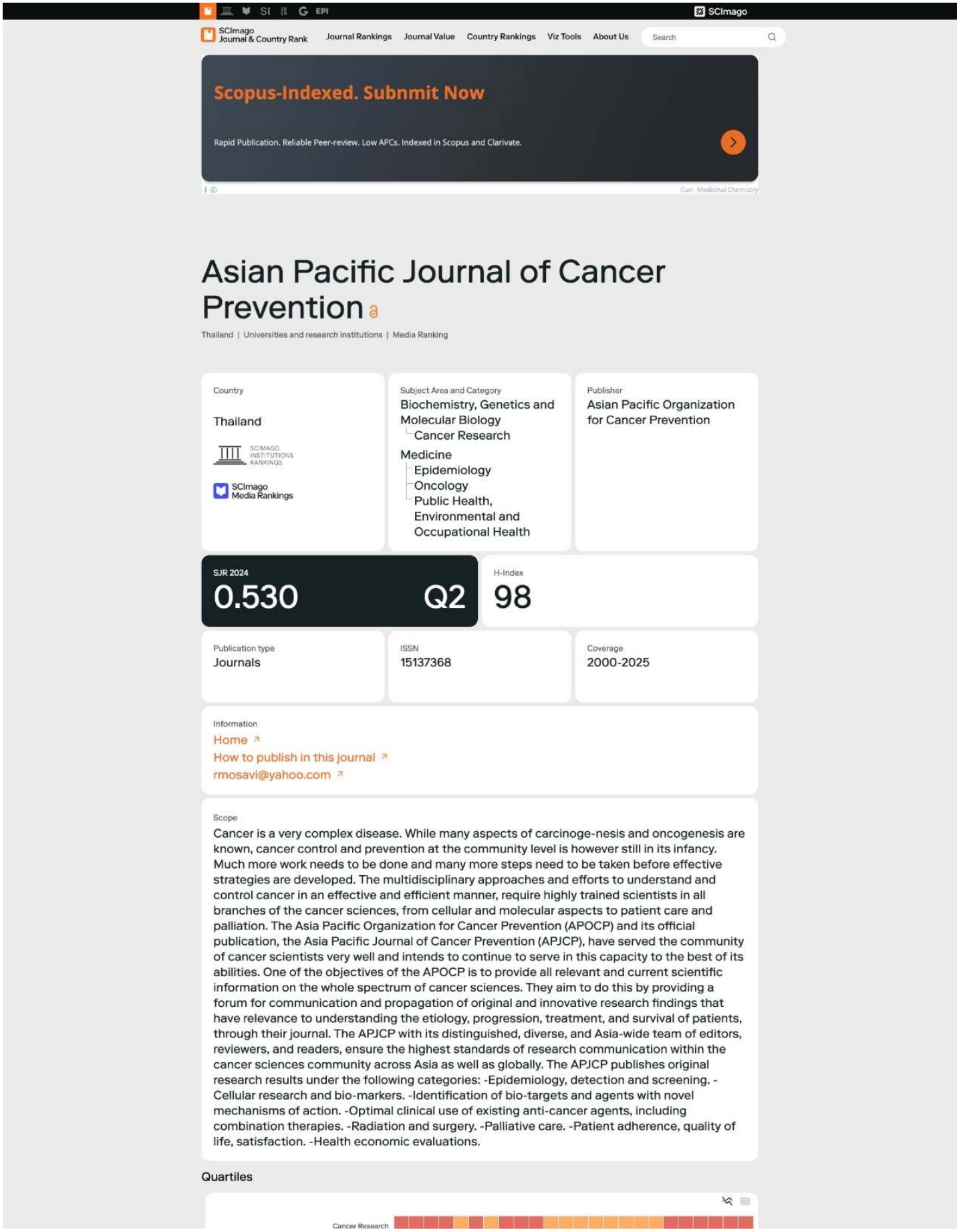
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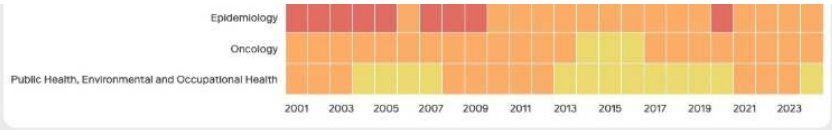

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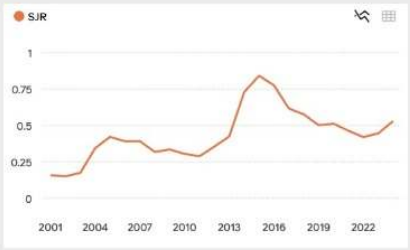

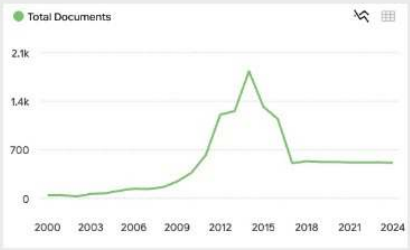

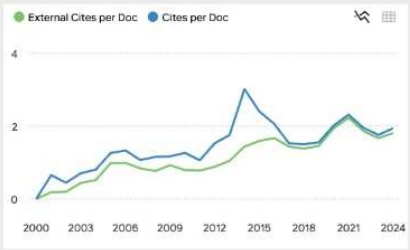

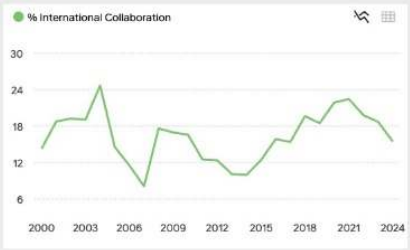

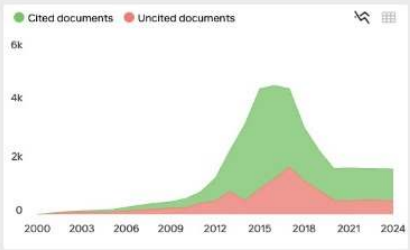

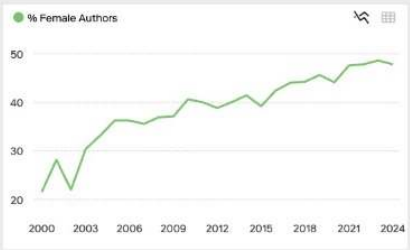

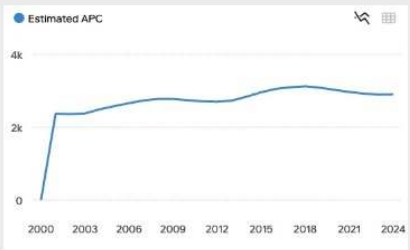

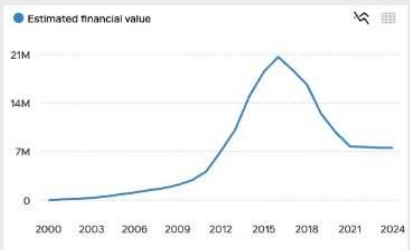

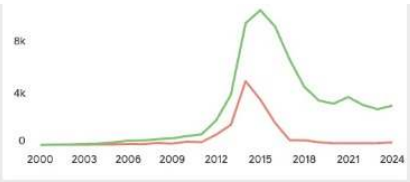
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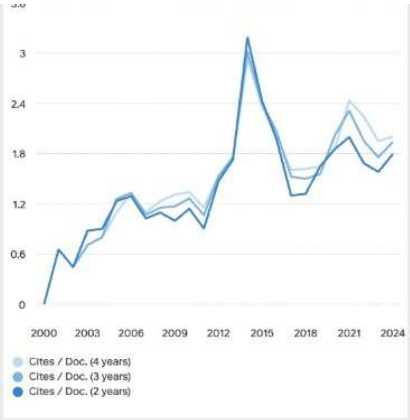
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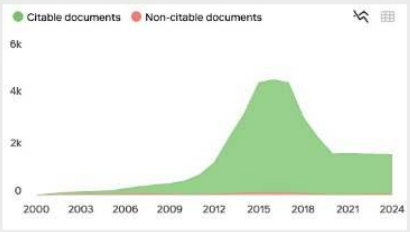
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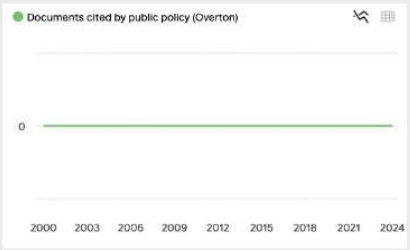
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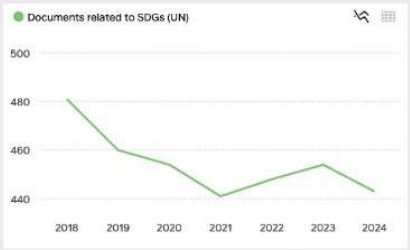
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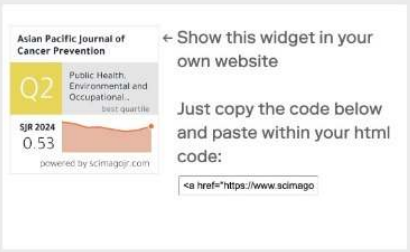
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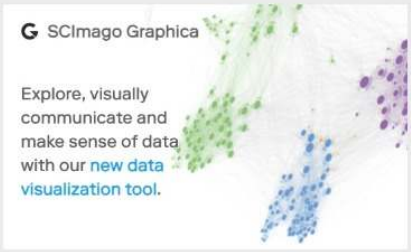
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Marzieh Nasiri
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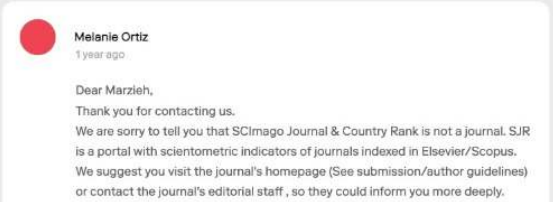
Please accept me as the author of the article in this journal as an editor in the field of telomerase gene inhibition and targeted cancer therapy. Thank you.
reply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1 year ago
Dear Marzieh,
Thank you for contacting us.
We are sorry to tell you that SCImago Journal & Country Rank is not a journal. SJR is a portal with scientometric indicators of journals indexed in Elsevier/Scopus.
We suggest you visit the journal's homepage (See submission/author guidelines) or contact the journal's editorial staff , so they could inform you more deeply.

D

Dian Islami Putri H
1 year ago

Dear Editor, why the Country on the website of Asian Pacific Journal of Cancer Prevention is different from the country mentioned in Scimago?

which is on the website mentioned Iran, while the Scimago mentioned Thailand.

thank you

reply

Melanie Ortiz
1 year ago

Dear Dian,
Thank you for contacting us.
SJR is a portal with scientometric indicators of journals indexed in Scopus and all the metadata (including the journal's country) have been provided by Scopus /Elsevier. Please note that Scopus takes into consideration the country of the journal's publisher.
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Best Regards,
SCImago Team

K

Kevin Tandarto
2 years ago

Hello I want to clarify if this journal is Q2 Scopus? I just check scopus journal and the highest quartile is 50th pecentile, which is Q2?

reply

Melanie Ortiz
2 years ago

Dear Kevin, thank you very much for your request. You can consult the Quartile information just above. Best Regards, SCImago Team

L

Lam van Ngo
2 years ago

I would like to ask if this journal is currently in the Scopus system. Thanks a lot

reply

Melanie Ortiz
2 years ago

Dear Lam, thank you very much for your comment. We suggest you consult the Scopus database directly. Keep in mind that the SJR is a static image (the update is made one time per year) of a database (Scopus) which is changing every day. The Scopus' update list can also be consulted here:
https://www.elsevier.com/solutions/scopus/how-scopus-works/content
Best Regards, SCImago Team

D

Davoud Jafari
3 years ago

Thank you for your attention. The best quartile for the Journal of "Asian Pacific Journal of Cancer Prevention" in many cities such as Scopus, Academic Accelerator, etc. is Q2 but in your prestigious cit is Q3 and is not updated. We kindly request to recheck it.

Sincerely Yours
Dr. Jafari

reply

Melanie Ortiz
3 years ago

Dear Davoud, Thank you for contacting us.
As you probably already know, our data come from Scopus, they annually send us an update of the data. This update is sent to us around April / May every year. The calculation of the indicators is performed with the copy of the Scopus database provided to us annually. However, the methodology used concerning the distribution of Quartiles by Scopus is different from the one used by SCImago.
For every journal, the annual value of the SJR is integrated into the distribution of SJR values of all the subject categories to which the journal belongs. There are more than 300 subject categories. The position of each journal is different in any journal, in particular. The distribution by Quartiles cannot be considered over the journals' total amount within a Category. In the case of SCImago, the distribution has to be considered with the formula Highest-SJR minus Lowest-SJR divided into four.
Best Regards,
SCImago Team

Academic Writer
6 years ago

My paper got accepted in Asian Pacific Journal of Cancer Prevention. Should I proceed it further for publication? Can you please let me know about updated impact factor the journal 2019/2020?

reply

Melanie Ortiz
6 years ago

Dear Sir/Madam,
thank you for contacting us.
Unfortunately, SCImago can not help you with that decision. SCImago Journal and Country Rank uses Scopus data, our impact indicator is the SJR. We suggest you consult the Journal Citation Report for other indicators (like Impact Factor) with a Web of Science data source.
Best Regards. SCImago Team

Md. Abdul Aziz
6 years ago

How can I know the latest impact factor?

reply

Melanie Ortiz
6 years ago

Dear Abdul, thank you very much for your comment.
SCImago Journal and Country Rank uses Scopus data, our impact indicator is the SJR. Check out our web to localize the journal. We suggest you to consult the Journal Citation Report for other indicators (like Impact Factor) with a Web of Science data source. Best Regards, SCImago Team

Firda Ridhayanti

Dear sir.
How long we can get Letter Of Submission after submit the article? Or LOS is autoreply?
Thanks

reply

Melanie Ortiz
6 years ago

Dear Firda,
thank you for contacting us.
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N

NAEEM
6 years ago

recent years impact factor of this journal.

reply

Melanie Ortiz
6 years ago

Dear user, SCImago Journal and Country Rank uses Scopus data, our impact indicator is the SJR. Check our web to locate the journal. We suggest you to consult the Journal Citation Report for other indicators (like Impact Factor) with a Web of Science data source. Best Regards, SCImago Team

S

Shilpa
6 years ago

Impact factor of this journal

reply

Melanie Ortiz
6 years ago

Dear user, SCImago Journal and Country Rank uses Scopus data, our impact indicator is the SJR. Check our page to locate the journal. We suggest you consult the Journal Citation Report for other indicators (like Impact Factor) with a Web of Science data source. Best Regards, SCImago Team

M

Mohammed H. Hassan
8 years ago

What about the impact factor of this journal and is it indexed on thombson rheuter?

reply

Z

Zuhair Allebban
8 years ago

Dear Sir,
I would like to know the reason for the long time it takes the journal to process the submitted manuscript. Such respected journal,APJCP should have a time schedule for processing any submitted manuscript even though there are technical, number of participating reviewers, number of submitted manuscript, others. I think this is

important issue in order to compete and improve its standing in the field which is in an increasing number of specialty journals with my best regards.

Zuhair Allebban, BVMS, MS, PhD, MB(ASCP), M(ASCP)
Professor of Pathology
College of medicine/Kufa University
Najaf, IRAQ

reply

M

Mosavi-Jarrahi
7 years ago

Hi Zuhair,
The journal try its best to have a decision in any manuscript as soon as it can, however, if a manuscript is passed the scope and novelty screening and go to review process, how fast is processed depends on how fast reviewers are ready to review. In many cases, reviewers either decline or even accept but do not do the review. This is out of journal management power. What the journal can do is to send a reminder and on some occasion, these reminder does jot really help and sometimes even backfire.

APJCP can have a decision on a submitted manuscript when an editor and at least two reviewers have their comment and recommendation reached to the editorial office.

Best

Elena Corera
8 years ago

Dear Zuhair, in the link below you will find the information corresponding to the author's instructions of this journal. Best regards, SCImago Team
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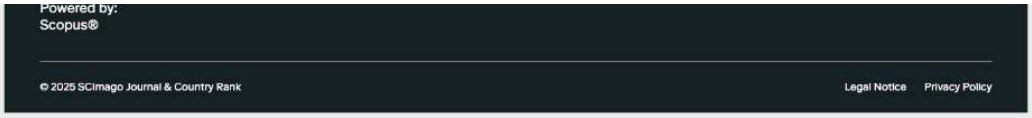
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Article

2-Methoxyestradiol-3,17-*O*,*O*-bis-sulfamate (STX140) Inhibits Proliferation and Invasion via Senescence Pathway Induction in Human BRAFi-Resistant Melanoma Cells

Ylana Adami Franco [1,†], Manoel Oliveira de Moraes, Jr. [1,†], Larissa A. C. Carvalho [1], Wolfgang Dohle [2], Renaira Oliveira da Silva [1], Isabella Harumi Yonehara Noma [1], Keli Lima [3], Barry V. L. Potter [2], João A. Machado-Neto [3] and Silvya Stuchi Maria-Engler [1,*]

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3 Department of Pharmacology, Biomedical Sciences Institute, University of São Paulo, Avenida Professor Lineu Prestes, Butantã 05508-000, São Paulo, Brazil; kelilima@usp.br (K.L.); jamachadoneto@usp.br (J.A.M.-N.)
* Correspondence: silvya@usp.br
† These authors contributed equally to this work.

Abstract: The endogenous estradiol derivative 2-Methoxyestradiol (2-ME) has shown good and wide anticancer activity but suffers from poor oral bioavailability and extensive metabolic conjugation. However, its sulfamoylated derivative, 2-methoxyestradiol-3,17-*O*,*O*-bis-sulfamate (STX140), has superior potential as a therapeutic agent, acts by disrupting microtubule polymerization, leading to cell cycle arrest and apoptosis in cancer cells and possesses much better pharmaceutical properties. This study investigated the antiproliferative and anti-invasive activities of STX140 in both SKMEL-28 naïve melanoma (SKMEL28-P) cells and resistant melanoma cells (SKMEL-28R). STX140 inhibited cell proliferation in the nanomolar range while having a less pronounced effect on human melanocytes. Additionally, STX140 induced cell cycle arrest in the G2/M phase and sub-G1, reduced migration, and clonogenic potential in monolayer models, and inhibited invasion in a 3D human skin model with melanoma cells. Furthermore, STX140 induced senescence features in melanoma and activated the senescence machinery by upregulating the expression of senescence genes and proteins related to senescence signaling. These findings suggest that STX140 may hold potential as a therapeutic agent for melanoma treatment.

Keywords: STX140; estradiol analogue; melanoma; senescence; B-raf-inhibitor resistance

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1. Introduction

Melanoma is one of the most aggressive types of cancer that arises from melanin-producing cells, the melanocytes [1]. Although it has a low incidence rate compared to other types of skin cancers, it manifests a relatively high mortality number representing a huge public health problem throughout the world [2,3]. The worst prognosis given to patients with melanoma is mainly due to its typical characteristic of evasion of the immune system and the high rate of cell proliferation and metastasis [4,5].

Melanoma's high proliferative rate arises mainly from alteration in the MAPK (Mitogen-Activated Protein Kinase) pathway in around 90% of patients, and essentially, the $BRAF^{V600E}$ point mutation represents about 50% of cutaneous melanoma [6–8]. Thus, tumor cells with the $BRAF^{V600E}$ mutation usually maintain the active conformation of this intracellular receptor, constitutively activating MAPK signaling, which is responsible for stimulating cell survival, proliferation, migration, apoptosis evasion, and metastasis [9,10]. Therefore,

the MAPK pathway is currently the primary target in melanoma treatment, mainly with BRAF-inhibitors (BRAFi), such as vemurafenib [11,12].

Although the development of BRAFi has revolutionized melanoma treatment increasing overall survival (OS, 14–17 months) and response rate (RR, 48–53%) over dacarbazine (5.6–7.8 m; 5–20%, respectively), it is expected that in a 6- to 12-month period, patients experience a therapeutic relapse due heterogeneity that gives rise to intrinsic and acquired resistant cells that repopulate the tumor [13,14]. Furthermore, among the various resistance phenotypes, the hyperactivation of compensatory pathways such as PI3K-AKT-mTOR, overexpression of tyrosine kinase receptors, BRAF/CRAF isoform switch, and metabolic reprogramming are some challenges [15]. More recently, clinical trials demonstrated that polytherapy with BRAFi associated with MEK inhibitors and immune checkpoint modulators could increase OS (29.8 months) and response rate (63%), postponing resistance phenotype appearance [5,16]. Despite these recent efforts, new therapeutic alternatives aiming to overcome BRAFi resistance mechanisms that target metastatic melanoma are still an open public health demand [11].

In this context, a major endogenous metabolite of 17-beta-estradiol, 2-methoxyestradiol (2-ME), has been studied in vitro, in vivo, and in clinical trials against several types of cancer, including melanoma. Our previous study reported that 2-ME activity can induce cell death, it acts as an antiangiogenic compound and as a senescence inductor [17]. In addition, other studies showed that 2-ME also reversed metabolic-related pro-carcinogenic phenotype in osteosarcoma cells [18], inhibited skin injuries induced by radiation exposure [19], increased the infiltration of cytotoxic CD8+ T lymphocytes and the expression of PD-L1 in B16 tumors, in which authors suggest enhancing the efficacy of PD-1 blockade immunotherapy [20]. However, despite promising results, 2-ME has shown limited application due to its poor oral bioavailability (<2%) and extensive bioconjugation in vivo [21,22].

Therewith, the 2-ME derivative, 2-methoxyestradiol-3,17-*O*,*O*-bis-sulfamate (commonly referred to as STX140, Figure 1) has demonstrated excellent oral bioavailability (>85%) [23–26]. In addition, STX140 also demonstrated an estradiol-independent induction of angiogenesis, along with an in vitro and in vivo multitargeting anticancer activity, including breast and prostate cancer models [27–30]. Recently, STX140 was shown to have excellent in vivo activity in an animal model of multiple sclerosis [31]. However, STX140's potential action against melanoma model cells, especially those with the resistant BRAFi phenotype, still needs to be addressed. Therefore, the present work analyzes the senescence-inducing activity and antiproliferative and anti-invasive effects of STX140 against metastatic melanoma with BRAF mutation and BRAFi-resistant cells.

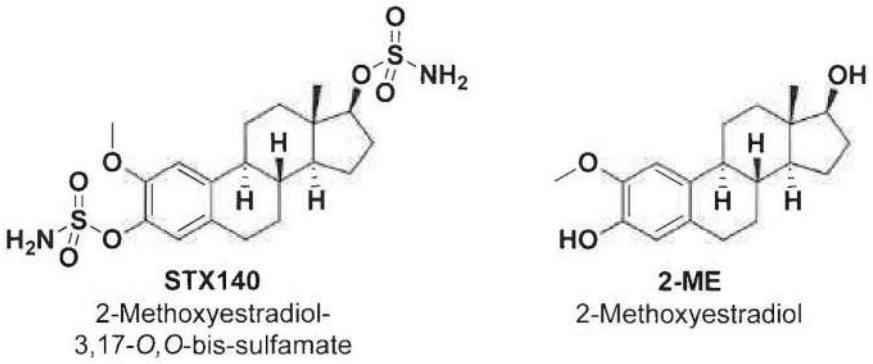

Figure 1. Chemical structures of STX140 and 2-ME.

2. Results

2.1. STX140 Showed Antiproliferative and Anti-Invasive Activity in Melanoma Cells

As evidenced in the curves of Figure 2a and Table 1, STX140 demonstrated cytotoxicity in the nanomolar range for all cell lines tested, being non-selective for resistant or parental melanomas and having a less pronounced effect on melanocytes (IC_{50} = 222.3 nM). The compound potency against melanoma cells increased time-dependently around 30–40%, but only in 72 h-incubation (61.9 and 68.2 nM for SKMEL-28P and -28R, respectively), with no pronounced variation in IC_{50} between 24 h (95.3 and 112.3 nM for SKMEL-28P and -28R, respectively) and 48 h (114.9 and 101.0 nM for SKMEL-28P and -28R, respectively). Considering prolonged and chronic exposure to STX140, the clonogenic evaluation showed inhibition of the clonogenicity potential of resistant melanoma from 50 nM up and for both melanoma (parental and resistant) cells from 100 nM, with total clonogenic inhibition at 200 nM.

Table 1. STX140 antiproliferative activity quantification.

	SKMEL-28P	SKMEL-28R	Melanocyte	Fibroblasts
24 h	95.3	112.3	222.3	133.5
48 h	114.9	101.0	ND	ND
72 h	61.9	68.2	ND	ND

Half-maximal inhibitory concentration (IC_{50}) values are expressed in nanomolar (nM). ND = not determined.

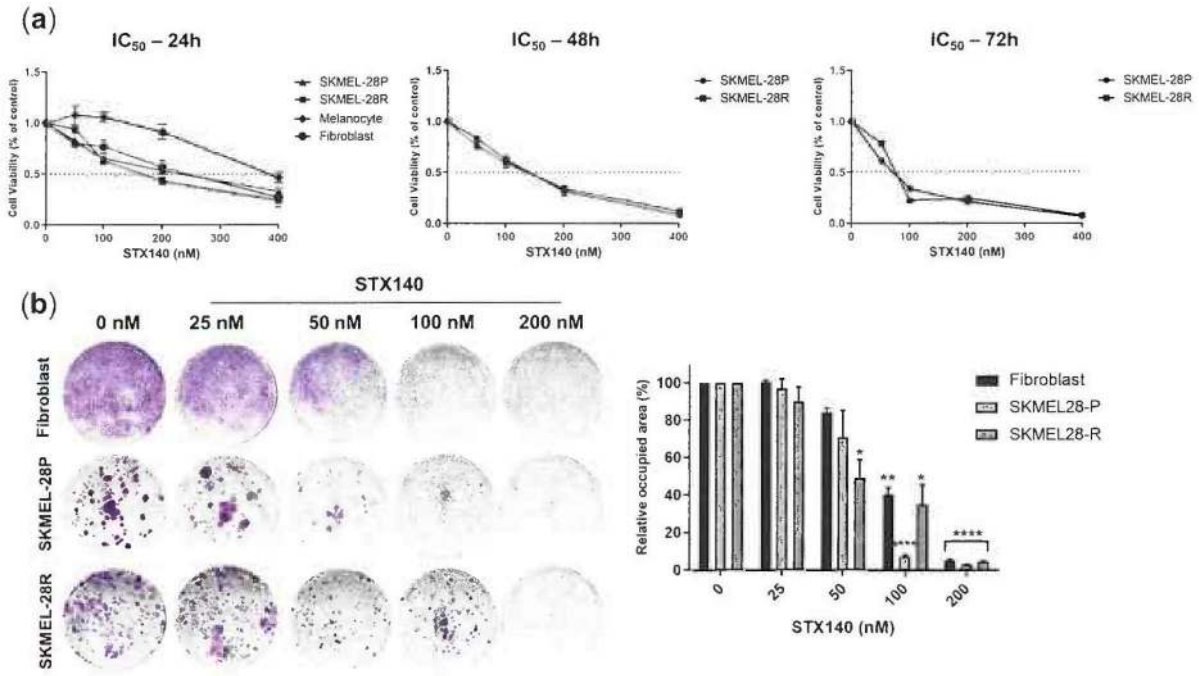

Figure 2. Antiproliferative activity of STX140 against human melanoma cells. (**a**) Cell viability evaluation of melanoma and primary cells for IC_{50} estimation; (**b**) clonogenic assay. Results are expressed as the mean of three independent experiments (n = 3) ± S.D.; significance is indicated by * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$. Representative micrographs from inverted microscope (4×).

In addition, STX140 (100 nM) also demonstrated a significant ability to inhibit cell migration after 24 and 48 h of incubation, with a more pronounced effect for SKMEL-28P in the monolayer model (Figure 3). In the three-dimensional skin model (Figure 4), STX140 (500 nM) demonstrated cytotoxicity with inhibition of SKMEL-28P proliferation when compared to the untreated control, in addition to inhibition of invasion of the dermal layer by SKMEL28-R compared to the untreated control. However, STX140 treatment did not appear to affect tissue formation when evaluating non-melanoma skin (Figure 4a). Furthermore, STX140 (100 nM) caused a significant decrease in IL-8 quantification only in SKMEL28-R, compared to the untreated control (Figure 4b). IL-6 was not affected by STX140 at the tested concentration.

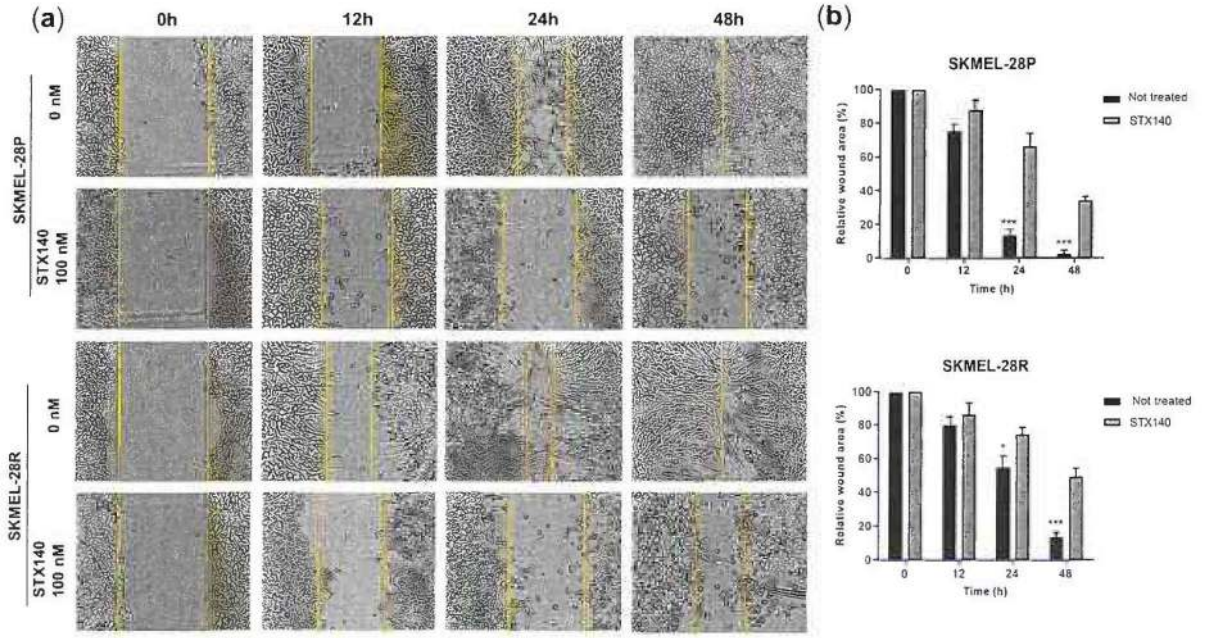

Figure 3. Antimigration activity of STX140 against human melanoma cells. (**a**) Representative micrographs from inverted microscope (20×); (**b**) Graphical representation, results are expressed as mean of three independent experiments ($n = 3$) ± S.D.; significance is indicated by * $p < 0.05$ and *** $p < 0.001$. Yellow lines represent the edge of the cell-wound.

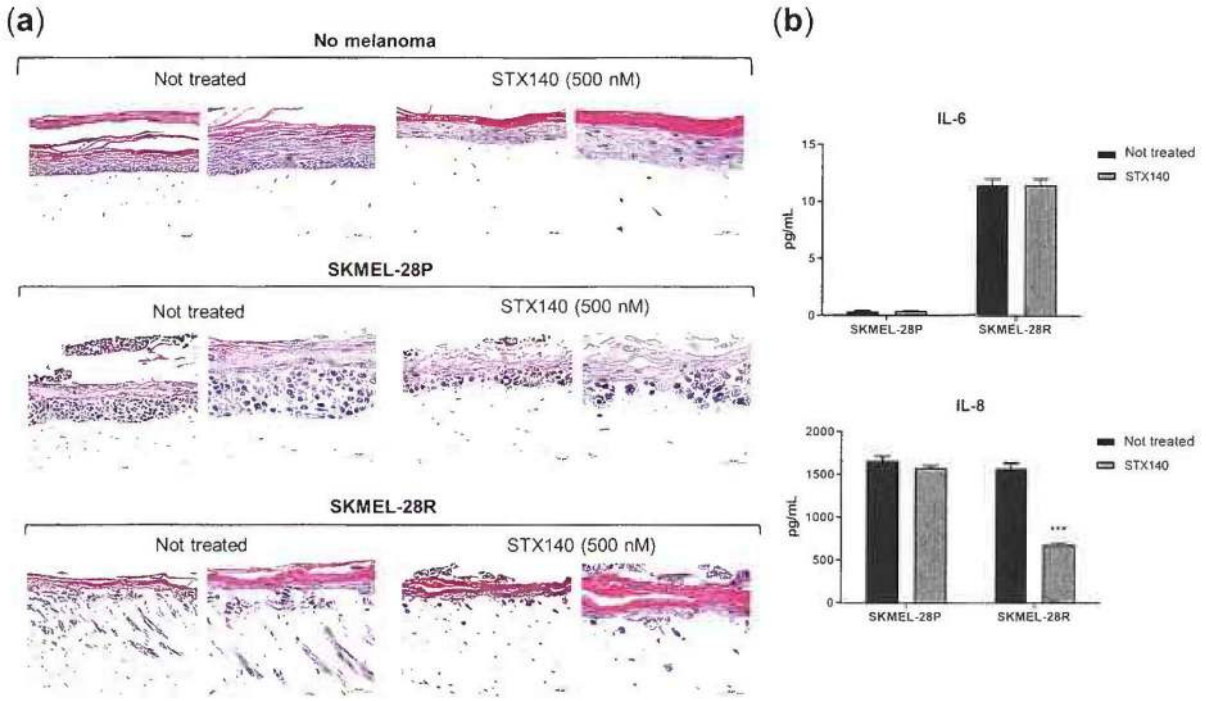

Figure 4. Anti-invasion activity of STX140 against human melanoma cells. (**a**) Reconstructed human skin with melanoma micrographs from inverted microscope (20× and 40×, respectively) dyed with hematoxylin/eosin (H&E); (**b**) Interleukin quantification in media from the 3D reconstructed human skin samples, STX140 (100 nM), results are expressed as the mean of three independent experiments ($n = 3$) ± S.D.; significance is indicated by *** $p < 0.001$.

2.2. *STX140 Treatment Led to Cell Cycle Arrest*

In order to study cell death induction caused by STX140 (Figure 5), we monitored the externalization of phosphatidylserine as a marker of early apoptosis which, combined with cell membrane fragmentation marked by positive nucleus fluorescence with propidium iodide (PI), indicated late apoptosis characteristics. Results showed that only 500 nM of STX140 was able to increase significantly ($p < 0.05$) the number of cells in early apoptosis in SKMEL-28P and -R, with no cells in late apoptosis.

Concerning cell cycle progression, although STX140 did not show remarkable apoptosis-triggering potential, this compound was able to cause cell cycle arrest at 200–500 nM concentrations with a significant increase ($p < 0.001$) in the number of cells in the G2/M phase compared to the untreated control in both parental and resistant melanoma cells (Figure 6). In addition, the significant increase ($p < 0.05$) in the percentage of cells in the sub-G1 population, commonly associated with DNA fragmentation, is also worth mentioning, caused by STX140 only at 500 nM and slightly more severe in SKMEL-28P.

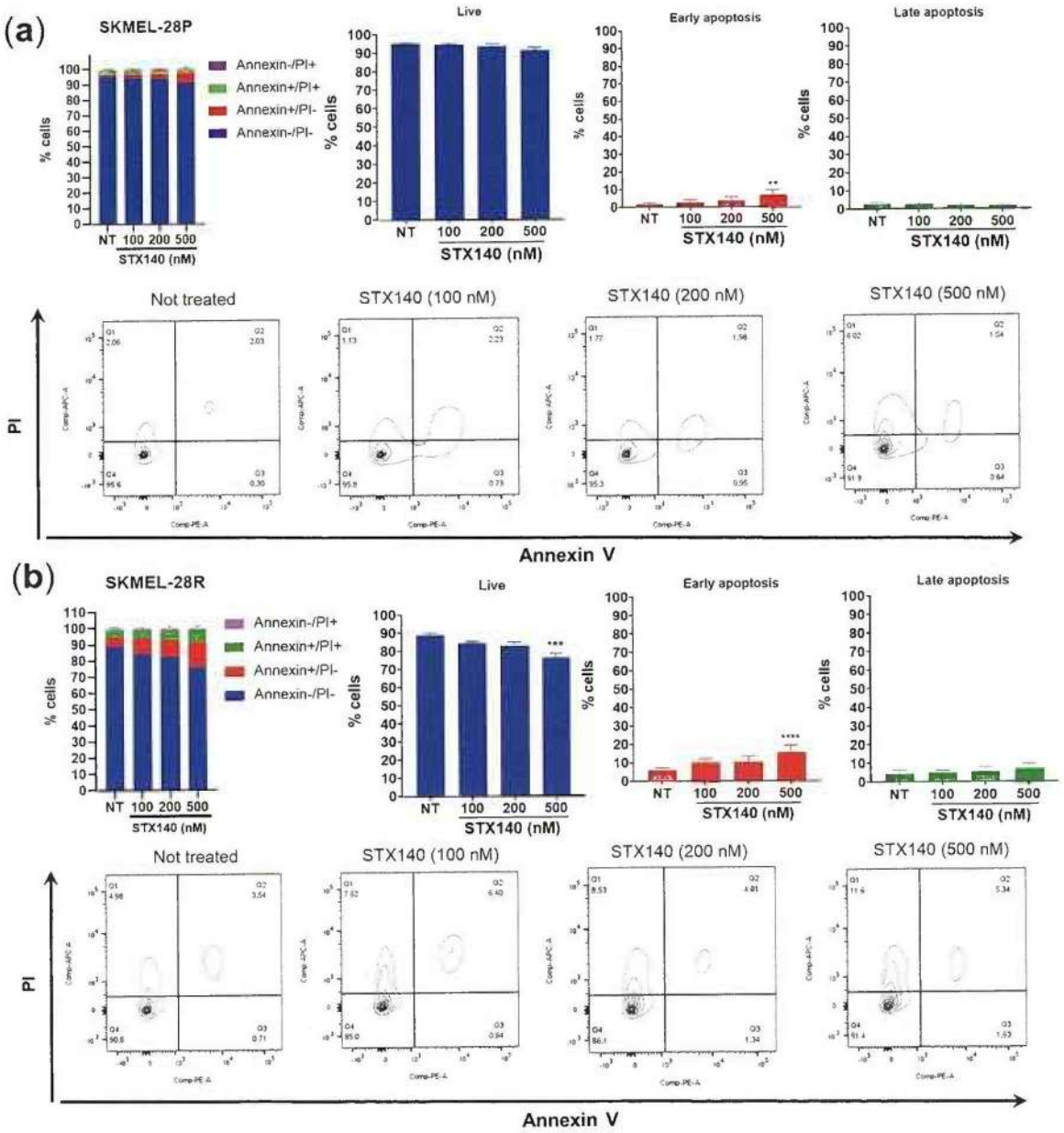

Figure 5. Cell death evaluation by STX140 activity against human melanoma cells. (**a**) SKMEL-28P; (**b**) SKMEL-28R. Graphical results are expressed as the mean of three independent experiments ($n = 3$) ± S.D.; significance is indicated by ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.001$. PI = propidium iodide; NT = not treated cells.

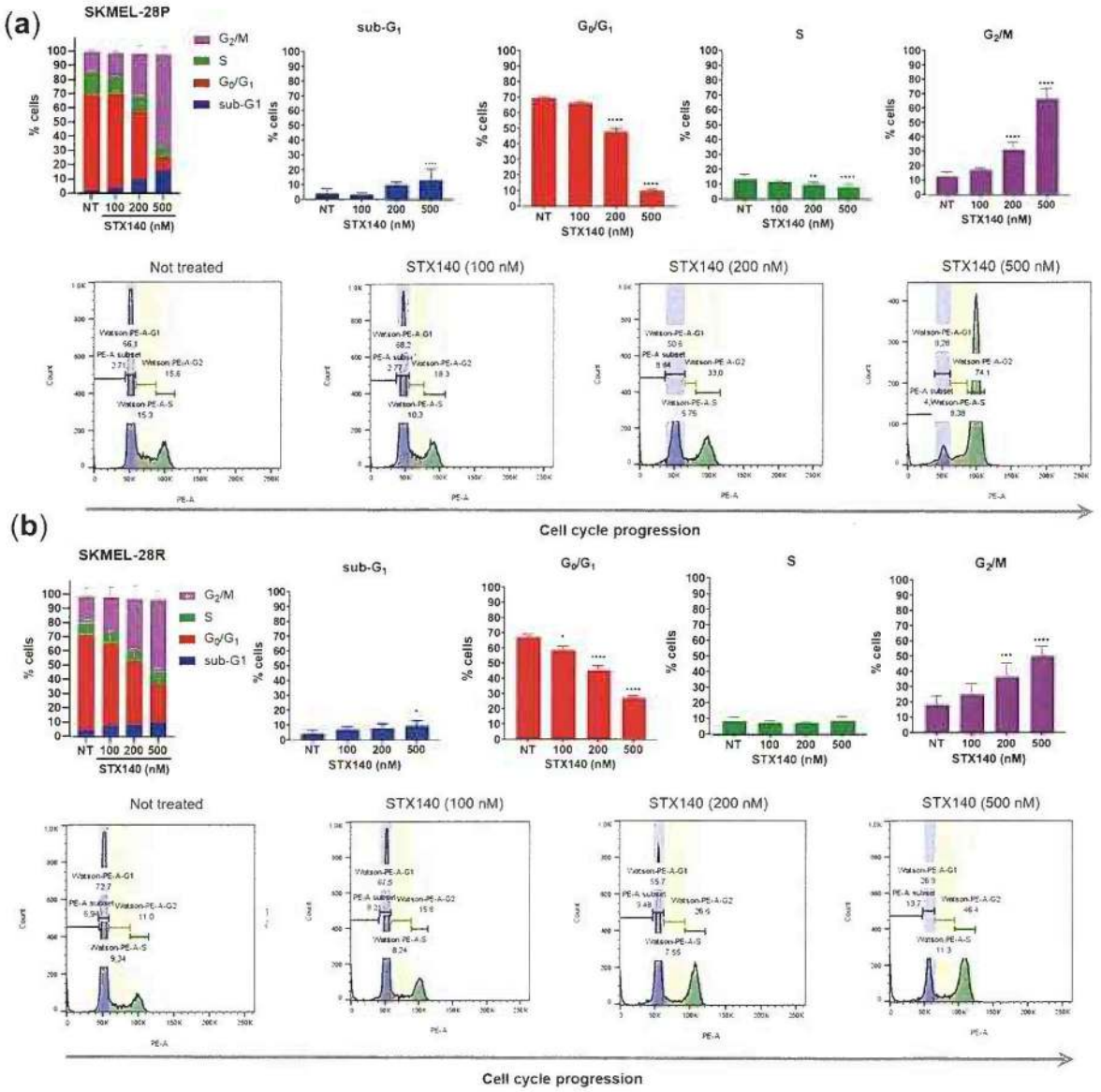

Figure 6. Cell cycle evaluation by STX140 activity against human melanoma cells. (**a**) SKMEL-28P; (**b**) SKMEL-28R. Graphical results are expressed as the mean of three independent experiments ($n = 3$) ± S.D.; significance is indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. NT = not treated cells.

2.3. STX140 Triggered Senescence Phenotype

Since parental and resistant melanoma cells suffered an antiproliferative activity with a cycle arrest in the G2/M phase and possible DNA damage with no apoptosis features, we evaluated the ability of STX140 to trigger senescence mechanisms. As evidenced in Figure 7a using the cell senescence dye, there was a significant increase ($p < 0.01$) concerning the untreated control by treatment with STX140 (100 nM), SA-β-gal stained in around 60% of SKMEL28-P and -R, by fluorescence microscopy. In addition, we observed melanoma cell morphology alterations when treated with STX140. These cells lost the characteristic stellate morphology into an acquired more spread-out shape.

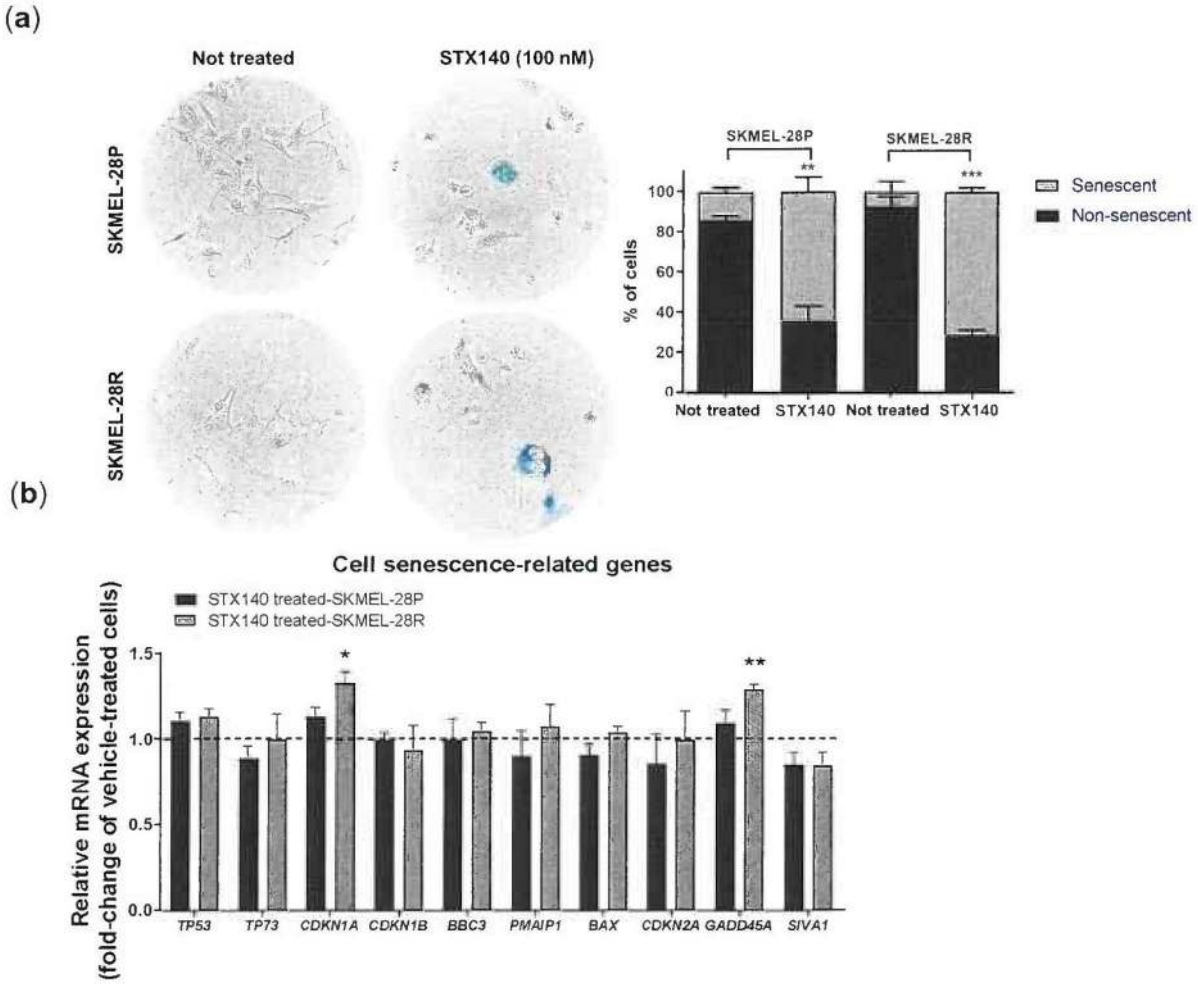

Figure 7. STX140 (100 nM) treatment induces senescence profile in human melanoma cells. (**a**) β-galactosidase staining with statistical quantification; (**b**) Cell senescence-related genes modulation; Results are expressed as the mean of three independent experiments ($n = 3$) ± S.D.; relative mRNA expression is compared to not treated cells (represented as the dash lines), significance is indicated by * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Representative micrographs from inverted microscope (10×).

Thus, associated with morphological and functional changes, the impact of STX140 on modulating the expression of genes related to senescence and apoptosis phenotypes was evaluated. As seen in Figure 7b, there was no alteration in the gene expression of SKMEL-28P. Also, *TP53*, *TP73*, *CDKN1B*, *CDKN2A*, *BBC3*, *PMAIP1*, *BAX*, and *SIVA1* genes remained unchanged in SKMEL-28R cell line. However, STX140 (100 nM) significantly ($p < 0.05$) increased *CDKN1A* and *GADD45A* expression in resistant melanoma cells.

Further, autophagy, apoptosis, and senescence signaling proteins were also quantified. STX140 could not significantly modulate the activity of PARP1 (apoptosis- and DNA damage-related) along with LC3B and SQSMT1/p62 (autophagy-related). However, compared to the negative control, STX140 induced significantly ($p < 0.05$) an elevated expression of p21 (only in SKMEL-28R) and γH2AX expression (in both SKMEL28-P and -R), as demonstrated in Figure 8.

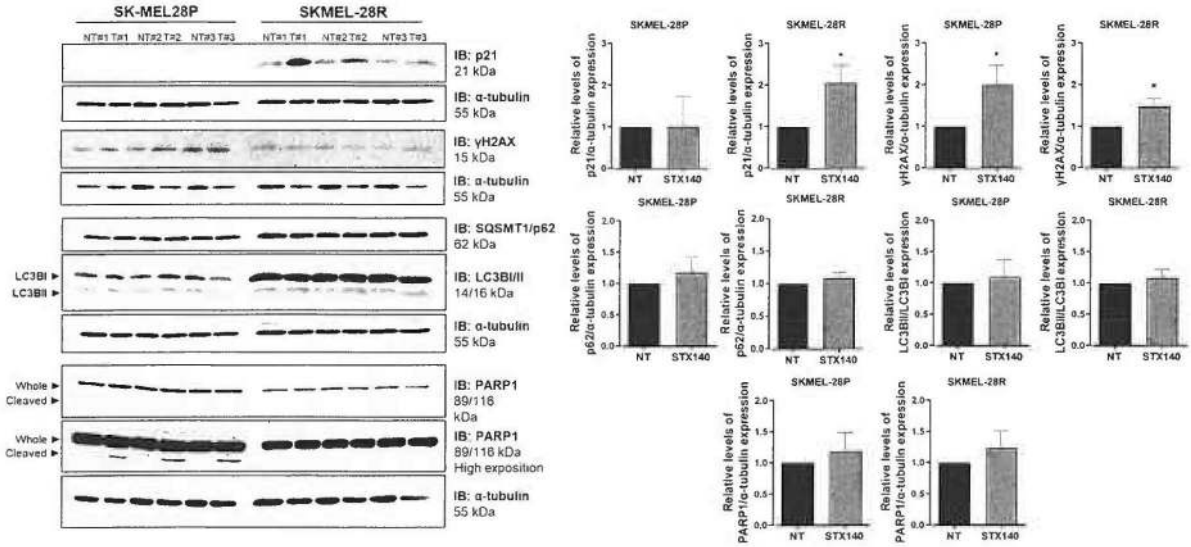

Figure 8. Cell death and senescence-related signaling modulation by STX140 (100 nM) activity in human melanoma cells. Results are expressed as mean of three independent experiments ($n = 3$) ± S.D.; significance is indicated by * $p < 0.05$. NT = no treated cells.

3. Discussion

STX140, classified as a non-estrogenic steroidal bis-sulfamate, has shown antitumor therapeutic potential in vivo and in vitro reported in several studies [24]. STX140 has been shown to disrupt microtubule polymerization when interacting with tubulin leading to cell cycle arrest and apoptosis. In a mouse model of breast cancer, orally administered STX140 outperformed the chemotherapy drug paclitaxel, which also disrupts microtubule dynamics by reducing tumor size and preventing metastasis formation without showing neurotoxicity [29,30]. Also, STX140 showed efficacy when administered orally in both MCF-7^{WT} and MCF-7^{DOX} breast cancer xenograft models, in contrast to paclitaxel and 2-methoxyestradiol [23]. Currently, there are no clinical trials ongoing with STX140, but the compound has already shown many promising results in preclinical studies in oncology [31,32] and also elsewhere, in particular its recent in vivo activity on preventing experimental autoimmune encephalitis [33].

Considering in vitro models, since STX140 is a 2-ME analog, it is worth mentioning that our group previously reported the 2-ME activity against melanoma cells using a bi- and three-dimensional model. Thus, 2-ME inhibited the proliferation, clonogenicity, and invasive potential of $BRAF^{V600E}$ mutated and BRAFi-resistant cells. In the potency evaluation assay, the IC_{50} for the SKMEL-28 cell line was 2.74 μM for 3 days, 6.81 μM for 5 days, and 4.41 μM for 7 days of treatment incubation [17]. As shown here, the 2-ME analog STX140 showed that it could reduce cell proliferation and clonogenicity potential at a nanomolar potency against melanoma cells that harbor $BRAF^{V600E}$ point mutation and its BRAFi-resistant variation (Table 1).

In a study with ovarian, prostate, and breast cancer cells, STX140 inhibited tumor viability with IC_{50} values of 280, 260, and 250 nM, respectively [34]. These values corroborated those presented herein and showed slightly more sensitivity in melanoma cell lines to the effect of STX140, with lower IC_{50} values.

In addition, STX140 demonstrated an inhibition of the migration and invasion capacity of melanoma cells, mainly in SKMEL-28R, as seen in a wound-healing assay, reconstructed skin model, and inhibition of IL-8 expression. Analyzing the results of cell death characterization by Annexin-FITC and PI, we conclude that migration and invasion inhibition is not due to the induction of cell death since STX140 does not significantly induce death features

(apoptosis/necrosis) in melanoma cells. Since other authors have shown that senescent cells commonly block intrinsic and extrinsic apoptosis signaling via antiapoptotic Bcl-2 family proteins [35–37], these results also contribute to the hypothesis that STX140 acts to prevent cell proliferation, migration, and invasiveness phenotype via senescence mechanisms.

Therefore, we evaluated if STX140 induces senescence features in melanoma, as senescence cells highly express the lysosomal enzyme β-galactosidase, classically marked positively using X-gal dye at pH 6 [38,39]. As mentioned before, STX140 led to positive blue staining to SA-β-gal and morphology characteristics such as a flattened cytoskeleton with a prominent nucleus and scattered cytoplasm. In addition to these parameters, quantitative PCR and blotting analysis demonstrated that STX140 upregulated the expression of the senescence gene and proteins related to senescence signaling. GADD45 stimulates proteins that have products that inhibit proliferation in tumor cells and, in response to different stresses, can induce senescence through interaction with p21 [40]. CDKN1A has p21 protein as a product, a potent cyclin-dependent kinase inhibitor that blocks cell cycle progression, and is a highly elevated protein in senescent cells in response to DNA injury. Furthermore, p21 is part of one of the p53/p21 signaling, a crucial main senescence pathway [41,42].

In a cell cycle evaluation, STX140 showed similar results compared to its parent 2-ME. 2-ME and STX140 demonstrated cell cycle arrest in the G2/M phase in human melanoma cells, but STX140 had activity in the nanomolar range. Although cell cycle arrest in G2/M was demonstrated as a microtubule inhibition consequence of 2-ME activity [17], it seems like STX140 is causing G2/M and sub-G1 arrest in response to DNA damage, triggering the senescent machinery activation. Studies have shown that other pharmacological compounds, such as aurora kinases inhibitors (AURK) and PLK1 inhibitors, can induce senescence features by stopping the passage of the G2 phase to the mitosis phase, consequently blocking the G2/M progression of the cell cycle via p21 and γH2AX [43,44].

Beyond the classical cell senescence induction through progressive telomere shortening in response to cell aging and genomic instability prevention, cells can also trigger an irreversible premature senescence signaling that can act as a tumor-suppressive function [45]. Double-stranded DNA breaks are powerful senescent signaling activators, usually inducing activation of H2AX aiming to recruit DNA damage response components [46]. DNA damage response also can act via the cyclin-dependent kinase inhibitor 1A (CDKN1A), triggering the p21 protein and resulting in cell cycle arrest. Even though these molecules related to cell cycle inhibition alone do not necessarily attempt to trigger senescence, these alterations together are an essential part of the hallmarks of the cellular senesce pathway [45,47–50]. In this context, STX140 activity modulated the expression of some of the hallmarks of the cellular senescence pathway, such as upregulating *CDKN1A* and *GADDA45* genes in addition to the elevation of the expression of γH2AX (a phosphorylated form of the histone variant H2AX) and p21 proteins, p53-independently, mostly seen in resistant human melanoma cells.

Moreover, although senescence signaling usually upregulates IL-6 and IL-8 activity in an autocrine manner part of the pro-inflammatory factors termed the senescence-associated secretory phenotype (SASP) [49–51], in melanoma cells, these interleukins are commonly associated with high tumor growth and invasiveness linked to poor prognosis [51,52]. Jobe et al. [53] showed that simultaneous blocking of IL-6 and IL-8 could represent a total inhibition of human melanoma invasiveness, which means that IL-8 downregulation could represent an STX140 anti-invasion pathway activity. Also, patients with a decrease in serum levels of IL-8 could reflect and predict response to anti-PD-1 treatment in melanoma and non-small cell lung cancer [54].

Even though the mechanism of action of STX140 was not tested entirely in non-melanoma cells, we analyzed STX140 cytotoxicity in the 3D reconstructed human skin model. The reconstructed human skin includes a group of samples with no melanoma (Figure 2a) consisting of three primary non-cancer cells (e.g., fibroblasts, keratinocytes, and melanocytes). This group exhibited no STX140 toxicity or morphological changes in the reconstructed human skin tissue.

Moreover, treatment with drugs that induce senescence and inhibit cell proliferation without triggering cell death currently presents an alternative to traditional cytotoxic therapies that can cause serious adverse effects in patients and often develop resistance [36]. The implication of using senotherapeutic drugs (compounds that modulate senescence) to treat cancer are being discussed currently and studied in various stages of development [55–57]. Also, further investigation should be necessary to understand the synergize potential of STX140 with other melanoma drug therapies used in clinics (such as BRAF and MEK inhibitors).

4. Materials and Methods

4.1. STX140 Synthesis, Cell Culture and Reagents

STX140 was synthesized according to the method of Leese et al. [58] (referred to as compound **21**).

Primary human melanocytes and fibroblasts were isolated from foreskin samples donated at the Hospital of the University of São Paulo (CEP/HU-USP 943/09, SISNEP CAAE 0062.0.198.000-9) as approved by the local ethics committee (CEP/FCF-USP 534). They were isolated and cultured according to the protocol previously described by our group [59].

Melanoma SKMEL-28 cell line ($BRAF^{V600E}$ mutation) was kindly donated by Dr. Marisol Soengas (Centro Nacional de Investigaciones Oncológicas, Madrid, Spain). Melanoma cells with BRAF resistance phenotype were prior generated according to Sandri et al. [59] procedure. The parental and vemurafenib-resistant SKMEL-28 melanoma cell lines with $BRAF^{V600E}$ mutation were used as a monolayer cell model. Cultivation was performed in DMEM (Dulbecco's modified Eagle's medium) for the SKMEL-28 cell supplemented with 10% fetal bovine serum (FBS) and antibiotics (25 µg/mL of ampicillin and 100 µg/mL of streptomycin). BRAFi-resistant SKMEL-28 cells were maintained in the presence of vemurafenib (3.0 µM, S1267, Selleck Chemicals, Houston, TX, USA). The cells were maintained in a humid incubator at 37 °C temperature under a 5% CO_2 atmosphere. All cell lines were checked for mycoplasma contaminations by PCR assay.

4.2. Cell Viability Evaluation

Cell viability was accessed by trypan blue (0.4%, 1:1 culture medium) (1.11732, Merck, Temecula, CA, USA) dye exclusion assay. Cells were seeded (2×10^4 cell/well) in a 24-well plate and treated with STX140 at 50 nM, 100 nM, 200 nM, and 400 nM concentrations for 24, 48, and 72 h of incubation. After dye addition, viable cells were counted in Neubauer chambers, considering colored cells as non-viable.

4.3. Clonogenic Assay

Cells were seeded in a 6-well cell culture plate (800 per well) and treated with STX140 at 25, 50, 100, and 200 nM concentrations twice a week during 10–17 days. After this period, cell colonies were stained with crystal violet (0.5% in a 1:1 water/methanol solution). The percentage of the area occupied by the colonies was quantified through a computational analysis.

4.4. Senescence Evaluation with β-Galactosidase Staining

Melanoma cells were seeded (2×10^4 cells/well) in 6-well plates and treated with STX140 (100 nM) concentration for 48 h-incubation. Cells were fixated and stained of senescence-associated β-galactosidase (SA-β-gal) was performed using the Senescence β-galactosidase Staining Kit (9860, Cell Signaling Technology, Danvers, MA, USA) following the manufacturer's instructions. Senescent and non-senescent cells were counted under the microscope in 6 different random fields for each group (treated and not treated).

4.5. Wound-Healing Assay

Melanoma cells were seeded (1×10^4 cells/well) in a 24-well plate and left to reach approximately 90% confluence. Using a 200 µL tip, a vertical scratch was made in the center of each well. Then, cells were washed with phosphate buffered saline (PBS) and treated with STX140 (100 nM) diluted in DMEM culture medium + 1% FBS. The wound-healing process was measured by acquiring twice-per-well scratch images in time 0-, 12-, 24-, and 48-h incubation. Cell-free areas were calculated using Image J 3.7 software.

4.6. IL-6 and IL-8 Quantification

The supernatant of melanoma cell culture prior treated with STX140 (100 nM) was collected and frozen at −80 °C. Interleukins IL-6 (QC193, R&D Systems®, Minneapolis, MN, EUA) and IL-8 (R&D Systems®, AF-208-NA) from samples were quantified by ELISA assay (R&D Systems®) and analyzed according to the instructions of the commercial kit manufacturers. Optical density was immediately measured at 450 nm in a 96-well microplate spectrophotometer detector. Interleukin concentration was determined by interpolation from the standard curve.

4.7. Flow Cytometry—Apoptosis Evaluation

Cell lines were seeded in a 6-well plate (2×10^5 cells/well) and treated with STX140 at 100, 200, and 500 nM concentrations in a 24-h incubation. After treatment, samples were washed twice and resuspended with ice-cold PBS, and stained with FITC Annexin V Apoptosis Detection Kit II (556570, BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's instructions. Fluorescence was measured in a flow cytometer equipment (FACS Cantho, BD Biosciences). For interpretation, cells marked as both FITC Annexin V+/PI+ were considered either in the late stage of apoptosis; FITC Annexin V+/PI− were considered in the early stage of apoptosis; FITC Annexin V−/PI+ were considered already dead or necrosis; both FITC Annexin V−/PI− are alive and not undergoing measurable apoptosis.

4.8. Flow Cytometry—Cell Cycle

Cell lines were seeded in a 6-well plate (2×10^5 cells/well) and treated with STX140 at 100, 200, and 500 nM concentrations in a 24-h incubation. Then, cell samples were washed with PSB solution and resuspended in a permeabilization and stain buffer (PBS containing 4 mg/mL of RNase A, triton x-100 0.1%, and 50 µg/mL of propidium iodide). After 30 min-incubation at room temperature in the dark, DNA quantification was measured by the sample's fluorescence read in a flow cytometer (FACS Cantho, BD Biosciences).

4.9. Gene Expression Evaluation—RT-PCR

Total RNA from samples was extracted using TRIzol reagent (Thermo Fisher Scientific, Rockford, IL, USA). A total of 1 µg of RNA was reverse transcribed into cDNA with the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). Real-time gene amplification was performed on the QuantStudio 3 Real-Time PCR System equipment and the Power SybrGreen reagent (Thermo Fisher Scientific) with specific primers (Table 2). Fifteen nanograms of each cDNA sample were used in the reactions and a negative control, with the addition of water (nuclease-free) in place of cDNA and performed for each pair of primers. HPRT1 and ACTB were used as reference genes. The relative expression was calculated using the formula $2^{-\Delta\Delta CT}$ (PMID:11846609) [60].

Table 2. Primers sequences and gene functions.

Gene	Sequence	Concentration
TP53	FW: GGCGCACAGAGGAAGAGAAT RV: GGAGAGGAGCTGGTGTTGTTG	300 nM
TP73	FW: GCACCACGTTTGAGCACCTCT RV: GCAGATTGAACTGGGCCATGA	300 nM
CDKN1A	FW: TGTCACTGTCTTGTACCCTTGT RV: GCCGGCGTTTGGAGTGGTAG	300 nM
CDKN1B	FW: ACTCTGAGGACACGCATTTGGT RV: TCTGTTCTGTTGGCTCTTTTGTT	300 nM
BBC3	FW: GACCTCAACGCACAGTACGAG RV: AGGAGTCCCATGATGAGATTGT	300 nM
PMAIP1	FW: CGCGCAAGAACGCTCAACC RV: CACACTCGACTTCCAGCTCTGCT	300 nM
BAX	FW: GAGCTGCAGAGGATGATTGC RV: CAGCTGCCACTCGGAAAA	300 nM
CDKN2A	FW: CACCGAATAGTTACGGTCGGA RV: CACGGGTCGGGTGAGAGTG	300 nM
GADD45A	FW: AAGGATGGATAAGGTGGGG RV: CTGGATCAGGGTGAAGTGG	300 nM
SIVA1	FW: TCTTCGAGAAGACCAAGCG RV: TGCCCAAGGCTCCTGATC	300 nM
ACTB	FW: AGGCCAACCGCGAGAAG RV: ACAGCCTGGATAGCAACGTACA	150 nM
HPRT1	FW: GAACGTCTTGCTCGAGATGTGA RV: TCCAGCAGGTCAGCAAAGAAT	150 nM

4.10. Reconstructed Human Skin (RHS)

The reconstructed human skin model was based on the previous description in Brohem et al. [61] with minor changes. In summary, dermis equivalent was constructed in a 6-well plate with fibroblast cells (1.5×10^5 cells/well) plus collagen type I (BD Biosciences). Melanoma cells (5×10^5 cells/well) were incorporated into the epidermis along with keratinocytes and melanocytes (2.5×10^5 and 0.8×10^4 cells/well, respectively). After 24 h-submersion, skin samples were transferred to the air-liquid interface and maintained for 10 days with DMEM/HAM's F-12 supplemented culture medium with 10% FBS. Then, 24 h prior to tissue collection and fixation (4% paraformaldehyde for 4 h and 70% alcohol for 24 h), RHS were treated with STX140 (500 nM). Tissue samples were stained with hematoxylin/eosin (H&E) for histological analysis.

4.11. Statistical Analysis

Data analysis was carried out using GraphPad Prism 8.0 software. Values are expressed as the mean ± standard deviation (S.D.) of three independent experiments (n = 3). Statistical significance (significant when $p < 0.05$) was analyzed using one-way analysis of variance (ANOVA).

5. Conclusions

This study demonstrates that STX140 has potent antiproliferative and anti-invasive effects in the nanomolar range on BRAFi-resistant melanoma cells while having a less pronounced effect on human melanocytes. Additionally, STX140 induced cell cycle arrest in the G2/M phase and sub-G1, reduced migration, and clonogenic potential in monolayer models, and inhibited invasion in a three-dimensional human skin model with melanoma cells. Moreover, STX140 induced senescence features in melanoma and activated the senes-

cence machinery by upregulating the expression of senescence genes, such as CDKN1A e GADD45A in resistant melanoma and proteins related to senescence signaling, such as p21 (only in SKMEL-28R) and γH2AX. Preclinical studies conducted on STX140 demonstrate its potential as a therapeutic agent for the treatment of other cancer types, and clinical trials are necessary to confirm its safety and effectiveness in humans. However, STX140's significant cytostatic and antiproliferative effects underline the need for continued research on this promising compound.

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References

1. Shain, A.H.; Bastian, B.C. From melanocytes to melanomas. *Nat. Rev. Cancer* **2016**, *16*, 345–358. [CrossRef] [PubMed]
2. Chhikara, B.S.; Parang, K. Global cancer statistics 2022: The trends projection analysis. *Chem. Biol. Lett.* **2023**, *10*, 451.
3. Siegel, R.L.; Miller, K.D.; Wagle, N.S.; Jemal, A. Cancer statistics, 2023. *CA Cancer J. Clin.* **2023**, *73*, 17–48. [CrossRef] [PubMed]
4. Aplin, A.E.; Kaplan, F.M.; Shao, Y. Mechanisms of resistance to RAF inhibitors in melanoma. *J. Investig. Dermatol.* **2011**, *131*, 1817–1820. [CrossRef]
5. Tang, T.; Eldabaje, R.; Yang, L. Current status of biological therapies for the treatment of metastatic melanoma. *Anticancer Res.* **2016**, *36*, 3229–3241.
6. Davies, H.; Bignell, G.R.; Cox, C.; Stephens, P.; Edkins, S.; Clegg, S.; Teague, J.; Woffendin, H.; Garnett, M.J.; Bottomley, W.; et al. Mutations of the BRAF gene in human cancer. *Nature* **2002**, *417*, 949–954. [CrossRef]
7. Pollock, P.M.; Harper, U.L.; Hansen, K.S.; Yudt, L.M.; Stark, M.; Robbins, C.M.; Moses, T.Y.; Hostetter, G.; Wagner, U.; Kakareka, J.; et al. High frequency of BRAF mutations in nevi. *Nat. Genet.* **2003**, *33*, 19–20. [CrossRef]
8. Platz, A.; Egyhazi, S.; Ringborg, U.; Hansson, J. Human cutaneous melanoma; a review of NRAS and BRAF mutation frequencies in relation to histogenetic subclass and body site. *Mol. Oncol.* **2008**, *1*, 395–405. [CrossRef]
9. Guo, Y.; Pan, W.; Liu, S.; Shen, Z.; Xu, Y.; Hu, L. ERK/MAPK signalling pathway and tumorigenesis. *Exp. Ther. Med.* **2020**, *19*, 1997–2007. [CrossRef]
10. Teixido, C.; Castillo, P.; Martinez-Vila, C.; Arance, A.; Alos, L. Molecular markers and targets in melanoma. *Cells* **2021**, *10*, 2320. [CrossRef]
11. Fedorenko, I.V.; Paraiso, K.H.T.; Smalley, K.S.M. Acquired and intrinsic BRAF inhibitor resistance in BRAF V600E mutant melanoma. *Biochem. Pharm.* **2011**, *82*, 201–209. [CrossRef]
12. Cheng, Y.; Zhang, G.; Li, G. Targeting MAPK pathway in melanoma therapy. *Cancer Metastasis Rev.* **2013**, *32*, 567–584. [CrossRef] [PubMed]
13. Chapman, P.B.; Hauschild, A.; Robert, C.; Haanen, J.B.; Ascierto, P.; Larkin, J.; Dummer, R.; Garbe, C.; Testori, A.; Maio, M.; et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N. Engl. J. Med.* **2011**, *364*, 2507–2516. [CrossRef] [PubMed]

14. Kudchadkar, R.R.; Smalley, K.S.; Glass, L.F.; Trimble, J.S.; Sondak, V.K. Targeted therapy in melanoma. *Clin. Dermatol.* **2013**, *31*, 200–208. [CrossRef]
15. Rebecca, V.W.; Herlyn, M. Nongenetic mechanisms of drug resistance in melanoma. *Annu. Rev. Cancer Biol.* **2020**, *4*, 315–330. [CrossRef]
16. Munhoz, R.R.; Postow, M.A. Combinatorial approaches to the treatment of advanced melanoma. *Hematol. Oncol. Clin.* **2021**, *35*, 145–158. [CrossRef]
17. Massaro, R.R.; Faião-Flores, F.; Rebecca, V.W.; Sandri, S.; Alves-Fernandes, D.K.; Pennachi, P.C.; Smalley, K.S.M.; Maria-Engler, S.S. Inhibition of proliferation and invasion in 2D and 3D models by 2-methoxyestradiol in human melanoma cells. *Pharm. Res.* **2017**, *119*, 242–250. [CrossRef]
18. Gorska-Ponikowska, M.; Kuban-Jankowska, A.; Daca, A.; Nussberger, S. 2-methoxyestradiol reverses the pro-carcinogenic effect of L-lactate in osteosarcoma 143B cells. *Cancer Genom. Proteom.* **2017**, *14*, 483–493.
19. Kim, J.; Nam, J.; Kim, A.; Park, M.; Lee, H.; Park, J.; Kim, J.; Lee, Y. 2-methoxyestradiol inhibits radiation-induced skin injuries. *Int. J. Mol. Sci.* **2022**, *23*, 4171. [CrossRef]
20. Hua, W.; Huang, X.; Li, J.; Feng, W.; Sun, Y.; Guo, C. 2-methoxyestradiol inhibits melanoma cell growth by activating adaptive immunity. *Immunopharm. Immunotoxicol.* **2022**, *44*, 541–547. [CrossRef]
21. Ireson, C.R.; Chander, S.K.; Purohit, A.; Perera, S.; Newman, S.P.; Parish, D.; Leese, M.P.; Smith, A.C.; Potter, B.V.L.; Reed, M.J. Pharmacokinetics and efficacy of 2-methoxyoestradiol and 2-methoxyoestradiol-bis-sulphamate *in vivo* in rodents. *Br. J. Cancer* **2004**, *90*, 932–937. [CrossRef]
22. Dahut, W.L.; Lakhani, N.J.; Gulley, J.L.; Arlen, P.M.; Kohn, E.C.; Kotz, H.; McNally, D.; Parr, A.; Parr, A.; Nguyen, D.; et al. Phase I clinical trial of oral 2-methoxyestradiol, an antiangiogenic and apoptotic agent, in patients with solid tumors. *Cancer Biol. Ther.* **2006**, *5*, 22–27. [CrossRef] [PubMed]
23. Newman, S.P.; Foster, P.A.; Stengel, C.; Day, J.M.; Ho, Y.T.; Judde, J.-G.; Lassalle, M.; Prevost, G.; Leese, M.P.; Potter, B.V.L.; et al. STX140 is efficacious *in vitro* and *in vivo* in taxane-resistant breast carcinoma cells. *Clin. Cancer Res.* **2008**, *14*, 597–606. [CrossRef] [PubMed]
24. Potter, B.V.L. Steroid sulfatase inhibition by aryl sulfamates: Clinical progress, mechanism, and future prospects. *J. Mol. Endocrinol.* **2018**, *61*, T233–T252. [CrossRef] [PubMed]
25. Thomas, M.P.; Potter, B.V.L. Estrogen *O*-sulfamates and their analogues: Clinical steroid sulfatase inhibitors with broad potential. *J. Steroid Biochem. Mol. Biol.* **2015**, *153*, 160–169. [CrossRef]
26. Thomas, M.P.; Potter, B.V.L. Discovery and development of the aryl *O*-sulfamate moiety for oncology and women's health. *J. Med. Chem.* **2015**, *58*, 7634–7658. [CrossRef]
27. Day, J.M.; Foster, P.A.; Tutill, H.J.; Newman, S.P.; Ho, Y.T.; Leese, M.P.; Potter, B.V.L.; Reed, M.J.; Purohit, A. BCRP expression does not result in resistance to STX140 in vivo, despite the increased expression of BCRP in A2780 cells in vitro after long-term STX140 exposure. *Br. J. Cancer* **2009**, *100*, 476–486. [CrossRef]
28. Ho, Y.T.; Newman, S.P.; Purohit, A.; Leese, M.P.; Potter, B.V.L.; Reed, M.J. The effects of 2-methoxyoestrogens and their sulphamoylated derivatives in conjunction with TNF-α on endothelial and fibroblast cell growth, morphology, and apoptosis. *J. Steroid Biochem.* **2003**, *86*, 189–196. [CrossRef]
29. Newman, S.P.; Leese, M.P.; Purohit, A.; James, D.R.C.; Rennie, C.E.; Potter, B.V.L.; Reed, M.J. Inhibition of *in vitro* angiogenesis by 2-methoxy- and 2-ethylestrogen sulfamates. *Int. J. Cancer* **2004**, *109*, 533–540. [CrossRef]
30. Jourdan, F.; Leese, M.P.; Dohle, W.; Hamel, E.; Ferrandis, E.; Newman, S.P.; Purohit, A.; Reed, M.J.; Potter, B.V.L. Synthesis, anti-tubulin, and antiproliferative SAR of analogues of 2- methoxyestradiol-3,17-*O*,*O*-bis-sulfamate. *J. Med. Chem.* **2010**, *53*, 2942–2951. [CrossRef]
31. Stengel, C.; Newman, S.P.; Leese, M.P.; Thomas, M.P.; Potter, B.V.L.; Reed, M.J.; Purohit, A.; Foster, P.A. The *in vitro* and *in vivo* activity of the microtubule disruptor STX140 is mediated by Hif-1 alpha and CAIX expression. *Anticancer Res.* **2015**, *35*, 5249–5261. [PubMed]
32. Meyer-Losic, F.; Newman, S.P.; Day, J.M.; Reed, M.J.; Kasprzyk, P.G.; Purohit, A.; Foster, A. STX140, but not paclitaxel, inhibits mammary tumour initiation and progression in C3(1)/SV40 T/t-antigen transgenic mice. *PLoS ONE* **2013**, *8*, e80305. [CrossRef] [PubMed]
33. Hosang, L.; Löhndorf, A.; Dohle, W.; Rosche, A.; Marry, S.; Diercks, B.P.; Müller-Kirschbaum, L.C.; Flügel, L.T.; Potter, B.V.L.; Odoardi, F.; et al. 2-Methoxyestradiol-3, 17-*O*, *O*-bis-sulfamate inhibits store-operated Ca^{2+} entry in T lymphocytes and prevents experimental autoimmune encephalomyelitis. *Biochim. Biophys. Acta Mol. Cell Res.* **2023**, *1870*, 119485. [CrossRef]
34. Foster, P.A.; Ho, Y.T.; Newman, S.P.; Leese, M.P.; Potter, B.V.L.; Reed, M.J.; Purohit, A. STX140 and STX641 cause apoptosis via the intrinsic mitochondrial pathway and down-regulate survivin and XIAP expression in ovarian and prostate cancer cells. *Anticancer Res.* **2009**, *29*, 3751–3758.
35. Wang, E. Senescent human fibroblasts resist programmed cell death, and failure to suppress Bcl-2 is involved. *Cancer Res.* **1995**, *55*, 2284–2292. [PubMed]
36. Sasaki, M.; Kumazaki, T.; Takano, H.; Nishiyama, M.; Mitsui, Y. Senescent cells are resistant to death despite low Bcl-2 level. *Mech. Ageing Dev.* **2001**, *122*, 1695–1706. [CrossRef] [PubMed]
37. Yosef, R.; Pilpel, N.; Tokarsky-Amiel, R.; Biran, A.; Ovadya, Y.; Cohen, S.; Vadai, E.; Dassa, L.; Shahar, E.; Condiotti, R.; et al. Directed elimination of senescent cells by inhibition of Bcl-w and Bcl-xL. *Nat. Commun.* **2016**, *7*, 11190. [CrossRef]

38. Lee, B.Y.; Han, J.A.; Im, J.S.; Morrone, A.; Johung, K.; Goodwin, E.C.; Kleijer, W.J.; DiMaio, D.; Hwang, E.S. Senescence-associated β-galactosidase is lysosomal β-galactosidase. *Aging Cell* **2006**, *5*, 187–195. [CrossRef]
39. Ewald, J.A.; Desotelle, J.A.; Wilding, G.; Jarrard, D.F. Therapy-induced senescence in cancer. *J. Natl. Cancer Inst.* **2010**, *102*, 1536–1546. [CrossRef]
40. Zhang, L.; Yang, Z.; Liu, Y. GADD45 proteins: Roles in cellular senescence and tumor development. *Exp. Biol. Med.* **2014**, *239*, 773–778. [CrossRef]
41. Minamino, T.; Orimo, M.; Shimizu, I.; Kunieda, T.; Yokoyama, M.; Ito, T.; Nojima, A.; Nabetani, A.; Oike, Y.; Matsubara, H.; et al. A crucial role for adipose tissue p53 in the regulation of insulin resistance. *Nat. Med.* **2009**, *15*, 1082–1087. [CrossRef]
42. Stivala, A.L.; Cazzalini, O.; Prosperi, E. The cyclin-dependent kinase inhibitor p21CDKN1A as a target of anti-cancer drugs. *Curr. Cancer Drug Targets* **2012**, *12*, 85–96. [CrossRef] [PubMed]
43. Katayama, H.; Sasai, K.; Kawai, H.; Yuan, Z.; Bondaruk, J.; Suzuki, F.; Fujii, S.; Arlinghaus, R.B.; Czerniak, B.A.; Sen, S. Phosphorylation by aurora kinase a induces MDM2-mediated destabilization and inhibition of p53. *Nat. Genet.* **2004**, *36*, 55–62.
44. Driscoll, D.L.; Chakravarty, A.; Bowman, D.; Shinde, V.; Lasky, K.; Shi, J.; Vos, T.; Stringer, B.; Amidon, B.; D'Amore, N.; et al. Plk1 inhibition causes post-mitotic DNA damage and senescence in a range of human tumor cell lines. *PLoS ONE* **2014**, *9*, e111060. [CrossRef]
45. Muñoz-Espín, D.; Serrano, M. Cellular senescence: From physiology to pathology. *Nat. Rev. Mol.* **2014**, *15*, 482–496. [CrossRef] [PubMed]
46. Ayrapetov, M.K.; Gursoy-Yuzugullu, O.; Xu, C.; Xu, Y.; Price, B.D. DNA double-strand breaks promote methylation of histone h3 on lysine 9 and transient formation of repressive chromatin. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 9169–9174. [CrossRef] [PubMed]
47. Chicas, A.; Wang, X.; Zhang, C.; McCurrach, M.; Zhao, Z.; Mert, O.; Dickins, R.A.; Narita, M.; Zhang, M.; Lowe, S.W. Dissecting the unique role of the retinoblastoma tumor suppressor during cellular senescence. *Cancer Cell* **2010**, *17*, 376–387. [CrossRef]
48. Wang, L.; Lankhorst, L.; Bernards, R. Exploiting senescence for the treatment of cancer. *Nat. Rev. Cancer* **2022**, *22*, 340–355. [CrossRef]
49. Takahashi, A.; Imai, Y.; Yamakoshi, K.; Kuninaka, S.; Ohtani, N.; Yoshimoto, S.; Hori, S.; Tachibana, M.; Anderton, E.; Takeuchi, T.; et al. DNA damage signaling triggers degradation of histone methyltransferases through APC/CCDH1 in senescent cells. *Mol. Cell* **2012**, *45*, 123–131. [CrossRef]
50. Hernandez-Segura, A.; Nehme, J.; Demaria, M. Hallmarks of cellular senescence. *Trends Cell Biol.* **2018**, *28*, 436–453. [CrossRef]
51. Lázár-Molnár, E.; Hegyesi, H.; Tóth, S.; Falus, A. Autocrine and paracrine regulation by cytokines and growth factors in melanoma. *Cytokine* **2000**, *12*, 547–554. [CrossRef]
52. Waugh, D.J.J.; Wilson, C. The interleukin-8 pathway in cancer. *Clin. Cancer Res.* **2008**, *14*, 6735–6741. [CrossRef] [PubMed]
53. Jobe, N.P.; Rosel, D.; Dvorankova, B.; Kodet, O.; Lacina, L.; Mateu, R.; Smetana, K.; Brabek, J. Simultaneous blocking of IL-6 and IL-8 is sufficient to fully inhibit CAF-induced human melanoma cell invasiveness. *Histochem. Cell Biol.* **2016**, *146*, 205–217. [CrossRef]
54. Sanmamed, M.F.; Perez-Gracia, J.L.; Schalper, K.A.; Fusco, J.P.; Gonzalez, A.; Rodriguez-Ruiz, M.E.; Oñate, C.; Perez, G.; Alfaro, C.; Martín-Algarra, S.; et al. Changes in serum interleukin-8 (IL-8) levels reflect and predict response to anti-PD-1 treatment in melanoma and non-small-cell lung cancer patients. *Ann. Oncol.* **2017**, *28*, 1988–1995. [CrossRef] [PubMed]
55. Prasanna, P.G.; Citrin, D.E.; Hildesheim, J.; Ahmed, M.M.; Venkatachalam, S.; Riscuta, G.; Xi, D.; Zheng, G.; Deursen, J.V.; Goronzy, J.; et al. Therapy-induced senescence: Opportunities to improve anticancer therapy. *J. Natl. Cancer Inst.* **2021**, *113*, 1285–1298. [CrossRef]
56. Zhang, L.; Pitcher, L.E.; Yousefzadeh, M.J.; Niedernhofer, L.J.; Robbins, P.D.; Zhu, Y. Cellular senescence: A key therapeutic target in aging and diseases. *J. Clin. Investig.* **2022**, *132*, e158450. [CrossRef] [PubMed]
57. Schmitt, C.A.; Wang, B.; Demaria, M. Senescence and cancer—Role and therapeutic opportunities. *Nat. Rev. Clin. Oncol.* **2022**, *19*, 619–636. [CrossRef] [PubMed]
58. Leese, M.P.; Leblond, B.; Smith, A.; Newman, S.P.; Di Fiore, A.; De Simone, G.; Supuran, C.T.; Purohit, A.; Reed, M.J.; Potter, B.V.L. 2-Substituted estradiol bis-sulfamates, multitargeted antitumor agents: synthesis, *in vitro* SAR, protein crystallography, and *in vivo* activity. *J. Med. Chem.* **2006**, *49*, 7683–7696. [CrossRef] [PubMed]
59. Sandri, S.; Faião-Flores, F.; Tiago, M.; Pennacchi, P.C.; Massaro, R.R.; Alves-Fernandes, D.K.; Berardinelli, G.N.; Evangelista, A.F.; de Lima Vazquez, V.; Reis, R.M.; et al. Vemurafenib resistance increases melanoma invasiveness and modulates the tumor microenvironment by mmp-2 upregulation. *Pharm. Res.* **2016**, *111*, 523–533. [CrossRef]
60. Liu, W.; Meng, J.; Cui, J.; Luan, Y. Characterization and function of microRNA's in plants. *Front. Plant Sci.* **2017**, *8*, 2200. [CrossRef]
61. Brohem, C.A.; Cardeal, L.B.S.; Tiago, M.; Soengas, M.S.; Barros, S.B.M.; Maria-Engler, S.S. Artificial skin in perspective: Concepts and applications. *Pigment Cell Melanoma Res.* **2011**, *24*, 35–50. [CrossRef] [PubMed]

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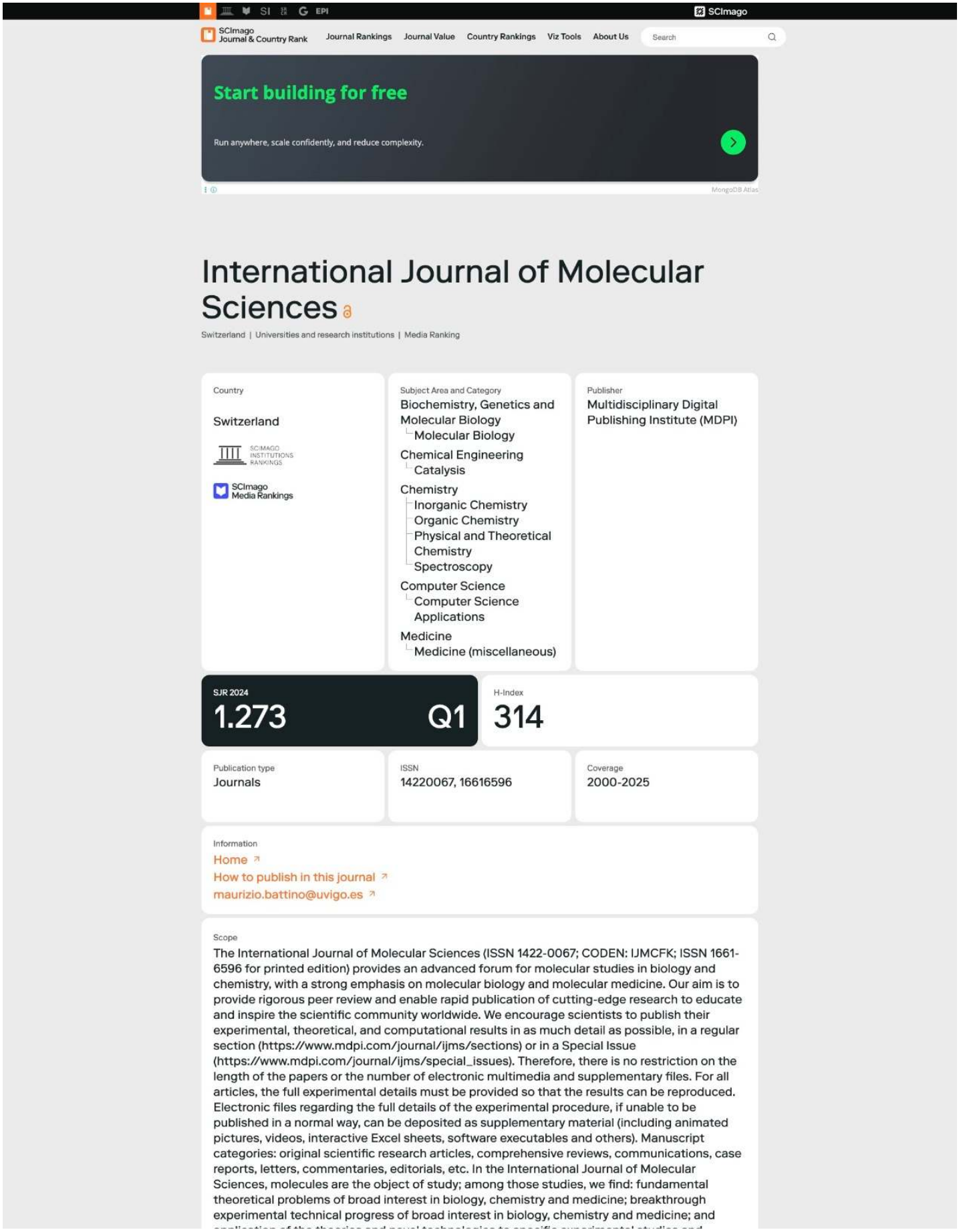
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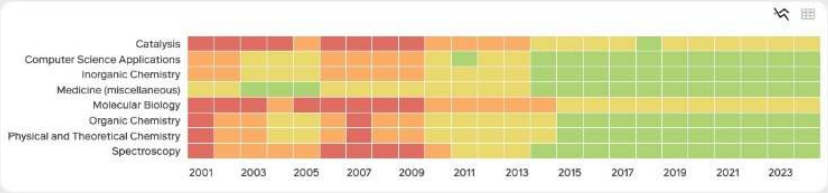

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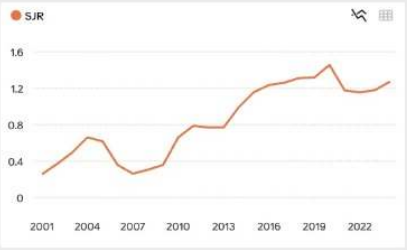

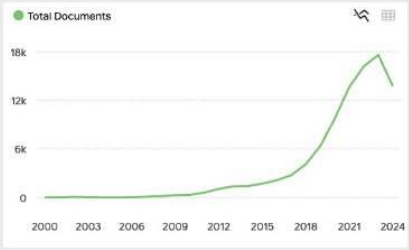

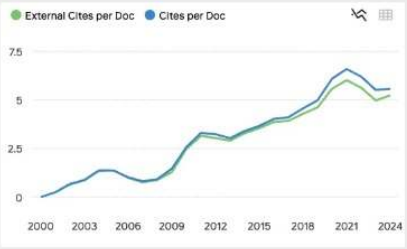

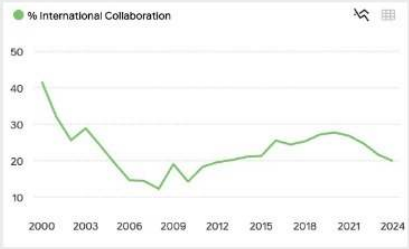

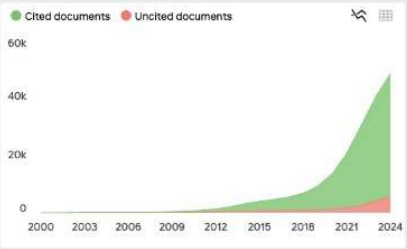

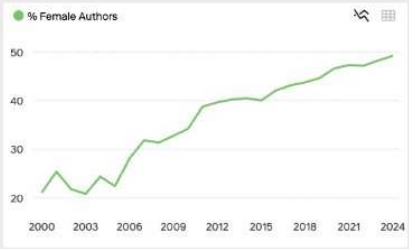

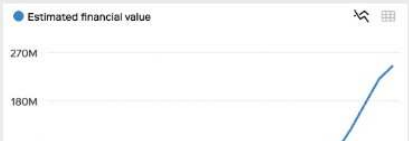

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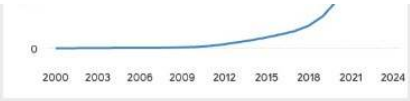
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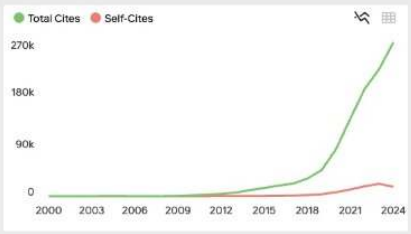
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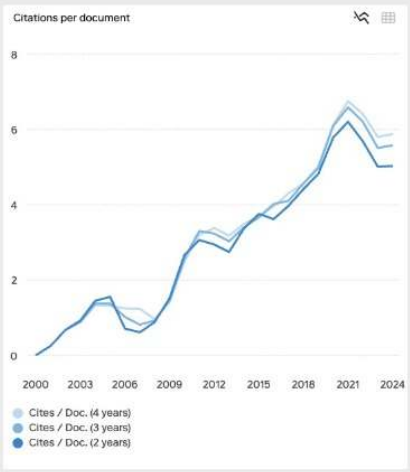
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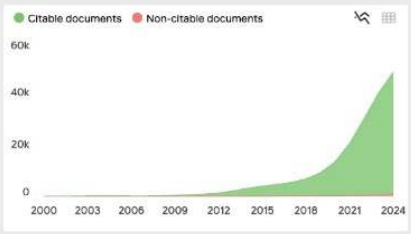
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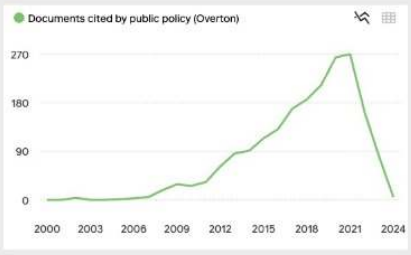
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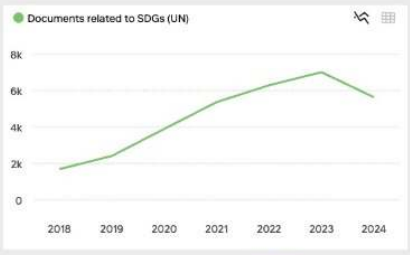
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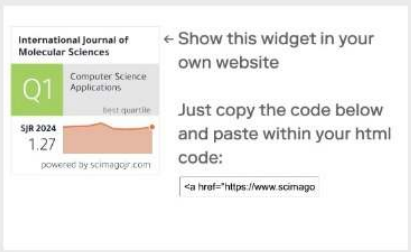
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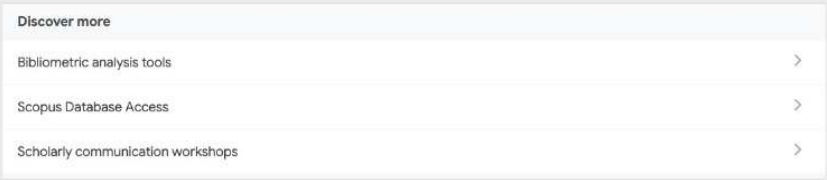
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Thank you
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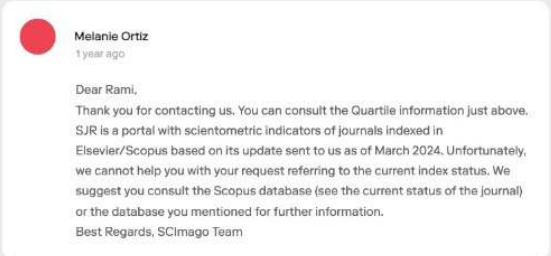
Melanie Ortiz
1 year ago
Dear Rami,
Thank you for contacting us. You can consult the Quartile information just above.
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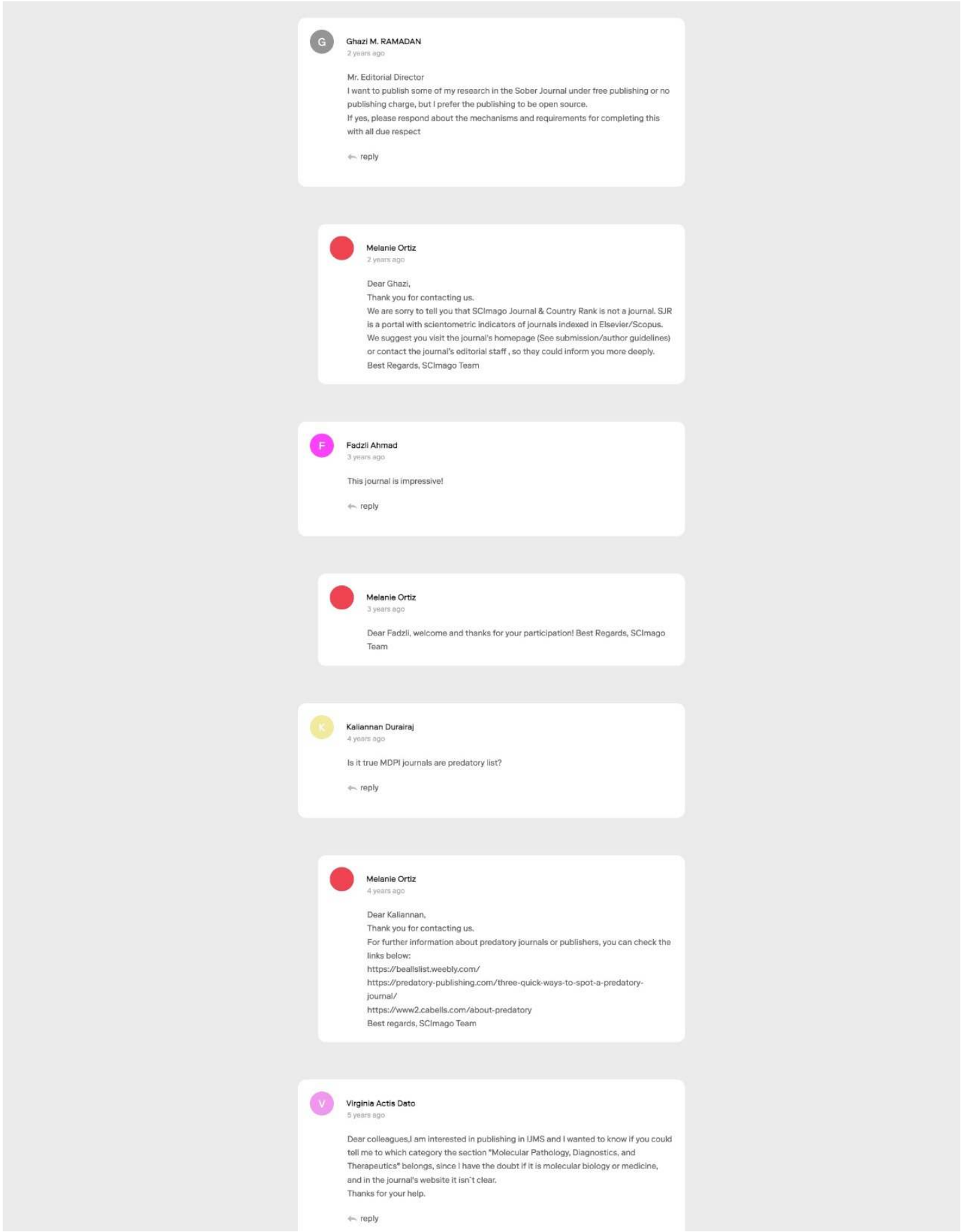
Best Regards, SCImago Team



Ghazi M. RAMADAN
2 years ago

Mr. Editorial Director
I want to publish some of my research in the Sober Journal under free publishing or no publishing charge, but I prefer the publishing to be open source.
If yes, please respond about the mechanisms and requirements for completing this with all due respect

reply

Melanie Ortiz
2 years ago

Dear Ghazi,
Thank you for contacting us.
We are sorry to tell you that SCImago Journal & Country Rank is not a journal. SJR is a portal with scientometric indicators of journals indexed in Elsevier/Scopus.
We suggest you visit the journal's homepage (See submission/author guidelines) or contact the journal's editorial staff , so they could inform you more deeply.
Best Regards, SCImago Team

Fadzli Ahmad
3 years ago

This journal is impressive!

reply

Melanie Ortiz
3 years ago

Dear Fadzli, welcome and thanks for your participation! Best Regards, SCImago Team

Kaliannan Durairaj
4 years ago

Is it true MDPI journals are predatory list?

reply

Melanie Ortiz
4 years ago

Dear Kaliannan,
Thank you for contacting us.
For further information about predatory journals or publishers, you can check the links below:
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https://predatory-publishing.com/three-quick-ways-to-spot-a-predatory-journal/
https://www2.cabells.com/about-predatory
Best regards, SCImago Team

Virginia Actis Dato
5 years ago

Dear colleagues,I am interested in publishing in IJMS and I wanted to know if you could tell me to which category the section "Molecular Pathology, Diagnostics, and Therapeutics" belongs, since I have the doubt if it is molecular biology or medicine, and in the journal's website it isn't clear.
Thanks for your help.

reply

Melanie Ortiz
5 years ago

Dear Virginia, thank you very much for your comment. Unfortunately, we cannot help you with your request, we suggest you contact the journal's editorial staff so they could inform you more deeply. Best Regards, SCImago Team

Mikhail
5 years ago

Dear colleagues,
could you explain why on the website of your Journal JCR category rank: 74/297 (Q1) in 'Biochemistry

reply

Melanie Ortiz
5 years ago

Dear Mikhail,
Thank you for contacting us.
We are sorry to tell you that SCImago Journal & Country Rank is not a journal. SJR is a portal with scientometric indicators of journals indexed in Elsevier/Scopus. As SCImago Journal and Country Rank uses Scopus data, our impact indicator is the SJR. We suggest you consult the Journal Citation Report for other indicators (like Impact Factor) with a Web of Science data source.
Best Regards, SCImago Team

Dr Andleeb
5 years ago

Does the journal provide any discount? I want to publish a review in Ijms. Anyone?

reply

Melanie Ortiz
5 years ago

Dear Dr Andleeb,
thank you for contacting us.
Unfortunately, we cannot help you with your request, we suggest you visit the journal's homepage or contact the journal's editorial staff , so they could inform you more deeply.
Best Regards, SCImago Team

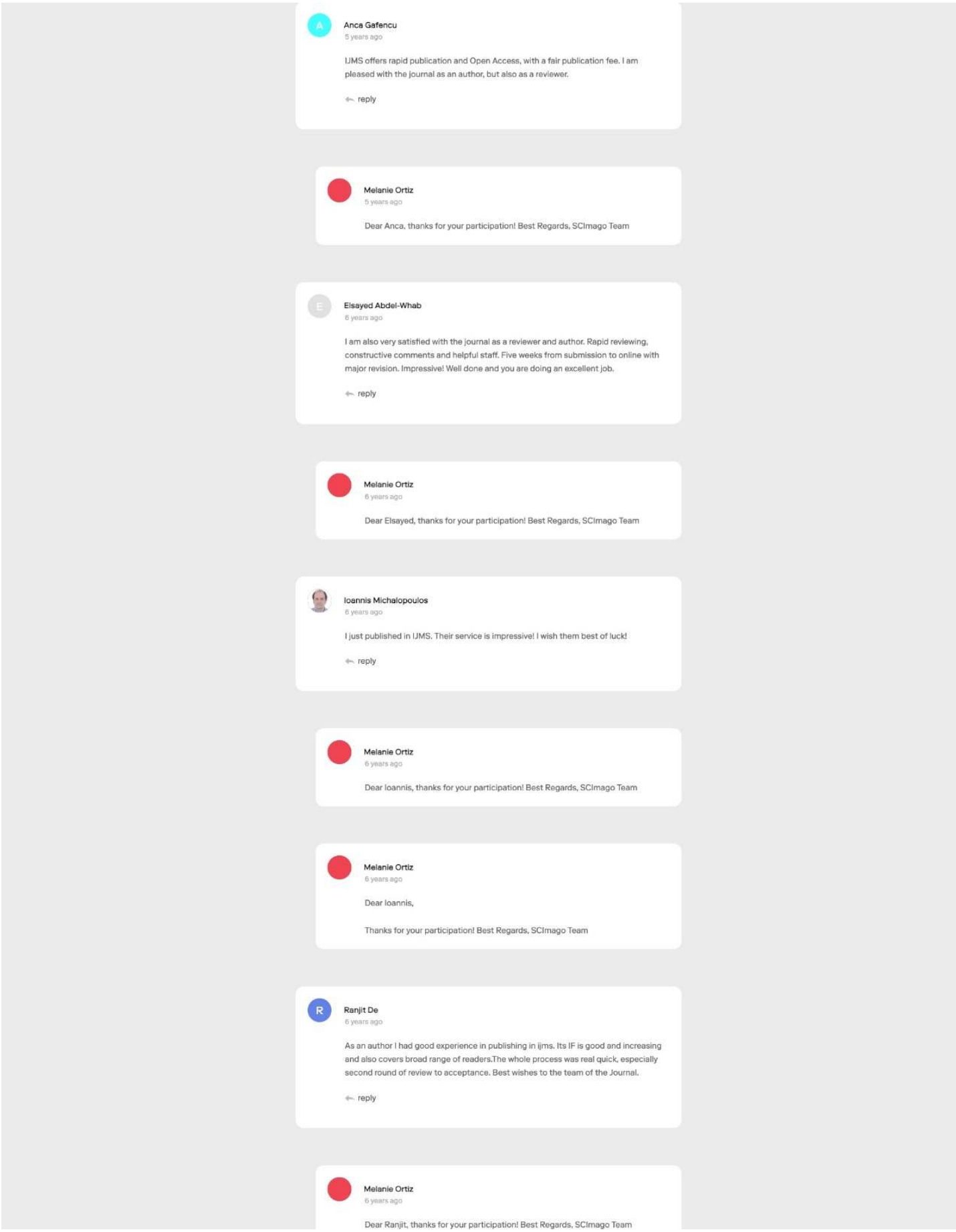
PARASTOO MEMARIAN
5 years ago

Hello. I would like to inquire some information about the special issue of this journal "namely, Cell-Biomaterial Interaction" which belongs to the section of "Materials Science". I would like to know if the journal is ranked Q1.

reply

Melanie Ortiz
5 years ago

Dear Sir/Madam, thank you very much for your request. You can consult that information just above.
Best Regards, SCImago Team



Anca Gafencu
5 years ago

IJMS offers rapid publication and Open Access, with a fair publication fee. I am pleased with the journal as an author, but also as a reviewer.

reply

Melanie Ortiz
5 years ago

Dear Anca, thanks for your participation! Best Regards, SCImago Team

Elsayed Abdel-Whab
6 years ago

I am also very satisfied with the journal as a reviewer and author. Rapid reviewing, constructive comments and helpful staff. Five weeks from submission to online with major revision. Impressive! Well done and you are doing an excellent job.

reply

Melanie Ortiz
6 years ago

Dear Elsayed, thanks for your participation! Best Regards, SCImago Team

Ioannis Michalopoulos
6 years ago

I just published in IJMS. Their service is impressive! I wish them best of luck!

reply

Melanie Ortiz
6 years ago

Dear Ioannis, thanks for your participation! Best Regards, SCImago Team

Melanie Ortiz
6 years ago

Dear Ioannis,

Thanks for your participation! Best Regards, SCImago Team

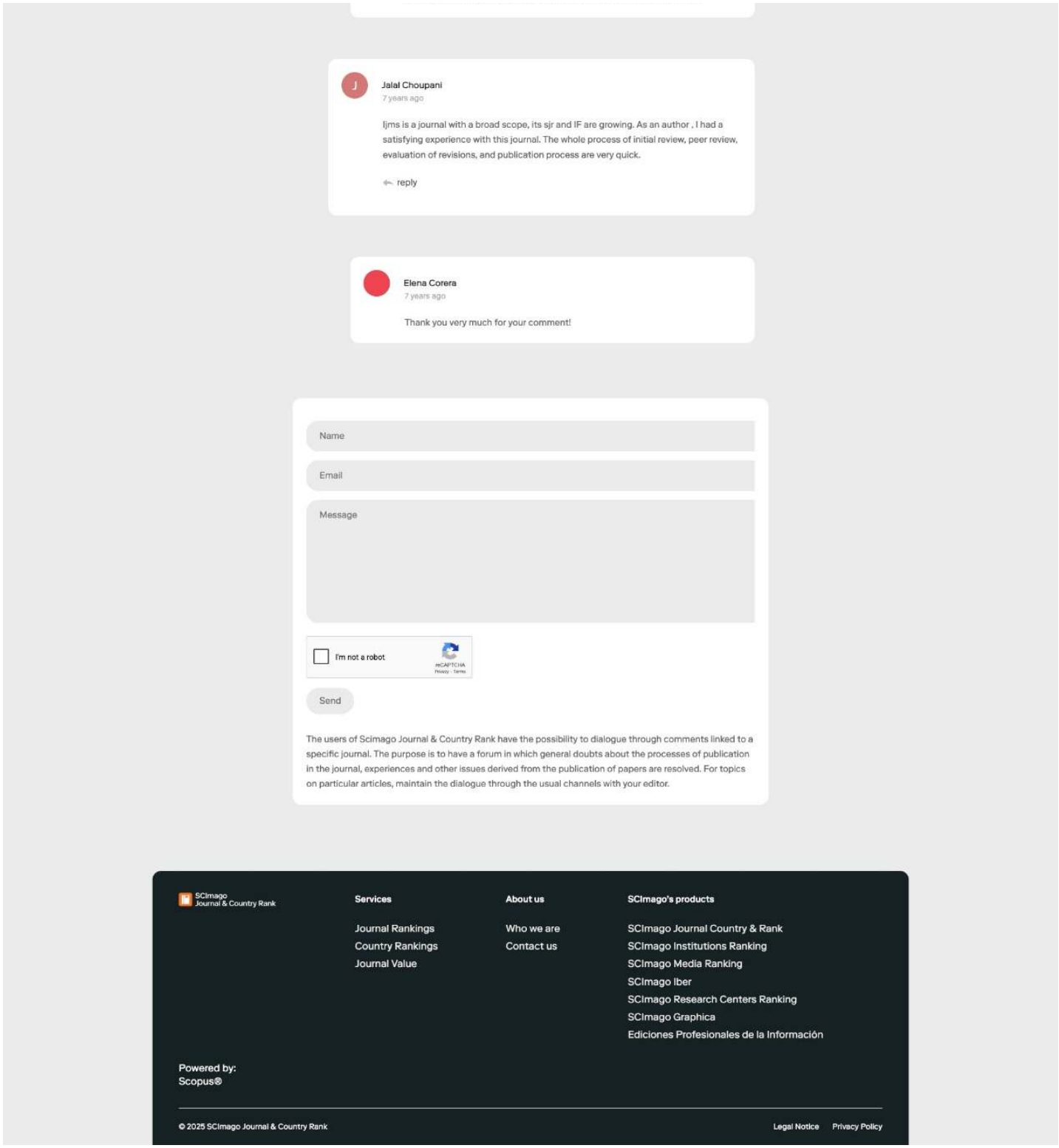
Ranjit De
6 years ago

As an author I had good experience in publishing in ijms. Its IF is good and increasing and also covers broad range of readers.The whole process was real quick, especially second round of review to acceptance. Best wishes to the team of the Journal.

reply

Melanie Ortiz
6 years ago

Dear Ranjit, thanks for your participation! Best Regards, SCImago Team


Jalal Choupani
7 years ago
Ijms is a journal with a broad scope, its sjr and IF are growing. As an author , I had a satisfying experience with this journal. The whole process of initial review, peer review, evaluation of revisions, and publication process are very quick.
reply
Elena Corera
7 years ago
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Association between the relative abundance of phyla actinobacteria, vitamin C consumption, and DNA methylation of genes linked to immune response pathways

Natália Yumi Noronha[1,2†], Isabella Harumi Yonehara Noma[3†], Rafael Fernandes Ferreira[4], Guilherme da Silva Rodrigues[2], Luzania dos Santos Martins[2], Lígia Moriguchi Watanabe[5], Marcela Augusta de Souza Pinhel[2,4], Isabelle Mello Schineider[2], Luísa Maria Diani[2], Daniela Carlos[6] and Carla Barbosa Nonino[5*]

[1]Department of Gynecology and Obstetrics, University Medical Center Groningen, Groningen, Netherlands, [2]Faculty of Medicine of Ribeirão Preto, Department of Internal Medicine, University of São Paulo, Ribeirão Preto, Brazil, [3]Department of Clinical and Toxicological Analyses, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil, [4]Department of Molecular Biology, Sao Jose do Rio Preto Medical School, São José do Rio Preto, Brazil, [5]Faculty of Medicine of Ribeirao Preto, Department of Health Sciences, University of São Paulo, Ribeirão Preto, Brazil, [6]Faculty of Medicine of Ribeirão Preto, Department of Biochemistry and Immunology, University of São Paulo, Ribeirão Preto, Brazil

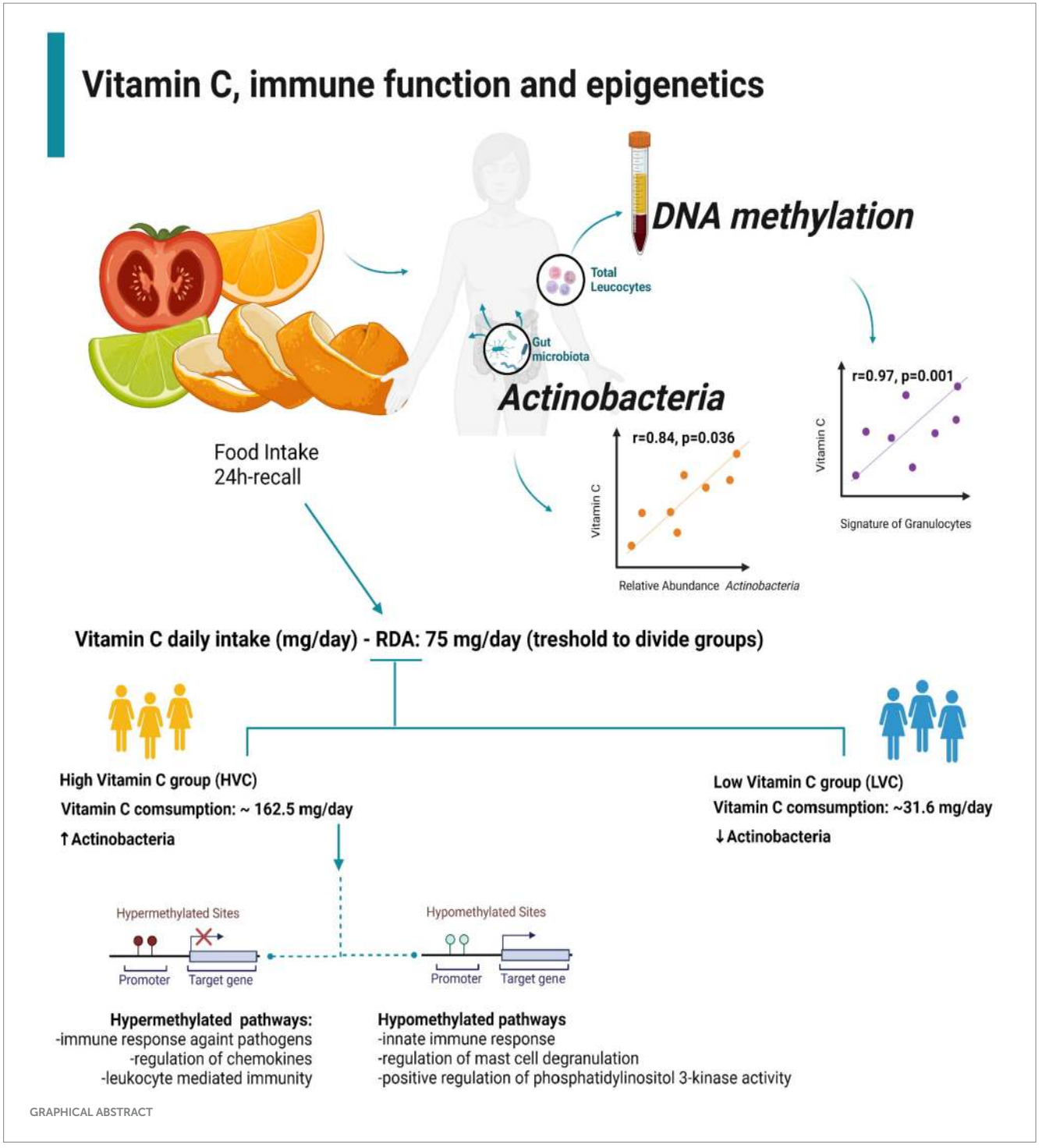
Introduction: There is an emerging body of evidence that vitamin C consumption can modulate microbiota abundance and can also impact DNA methylation in the host, and this could be a link between diet, microbiota, and immune response. The objective of this study was to evaluate common CpG sites associated with both vitamin C and microbiota phyla abundance.

Methods: Six healthy women participated in this cohort study. They were divided into two groups, according to the amount of vitamin C they ingested. Ingestion was evaluated using the 24-h recall method. The Illumina 450k BeadChip was used to evaluate DNA methylation. Singular value decomposition analyses were used to evaluate the principal components of this dataset. Associations were evaluated using the differentially methylated position function from the Champ package for R Studio.

Results and discussion: The group with higher vitamin C (HVC) ingestion also had a higher relative abundance of Actinobacteria. There was a positive correlation between those variables (r=0.84, p=0.01). The HVC group also had higher granulocytes, and regarding DNA methylation, there were 207 CpG sites commonly related to vitamin C ingestion and the relative abundance of Actinobacteria. From these sites, there were 13 sites hypomethylated and 103 hypermethylated. The hypomethylated targets involved the respective processes: immune function, glucose homeostasis, and general cellular metabolism. The hypermethylated sites were also enriched in immune function-related processes, and interestingly, more immune responses against pathogens were detected. These findings contribute to understanding the interaction between nutrients, microbiota, DNA methylation, and the immune response.

KEYWORDS

DNA methylation, Actinobacteria, vitamin C, immune response and nutrients, epigenetic



1 Introduction

In humans, the dominant phyla are *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Proteobacteria, Fusobacteria*, and *Verrucomicrobia* (1). Nutrients influence the microbial community by promoting or inhibiting the growth of microorganisms, indirectly influencing the host's metabolism and immune system (2). The gut microbiota synthesizes compounds as methyl and acetyl groups of vitamins. Consequently, it plays a role in DNA methylation and histone modifications, which influence physiological epigenetic changes. There is a growing body of evidence that nutrients can modulate the relative abundance of microbiota and that both nutrients and the diversity of bacteria in the microbiome can be related to DNA methylation (3). Especially vitamins can have an essential role in the microbiota's abundance (4). Vitamin C is known for its antioxidant properties, and it is well-established that it can improve immune function. Little is known about the relationship of vitamin C with the bacterial phyla and the effect of this interaction on the epigenome. There are few studies in humans integrating epigenetic data, vitamin C, and microbiota relative abundance and how they

are related. We hypothesize that vitamin C ingestion changed the microbiota, affecting DNA methylation. This study aimed to perform a pilot study to evaluate CpG sites related both to the relative abundance of specific bacterial phyla and vitamin C ingestion.

2 Materials and methods

2.1 Study design, ethics, and subjects

This was a case–control pilot study. The Ethics Committee of Ribeirão Preto Medical School approved the study protocol at the University of São Paulo, Brazil (process CAAE: 57459816.1.0000.5440). Informed consent was obtained from all individual participants, and the inclusion criteria are shown in Figure 1. The study was conducted according to the Declaration of Helsinki. Six women, aged 30.8 ± 2.2, were subdivided into two groups (Figure 1).

2.2 Inclusion and exclusion criteria

Adult women without declared diseases were included in the study. Non-inclusion criteria included the use of antibacterial therapy, anti-inflammatory medicine, antifungal and anti-allergic medicines, probiotics and prebiotics, lactation, smoking, drinking, chemotherapy, a history of gastrointestinal surgery, gastrointestinal disease, diabetes mellitus, hypothyroidism or hyperthyroidism, the practice of physical exercise and dietary interventions already in course, and the use of new medical therapy that could influence the intestinal microbiota. The included patients were healthy and had no severe diseases diagnosed during the study.

The groups were divided based on the recommended reference values for vitamin C ingestion, resulting in three participants in each group. Data collection commenced on 19 November 2013, and concluded on 29 June 2015. During this period, each woman underwent the 24-h dietary recall three times. It is important to note that the 24-h recall method was used to assess the participants' vitamin C consumption. This approach allowed us to evaluate their habitual vitamin C intake within the context of their daily dietary patterns. Blood collection and anthropometric measurements are only taken. Anthropometry, body composition, and resting metabolic rate were assessed following the protocol previously described by our group (5). Three 24-h dietary recalls were performed to evaluate the participants' food consumption, and the software Dietwin® was used to calculate it. The subjects were evaluated after 12 h of fasting. Total blood was collected to assess glycemia, lipidogram, and gene expression analysis. Stool samples were collected to analyze the microbiota relative abundance.

2.3 Relative abundance of intestinal microbiota

DNA was extracted using the kit QIAamp® Fast DNA Stool Mini. The primer sequences used for each phylum were Firmicutes (F-ATGTGGTTTAATTCGAAGCA, R-AGCTGACGACAACCATGCAC),Bacteroidetes(F-GTTTAATTCGATGATACGCGAG,R-TTAASCCGACACCTCACGG), Actinobacteria (F-TGTAGCGGTGGAATGCGC, R-AATTAAGCCACATGCTCCGCT), Proteobacteria (F-CATGACGTTACCCGCAGAAGAAG, R-CTCTACGAGACTCAAGCTTGC), Verrucomicrobia (F-TCAKGTCAGTATGGCCCTTAT, R-CAGTTTTYAGGATTTCCTCCGCC), and rRNA 16S universal (F-AACAGGATTAGATACCCTGGTAG, R-GGTTCTTCGCGTTGC

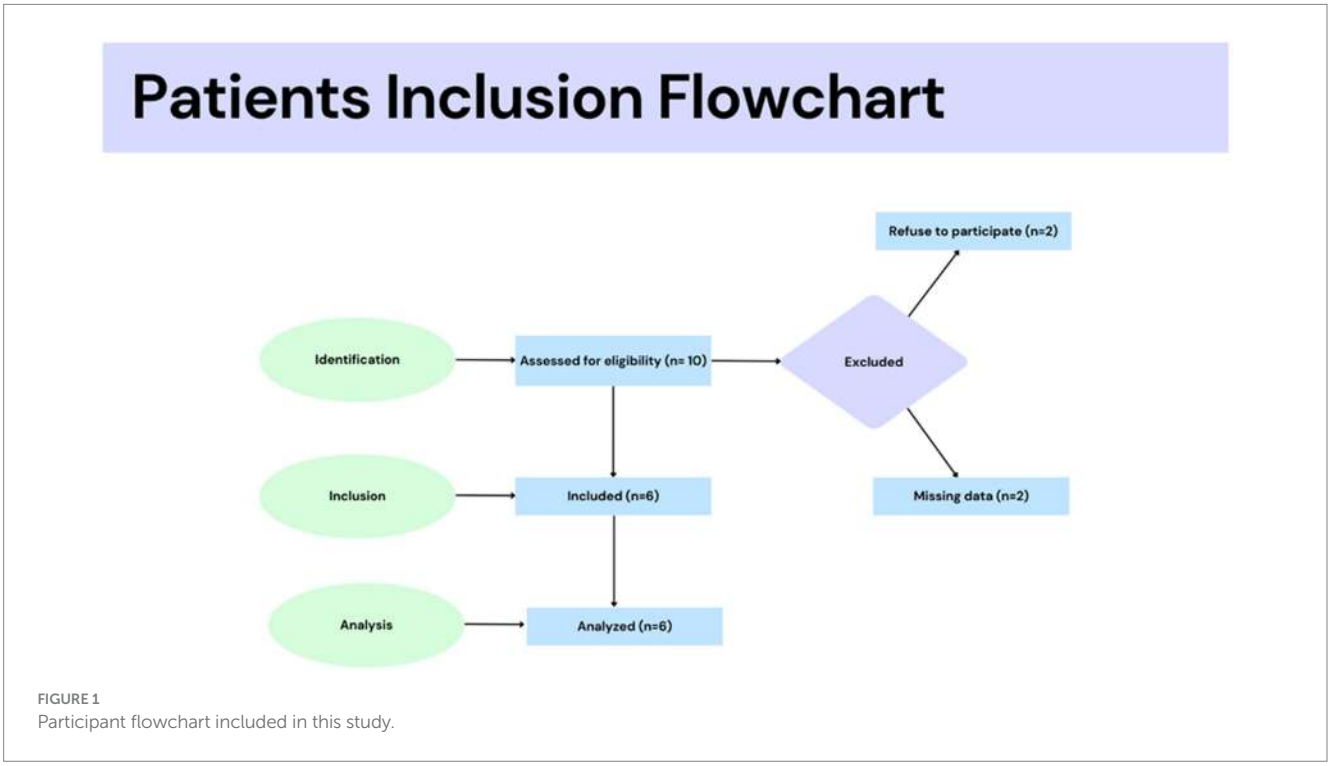


FIGURE 1
Participant flowchart included in this study.

ATC). The primers were at 10 μM. We used 20 ng of DNA in each well, ultra-pure water, and a master mix (SYBR Green, Promega®) with a final volume of 10 μL. After the reactions were performed, the results were analyzed using the Step One Plus® software (Thermo Fisher Scientific). We set the limit manually at 0.1. We use the $2^{-\Delta Ct}$ method to calculate the fold change and determine the relative abundance (6).

2.4 DNA extraction and methylation

DNA was extracted from whole peripheral blood using the Promega (Madison, Wisconsin, EUA) automated system with magnetic beads following the manufacturer's instructions (7). Integrity was evaluated by agarose gel. Quality was evaluated using NanoDrop (Thermo Fisher, Waltham, Massachusetts, EUA). DNA from total leucocytes were firstly bisulfite converted using the EZ DNA Methylation Kit (Zymo Research Corporation, Irvine, CA, United States), then 500 ng were hybridized in the Infinium Human Methylation 450 k (IHM450K) BeadChip (Illumina, Inc., San Diego, CA, United States) (8). After the protocol, images were obtained using the iScan system.

2.5 DNA methylation data analysis and enrichment

The analysis was performed using R version 3.6.2 and ChAMP, a user-friendly R package available on Bioconductor. Raw intensity data from the IHM450K idats files were loaded on the R package, ChAMP (9). After the normalizations of the myNorm function, 409,819 probes continued in the pipeline for further processing, the missing data were removed, and the KNN imputation was performed (10). White blood cell type estimation was performed with the Houseman method (11). The intensities of the database were obtained from purified cells, and the IHM450K contains 473 CpG sites as a reference for the cell types. The obtained proportions were used to correct beta values using the Refbase function from the Champ package. Single value decomposition (SVD) was performed to check the principal components of the data variation. Limma package was used to determine differentially methylated positions (DMPs) (12), and a p-value of <0.05 and an FDR of <0.05 were considered significant. Linear regressions were performed to verify the association with clinical parameters (12). We performed Gene Functional Enrichment using the STRING database, we used as input the gene symbol from the mapped differentially methylated sites (13).

2.6 Gene expression validation of classic targets

The collected blood sample was used for RNA extraction to evaluate the gene expression of the target genes: *TNF-α*, *NFKB*, *IL-6*, *TLR4*, and *PI3kR1*. The phenol–chloroform method was used for RNA extraction. Reverse transcription (RT) was performed using the high-capacity kit (Applied Biosystems) to obtain cDNA. Quantitative PCR (qPCR) was performed using the TaqMan (Thermo Fisher Scientific) inventoried assays for the target genes. The results were evaluated by the 2-ddct method, using *ACTB* and *GAPDH* as endogenous genes and women without obesity as the normalizing group.

2.7 Statistical analysis

Shapiro–Wilk tests were used to verify normality, t-tests were used to verify differences between groups, and Spearman's correlation was performed between vitamin C and phyla. For all analyses, we adopted a p-value of <0.05. Analysis was performed using the Statistica 7.0 software (StatSoft Statistica®).

3 Results

3.1 Sample

Six women (30.8 ± 2.2 years old) were included in this sample, and the group was divided by the recommendation for vitamin C ingestion (14). There were three women with high vitamin C (HVC) (30.7 ± 0.9 years old) and three with low vitamin C (LVC) (31.0 ± 2.9 years old) reference values. Table 1 shows all the collected clinical data. The two groups were similar for all evaluated parameters.

3.2 Phyla abundance and diversity

The HVC group had a higher relative abundance of Actinobacteria (Table 1). A strong positive correlation existed between the relative abundance of vitamin C and Actinobacteria ($r = 0.84$, $p = 0.018$). Table 1 shows the individual percentual relative abundance of the analyzed phyla. As expected, Bacteroides and Firmicutes were the most abundant groups in all women's intestinal microbiota.

3.3 Epigenetic analysis

Regarding the quality control steps, the SVD analysis revealed that vitamin C was the most important variable in the metafile and better explained the data variation ($p < 0.01$). It was significant in the first principal component (PC1). Regarding bacteria relative abundance, only Actinobacteria were significant ($p < 0.05$) and influenced data variability. Figure 2A shows the clusterization of samples before normalization. The occurrence of the two clusters confirmed that the two groups had significant DNA methylation differences. The distance between samples was calculated based on the identified principal components previously obtained by SVD analysis. The generated outputs are part of a series of quality control analyses in the Champ pipeline. The analysis of QC parameters revealed that all samples were suitable for downstream analysis.

The association analysis retrieved that the associated sites were located in 239 genes for vitamin C and 5,818 genes for Actinobacteria; from those, there were 207 common genes (Figure 2B, upper Venn diagram). There were 40 genes that did not show any DMPs and are not shown in the subsequent Venn diagram (Figure 2B, lower Venn diagram). These 207 genes were classified into three categories according to their methylation status (Figure 2B, lower Venn diagram).

10.3389/fnut.2024.1373499

TABLE 1 Means and standard deviation of the anthropometric profile, body composition, biochemical parameters, and relative abundance of intestinal microbiota phyla.

Parameters	HVC (n = 3)	LVC (n = 3)	Reference value
Vitamin C (mg/day)	165.2 ± 23.6	31.6 ± 7.5*	[16] 75 mg/day
Anthropometric profile			
Weight (kg)	62.8 ± 3.3	64.3 ± 4.2	
BMI (kg/m^2)	22.4 ± 0.7	22.3 ± 0.4	[17] 18.5–24.9 kg/m^2
AC (cm)	72.3 ± 4.5	76.1 ± 2.5	[18] 76.2 cm
Body composition			
FM (kg)	19.5 ± 1.6	18.2 ± 2.2	–
FM (%)	31.0 ± 1.0	28.3 ± 2.9	[19] 28.8–35.7
FFM(kg)	43.3 ± 1.8	46.1 ± 3.6	–
FFM (%)	69.0 ± 1.0	71.7 ± 2.9	–
Biochemical Parameters			
Glycemia (mg/dL)	82.0 ± 7.3	77.0 ± 5.7	[20] 70–99 mg/dL
CT(mg/dL)	159.7 ± 13.7	159.0 ± 22.6	[20] 0–199 mg/dL
HDL-c (mg/dL)	63.8 ± 5.2	52.3 ± 15.5	[20] 40–60 mg/dL
LDL-c (mg/dL)	73.7 ± 6.8	95.7 ± 13.8	[20] 0–99 mg/dL
Relative abundance (2^{-dct})			
FC *Firmicutes*	24.8 ± 14.6	21.0 ± 13.8	–
FC *Actinobacteria*	6.0 ± 3.4	0.4 ± 0.1*	–
FC *Bacteroides*	20.5 ± 17.2	20.4 ± 15.0	–
FC *Proteobacteria*	0.1 ± 0.1	0.3 ± 0.3	–
FC *Verrucomicrobia*	0.4 ± 0.6	0.1 ± 0.0	–

Values are shown in mean ± standard deviation; BMI: body mass index; AC: abdominal circumference; FM: fat mass; FFM: free fat mass; CT: total cholesterol; HDL-c: high-density lipoprotein cholesterol, low-density lipoprotein cholesterol; FC: fold change (15). Recommended dietary allowance (RDA) of 75 mg/day for adult women (16), normal BMI range. (17). In the study of Rexrode et al. (18), abdominal circumference higher than 76.2 cm was associated with higher cardiovascular risk. According to Branco et al. (17), the % of body fat between 28.8 and 35.7 is considered normal in the Brazilian population. (19) Reference values biochemical tests Szwarcwald et al. (19). –: no reference value.

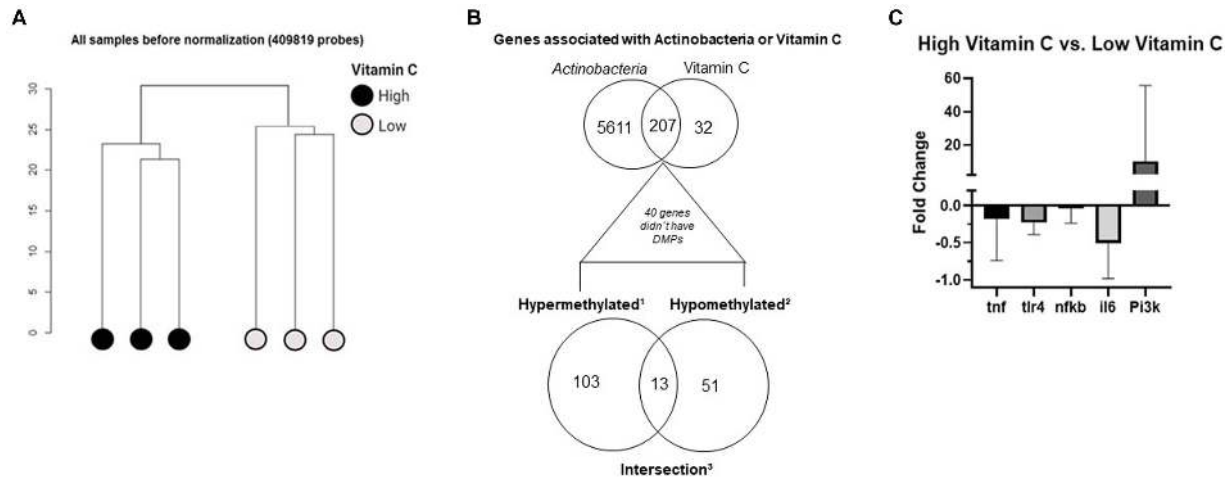


FIGURE 2
(A) Clusterization of raw DNA methylation data. **(B)** Venn diagram of common genes associated with vitamin C consumption and Actinobacteria relative abundance. [1]Genes with only hypermethylated sites (n = 103, lower Venn diagram—left circle). [2]Genes with only hypomethylated sites (n = 51, lower Venn diagram - right circle). [3]Genes with both hypermethylated sites and hypomethylated sites (n = 13; lower Venn diagram—intersection). **(C)** Gene expression analysis of markers involved in the inflammation process: the different tones of gray represent each target analyzed, the bars are the fold change of the high vitamin C group compared to the LVC group, fold changes higher than 1 represent upregulation in the high vitamin C group, and negative fold changes represent downregulation in the high vitamin C group.

TABLE 2 Functional enrichment of the hypomethylated sites in the promoter region.

	Term description	False discovery rate
GO:0048014	Tie signaling pathway	0.0081
GO:0046069	cGMP catabolic process	0.0113
GO:0002040	sprouting angiogenesis	0.0257
GO:0002092	positive regulation of receptor internalization	0.0257
GO:0002376	immune system process	0.0257
GO:0006878	cellular copper ion homeostasis	0.0257
GO:0007154	cell communication	0.0257
GO:0007626	locomotory behavior	0.0257
GO:0008542	visual learning	0.0257
GO:0023052	signaling	0.0257
GO:0030097	hemopoiesis	0.0257
GO:0043114	regulation of vascular permeability	0.0257
GO:0043304	regulation of mast cell degranulation	0.0257
GO:0043552	positive regulation of phosphatidylinositol 3-kinase activity	0.0257
GO:0046068	cGMP metabolic process	0.0257
GO:0050918	positive chemotaxis	0.0257
GO:0051897	positive regulation of protein kinase B signaling	0.0257
GO:0060627	regulation of vesicle-mediated transport	0.0257
GO:0070374	positive regulation of ERK1 and ERK2 cascade	0.0257
GO:0072012	glomerulus vasculature development	0.0257
GO:1900181	negative regulation of protein localization to the nucleus	0.0257
GO:1901215	negative regulation of neuron death	0.0257
GO:2000251	positive regulation of actin cytoskeleton reorganization	0.0257
GO:2000352	negative regulation of the endothelial cell apoptotic process	0.0257
GO:0014068	positive regulation of phosphatidylinositol 3-kinase signaling	0.0332
GO:1904589	regulation of protein import	0.0358
GO:0001505	regulation of neurotransmitter levels	0.0406
GO:0045087	innate immune response	0.0445

1. Genes with only hypermethylated sites ($n = 103$, lower Venn diagram - left circle).
2. Genes with only hypomethylated sites ($n = 51$, lower Venn diagram - right circle).
3. Genes with both hypermethylated sites and hypomethylated sites ($n = 13$; lower Venn diagram - intersection).

Based on the functional enrichment findings, we performed gene expression validation on some targets of interest (Figure 2C). We observed that the HVC group had, as expected, lower expression of the classical inflammation targets: *TNF-a*, *TLR4*, *NFKB*, and *IL-6* (GO:0070944, GO:0050829, GO:0002443) and higher expression of the *PI3K* target (enriched in the pathways GO:0043552 and GO:0014068).

The common genes ($n = 167$) that had differentially methylated CpGs were functionally enriched. The analysis was performed separately for the genes that had hypomethylated and hypermethylated sites (Tables 2, 3). The genes that had hypomethylated CpGs had targets involved in the respective processes: immune function, glucose homeostasis, and general cellular metabolism. The hypermethylated sites were also enriched in immune function-related processes, and interestingly, more immune responses against pathogens were detected.

Regarding cell fraction estimation, the group with HVC consumption had 30% more granulocytes (HVC: 0.68 ± 0.06; LVC:0.48 ± 0.02; $p = 0.0100$) (Figure 3). Furthermore, vitamin C and granulocytes had a strong positive correlation ($r = 0.97$, $p = 0.001$). Vitamin C and the relative abundance of Actinobacteria were also positively correlated ($r = 0.84$, $p = 0.036$).

4 Discussion

Our results showed a positive correlation between the relative abundance of Actinobacteria and the consumption of vitamin C. The same relationship was described by Xu et al. (20), who reported the same association in piglets. Vitamin C, known as ascorbic acid, is a potent antioxidant, helping to protect cells from the damage caused by free radicals, and a cofactor for a family of biosynthetic enzymes and gene regulators. It contributes to supporting various cellular

TABLE 3 Functional enrichment of the hypermethylated sites in the promoter region.

	Term description	False discovery rate
GO:0046903	Secretion	0.0118
GO:0070944	Neutrophil-mediated killing of bacterium	0.0118
GO:0045073	Regulation of the chemokine biosynthetic process	0.0202
GO:0032940	Secretion by cell	0.0220
GO:0044130	Negative regulation of the growth of symbionts in the host	0.0220
GO:0050829	Defense response to Gram-negative bacterium	0.0238
GO:0045055	Regulated exocytosis	0.0254
GO:0042108	Positive regulation of cytokine biosynthetic process	0.0360
GO:0002443	Leukocyte-mediated immunity	0.0422
GO:0050776	Regulation of immune response	0.0491

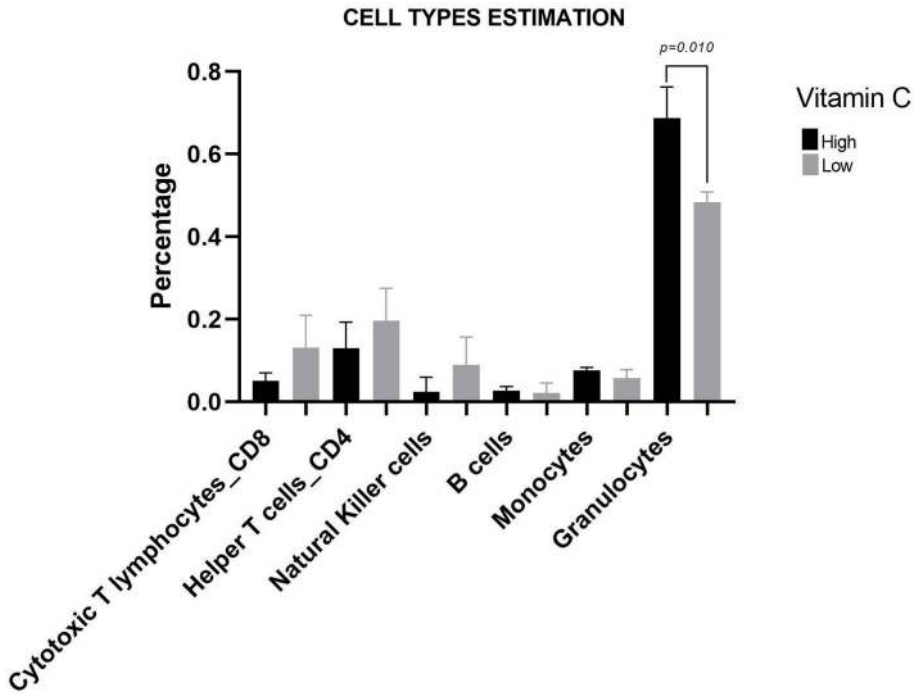


FIGURE 3
Cell type estimation graph based on the Houseman method. Results are presented as the mean, and the bars represent the standard deviation. *p*: *p*-value.

functions of the innate and adaptive immune systems. The role of ascorbate as a critical mediator by participating in epigenetic demethylation, as well as any genetic and environmental factors, such as gut microbiota, that influence ascorbate synthesis, absorption, transportation, and metabolism, could have significant consequences for the genome by modulating epigenetic control and consequences in health to control diseases (21). In addition, vitamin C serves as an essential cofactor for several monooxygenases and dioxygenases, including the 10–11 translocation (TET) family of enzymes, which participate in the process of DNA demethylation (22). Actinobacteria are Gram-positive and include three main anaerobe families (Bifidobacteria, Propionibacteria, and Corynebacteria) and one aerobe family (Streptomyces), with Bifidobacteria being the most common in the human gut. The Actinobacteria phylum represents a minority group of commensal bacteria but plays a role in the development and maintenance of gut homeostasis. Although it has been largely demonstrated that diet strongly influences gut microbiota composition, the relationship between diet and Actinobacteria is still unclear (23). Actinobacteria also modulate immune-inflammatory responses by inducing regulatory T-cells, and an unbalanced abundance has been observed in several pathological conditions (23). Indeed, there is growing evidence that bacterial phyla can exert a role in epigenetic regulation (3) in the pathways of the immune system and cell signaling.

Another study found an increase in Actinobacteria and Firmicutes after the administration of vitamin D (4), suggesting that the administration of vitamin D can modulate intestinal bacterial composition and increase the abundance of potentially

beneficial bacteria. Among the DNA methylation sites that were commonly associated with Actinobacteria and vitamin C consumption, it was observed that the immune response was the most important biological process in this dataset. The hypomethylation in the promoter regions in individuals with higher consumption of vitamin C may alter the pathways of the immune system process. Interestingly, the pathways of functional enrichment from hypermethylated genes are involved in the negative regulation of symbiont growth in the host, and neutrophil-mediated killing of bacteria. This aligns with the observation that the group with high vitamin C may modulate an abundance of Actinobacteria essential for maintaining a healthy gut microbiota and a balanced immune system.

Inhibition of DNA and histone methylation further promotes the effect of ascorbate on T-cell differentiation, which suggests a role for ascorbate in the active demethylation of DNA and histone in T-cell maturation (23). As in our results, the group with higher ingestion of vitamin C had pathways to immune function, signaling, and hypomethylation of degranulation. It was also reported to be involved in the differentiation of Th1 and Th17, as well as cytokine gene expression and CD8+ T cell regulation (24). HVC levels had a lower expression of *TNF-α*, *TLR4*, *NFKB,* and *IL-6* markers. According to a review that showed vitamin C in peripheral blood, monocytes decreased the generation of the pro-inflammatory cytokines *TNF-α* and *IL-6*. Furthermore, vitamin C has been shown to attenuate *NFKB* activation in neutrophils. Cytokines are related to pro- and anti-inflammatory responses, and vitamin C can modulate this complex (25). Toll-like receptor 4 (TLR4) is a pro-inflammatory group characterized by AP-1 and NFKB activation. Immediate response by the innate immune system requires class *PI3K* activation (26, 27), which aligns with our results due to the increase in PI3K and the activation of the innate immune response in the HVC group. The PI3K/AKT signaling pathway regulates glucose metabolism, facilitates glycolysis, and inhibits gluconeogenesis. This pathway directly influences glucose metabolism by phosphorylating various metabolic enzymes and transport regulators (28). In the present study, the gene expression of this target and the functional enrichment retrieved two GO terms related to the PI3K pathway; however, the literature does not have enough publications to discuss this finding.

We found a positive correlation between vitamin C and granulocytes. It has already been shown that a high-ascorbate diet increases circulating granulocyte numbers in peripheral blood (29). Granulocytes have a role in secondary infections and in the host defense mechanisms of polymicrobial infection (30). Therefore, vitamin C may change the immune response by increasing granulocytes and improving the response against secondary infections.

The limitations of this study are about sample size and no serology test was performed to measure vitamin C. The 24-h recall was used for the daily consumption analysis of vitamin C. This tool is often neglected in research. However, it is widely used in clinical practice. Therefore, it is possible to assess the intake of micronutrients and macronutrients through 24-h recall, an essential database tool. In conclusion, modulating the microbiota through the consumption of vitamin C can alter methylation patterns responsible for immune responses. Those who ingest more vitamin C seem to have hypermethylated genes related to the innate immune response.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/, GSE254646.

Ethics statement

The studies involving humans were approved by the Ethics Committee of Ribeirão Preto Medical School at the University of São Paulo, Brazil (process CAAE: 14275319.7.0000.5440). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

NN: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. IN: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. RF: Conceptualization, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. GR: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. LM: Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing. LW: Conceptualization, Data curation, Investigation, Supervision, Writing – original draft, Writing – review & editing. MP: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. IM: Conceptualization, Methodology, Writing – original draft, Writing – review & editing LD: Data curation, Methodology, Writing – review & editing. DC: Funding acquisition, Project administration, Resources, Writing – original draft, Writing – review & editing. CN: Funding acquisition, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano G, Gasbarrini A, et al. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms*. (2019) 7:14. doi: 10.3390/microorganisms7010014

2. Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. *Nat Rev Immunol*. (2016) 16:341–52. doi: 10.1038/nri.2016.42

3. D'Aquila P, Lynn Carelli L, De Rango F, Passarino G, Bellizzi D. Gut microbiota as important mediator between diet and DNA methylation and histone modifications in the host. *Nutrients*. (2020) 12:597. doi: 10.3390/nu12030597

4. Yang L, Balakrishnan B, Feng Z. Role of dietary nutrients in the modulation of gut microbiota: a narrative review. *Nutrients*. (2020) 12:381. doi: 10.3390/nu12020381

5. Cortes-Oliveira C, Nicoletti CF, de Souza Pinhel MA, de Oliveira BAP, Quinhoneiro DCG, Noronha NY, et al. UCP2 expression is associated with weight loss after hypocaloric diet intervention. *Eur J Clin Nutr*. (2017) 71:402–6. doi: 10.1038/ejcn.2016.185

6. Ip SC, Lin SW, Lai KM. An evaluation of the performance of five extraction methods: Chelex® 100, QIAamp® DNA blood Mini kit, QIAamp® DNA investigator kit, QIAsymphony® DNA investigator® kit and DNA IQ™. *Sci Justice*. (2015) 55:200–8. doi: 10.1016/j.scijus.2015.01.005

7. Bowden A, Fleming R, Harbison S. A method for DNA and RNA co-extraction for use on forensic samples using the Promega DNA IQ™ system. *Forensic Sci Int Genet*. (2011) 5:64–8. doi: 10.1016/j.fsigen.2009.11.007

8. Touleimat N, Tost J. Complete pipeline for Infinium ® human methylation 450K BeadChip data processing using subset quantile normalization for accurate DNA methylation estimation. *Epigenomics*. (2012) 4:325–41. doi: 10.2217/epi.12.21

9. Noronha NY, da Silva RG, de Souza Pinhel MA, Cazier JB, Watanabe LM, Menezes AN, et al. Sample preparation to bioinformatics analysis of DNA methylation: association strategy for obesity and related trait studies. *J Vis Exp*. (2022) 183:62598. doi: 10.3791/62598

10. Muller RL, Gerber L, Moreira DM, Andriole G, Hamilton RJ, Fleshner N, et al. Obesity is associated with increased prostate growth and attenuated prostate volume reduction by Dutasteride. *Eur Urol*. (2013) 63:1115–21. doi: 10.1016/j.eururo.2013.02.038

11. Tian Y, Morris TJ, Webster AP, Yang Z, Beck S, Feber A, et al. ChAMP: updated methylation analysis pipeline for Illumina BeadChips. Valencia a, editor. *Bioinformatics*. (2017) 33:3982–4. doi: 10.1093/bioinformatics/btx513

12. Morris TJ, Beck S. Analysis pipelines and packages for Infinium HumanMethylation450 BeadChip (450k) data. *Methods*. (2015) 72:3–8. doi: 10.1016/j.ymeth.2014.08.011

13. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res*. (2019) 47:D607–13. doi: 10.1093/nar/gky1131

14. Donadelli SP, Junqueira-Franco MVM, de Mattos Donadelli CA, Salgado W, Ceneviva R, Marchini JS, et al. Daily vitamin supplementation and hypovitaminosis after obesity surgery. *Nutrition*. (2012) 28:391–6. doi: 10.1016/j.nut.2011.07.012

15. Institute of Medicine (US). *Panel on dietary antioxidants and related compounds. Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids*. Washington, DC: National Academies Press (US) (2000).

16. Shrestha R, Upadhyay SK, Khatri B, Bhattarai JR, Kayastha M, Upadhyay MP. BMI, waist to height ratio and waist circumference as a screening tool for hypertension in hospital outpatients: a cross-sectional, non-inferiority study. *BMJ Open*. (2021) 11:e050096. doi: 10.1136/bmjopen-2021-050096

17. Branco BHM, Bernuci MP, Marques DC, Carvalho IZ, Barrero CAL, Oliveira FM de, et al. Proposal of a normative table for body fat percentages of Brazilian young adults through bioimpedanciometry. *J Exerc Rehabil* (2018) 14:974–979. doi: 10.12965/jer.1836400.200

18. Rexrode KM. Abdominal adiposity and coronary heart disease in women. *JAMA*. (1998) 280:1843. doi: 10.1001/jama.280.21.1843

19. Szwarcwald CL, Malta DC, Pereira CA, Figueiredo AW, Almeida, Machado IE, et al., Valores de referência para exames laboratoriais de colesterol, hemoglobina glicosilada e creatinina da população adulta brasileira. *Rev Bras Epidemiol* (2019);22, doi: 10.1590/1980-549720190002.supl.2,;e190002.supl.2

20. Xu J, Xu C, Chen X, Cai X, Yang S, Sheng Y, et al. Regulation of an antioxidant blend on intestinal redox status and major microbiota in early weaned piglets. *Nutrition*. (2014) 30:584–9. doi: 10.1016/j.nut.2013.10.018

21. Young JI, Züchner S, Wang G. Regulation of the epigenome by vitamin C. *Annu Rev Nutr*. (2015) 35:545–64. doi: 10.1146/annurev-nutr-071714-034228

22. Xue JH, Chen GD, Hao F, Chen H, Fang Z, Chen FF, et al. A vitamin-C-derived DNA modification catalysed by an algal TET homologue. *Nature*. (2019) 569:581–5. doi: 10.1038/s41586-019-1160-0

23. Binda C, Lopetuso LR, Rizzatti G, Gibiino G, Cennamo V, Gasbarrini A. Actinobacteria: a relevant minority for the maintenance of gut homeostasis. *Dig Liver Dis*. (2018) 50:421–8. doi: 10.1016/j.dld.2018.02.012

24. Ang A, Pullar JM, Currie MJ, Vissers MCM. Vitamin C and immune cell function in inflammation and cancer. *Biochem Soc Trans*. (2018) 46:1147–59. doi: 10.1042/BST20180169

25. Carr A, Maggini S. Vitamin C and immune function. *Nutrients*. (2017) 9:1211. doi: 10.3390/nu9111211

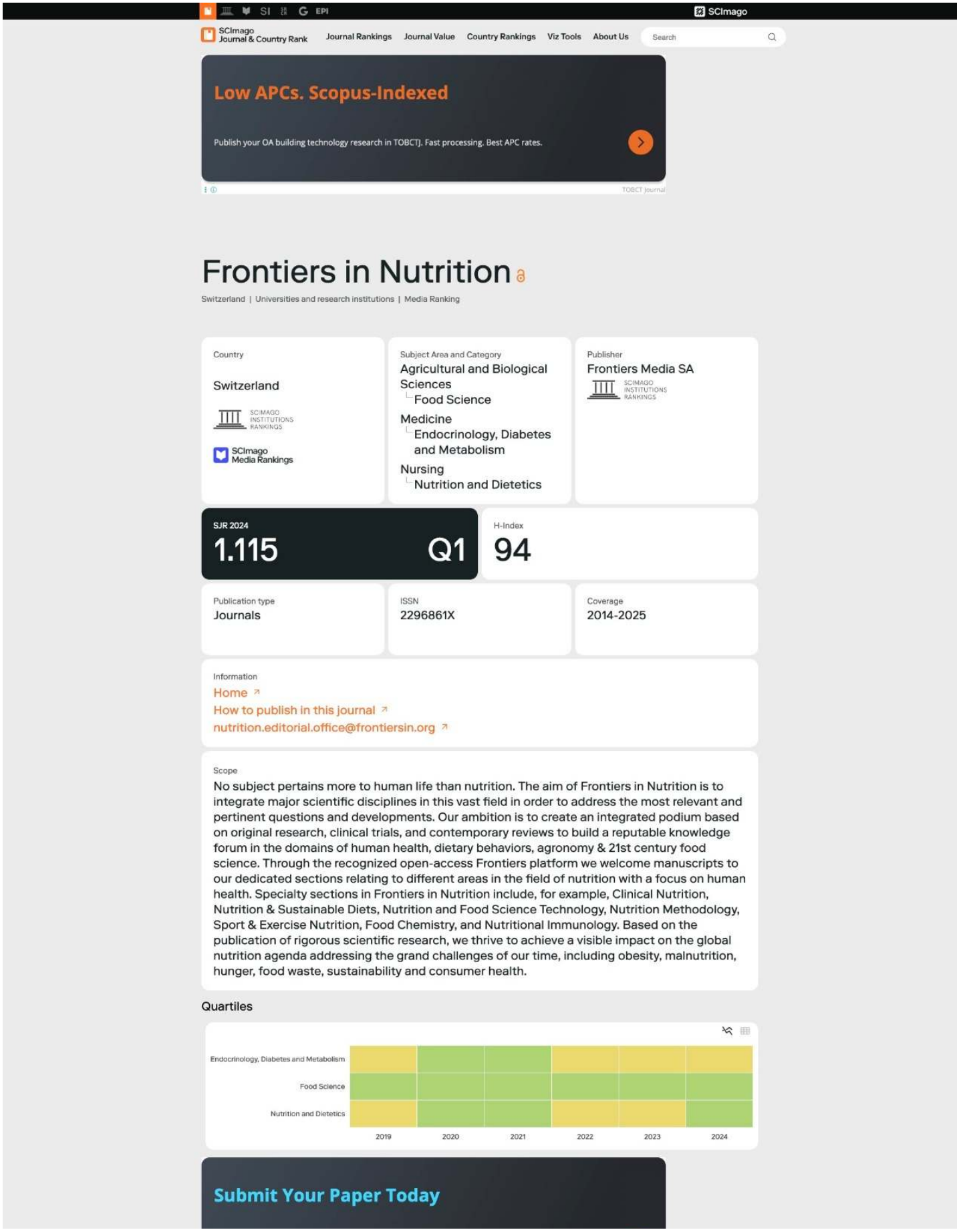
26. Hawkins PT, Stephens LR. PI3K signalling in inflammation. *Biochim Biophys Acta BBA*. (2015) 1851:882–97. doi: 10.1016/j.bbalip.2014.12.006

27. Fruman DA, Chiu H, Hopkins BD, Bagrodia S, Cantley LC, Abraham RT. The PI3K pathway in human disease. *Cell*. (2017) 170:605–35. doi: 10.1016/j.cell.2017.07.029

28. Wang W, Shi B, Cong R, Hao M, Peng Y, Yang H, et al. RING-finger E3 ligases regulatory network in PI3K/AKT-mediated glucose metabolism. *Cell Death Discov*. (2022) 8:372. doi: 10.1038/s41420-022-01162-7

29. Uchio R, Hirose Y, Murosaki S, Yamamoto Y, Ishigami A. High dietary intake of vitamin C suppresses age-related thymic atrophy and contributes to the maintenance of immune cells in vitamin C-deficient senescence marker protein-30 knockout mice. *Br J Nutr*. (2015) 113:603–9. doi: 10.1017/S0007114514003857

30. Wang B, Zhu L, Jia B, Zhao C, Zhang J, Li F, et al. Sepsis induces non-classic innate immune memory in granulocytes. *Cell Rep*. (2023) 42:113044. doi: 10.1016/j.celrep.2023.113044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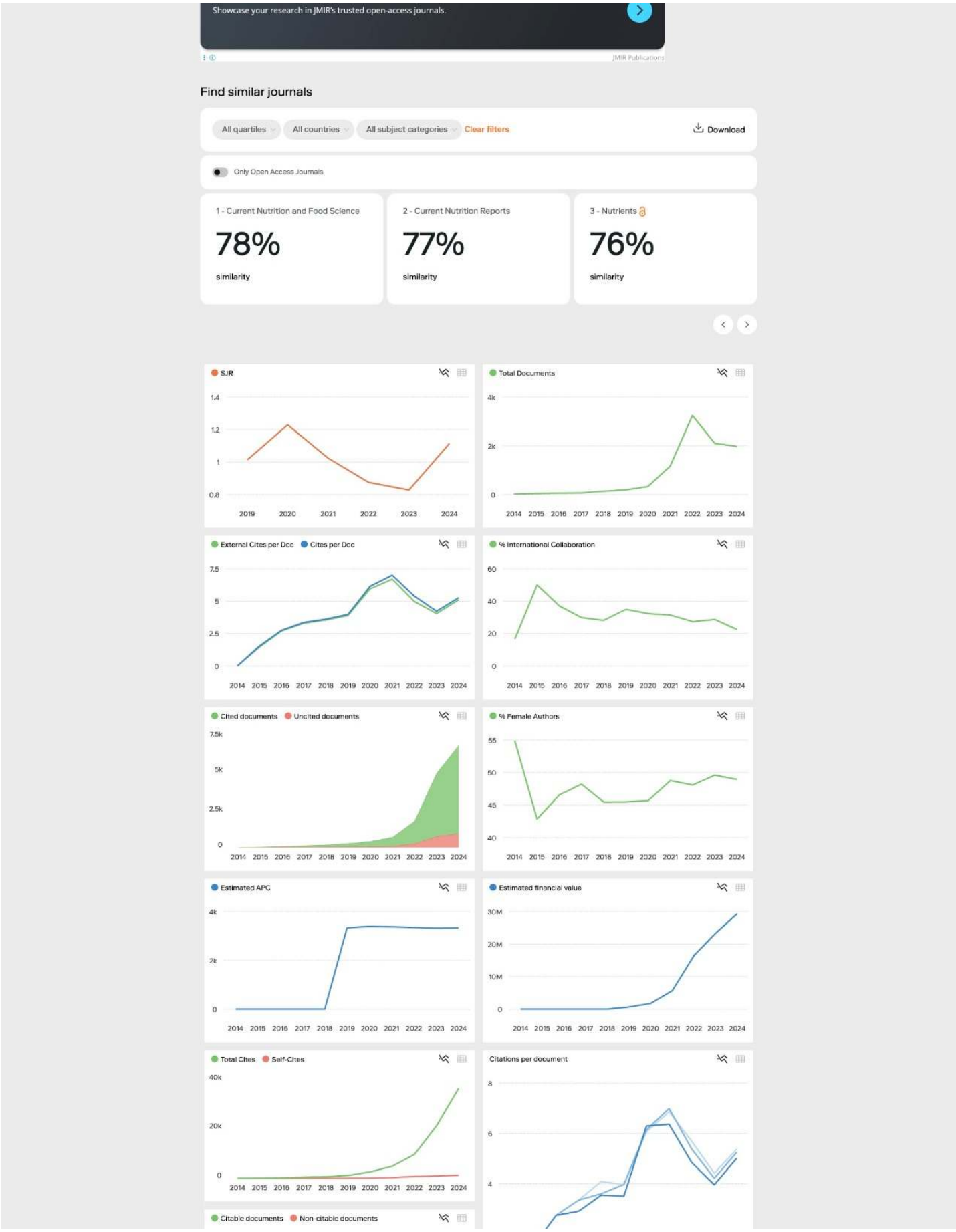
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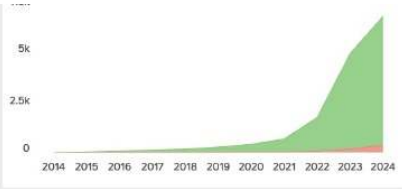
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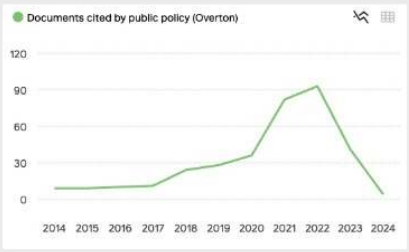
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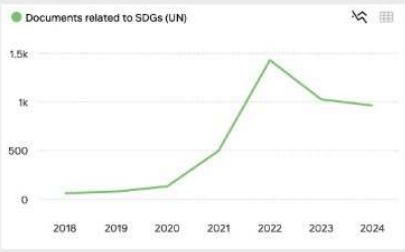
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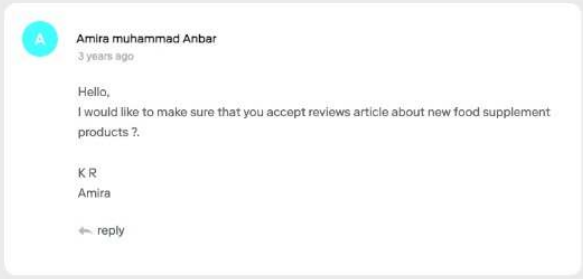
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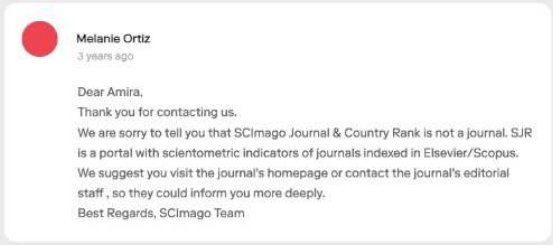
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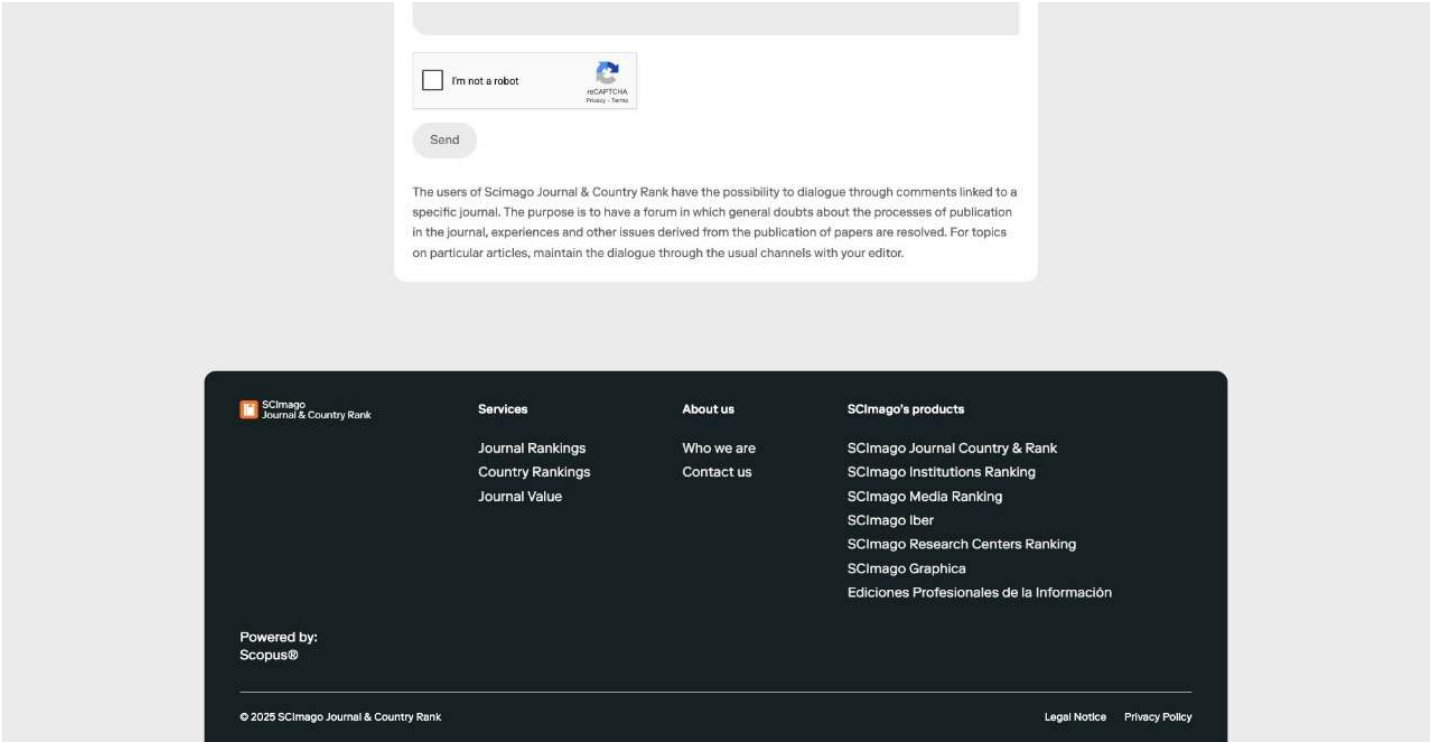
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E. 16. Evidence that the beneficiary is an author of scholarly articles in the field of science published in professional media

Maringa, Brazil, September 08, 2025

Beneficiary: Dr. Isabella Harumi Yonehara Noma

Form I-140: Petition for EB-1A Classification

To whom it may concern,

I am writing in enthusiastic support of an application by Dr. Isabella Harumi Yonehara Noma for an EB-1 visa, and to attest to her outstanding reputation as a scientist and researcher of excellence in the field of Melanoma, Molecular biology.

I am currently Associate Professor C in the Department of Clinical Analysis and Biomedicine at State University of Maringa (UEM). Current coordinator of the Graduate Program in Biosciences and Pathophysiology/UEM (Pharmacy/CAPES) and also from 2012-2016. Director of Graduate Studies at UEM from 2018-2022. Principal researcher in CNPq research groups, editor and reviewer of scientific journals. Coordinator of national and international projects funded by CNPq, CAPES, Fundacao Araucaria, DECIT/MS, National Institute of Health/NIH-USA, UK RESEARCH AND INNOVATION-UKRI, Medecins Sans Frontieres Operational Center Barcelona/MSF-OPB and Oxford University. I had supervised more than 100 students in their CBT, IC, master's, doctorate and post-doctorate studies and has published at least 131 scientific papers. I have experience in Pharmacy, with an emphasis on HPV and cervical cancer; sexually transmitted infections; and arboviruses.

I supervised Dr. Noma in her Master's degree, and she worked with HPV and infections. She produced her dissertation, and an article entitled: "Association of High-Risk Human Papillomavirus and Ureaplasma parvum Co-Infections with Increased Risk of Low-Grade Squamous Intraepithelial Cervical Lesions." Her work was an original contribution to the field of Melanoma and Molecular Biology, showing how high-risk HPV and other infections are able to increase the risk of low-grade squamous cervical lesions, with an important contribution in cervical cancer. Interestingly, this study is the first to be published about this relationship between HPV and other infections in cervical cancer. Moreover, this study had citations that impacted recent publications in the area in Frontiers, and MDPI journals, important to share knowledge.

Based on my long scientific career, and my vast experience in evaluating fellowships, grant applications, faculty candidates, graduate students' dissertations, and the research publications, I feel very confident in my ability to

evaluate Dr. Noma's scientific skills and judge her contributions to the field of Molecular biology and Cancer.

I became acquainted with Dr. Noma when she was a graduate student and Master candidate at the State University of Maringa. She performed the experimental work for her dissertation under my supervision, in the Health Science Program, at the Health Science Center. During that time, she utilized very creative approaches to conducting research and actively participated in the organization of multiple institutional activities, including the VI International Meeting of Biosciences and Physiopathology. After she graduated, she was recruited for her PhD to come to University of Sao Paulo to join in the School of Pharmaceutical Sciences, led by cancer famous researcher Dr. Silvya Stuchi, as well-known in her area in Brazil and other countries.

Unlike most researchers, her scientific and technical skills span the whole spectrum of research from basic fundamental biology to applied research. Owing to her training in multidisciplinary environments, Dr. Noma has shown the ability to integrate concepts of synthetic biology, molecular biology, systems biology, and oncology in the design and development of novel insights to therapeutic approaches for the treatment of human cancers.

Later in her career, she was awarded the prestigious grants from her project about PRDX2 and melanoma had high translational impact in the field of oncology in the wellknown congress in Melanoma area called Congress from the Society for Melanoma Research. Her writing and analytic skills are excellent.

In summary, Dr. Nomas's contributions to the cancer gene therapy communities are of significant relevance. She has extraordinary abilities and skills in developing new methodologies as well as applying previously described approaches. These characteristics, along with her remarkable communication skills, passion for science, and her appreciation of diversity, make her a valuable asset for the US scientific community. Without question, Dr. Noma has proven herself as an outstanding scientific researcher and I have no doubt that she will continue to make significant contributions to the cancer field and in so doing, benefit US public health.

I, therefore, strongly and unreservedly endorse Dr. Isabella's EB-1 visa petition to remain contributing substantially to her field in the United States. Please do not hesitate to contact me to confirm Dr. Isabella's credentials and qualifications.

Best Regards,

_____
Marcia Edilaine Lopes Consolaro, PhD
Associate Professor – Department of Clinical Analysis and Biomedicine
State University of Maringa (UEM)
Av. Colombo, 5790, 87020-900 Maringa, Parana, Brazil
Email: melconsolaro@gmail.com
Phone Number: +55 44 3011-5455)

F. Evidence of the display of the Petitioner’s work in the field in Public Exhibitions

CERTIFICATE *of* ATTENDANCE

THIS ACKNOWLEDGES THAT

ISABELLA HARUMI YONEHARA NOMA

HAS ATTENDED AND PRESENTED A POSTER ABSTRACT

At the

19th International Congress of the Society for Melanoma Research

OCTOBER, 17-20
2022

SIGNED Ashani Weeraratna, President
Society for Melanoma Research

ABSTRACTS

19th International Congress of the Society for Melanoma Research

Edinburgh, Scotland October 17-20, 2022

Oxidative metabolism contributes to tumor heterogeneity and BRAFi resistance in melanoma

Larissa A. Carvalho[1]; Isabella H. Noma[1]; Adriana Hiromi Uhera[1]; Ádamo Davi Diógenes Siena[1]; Luciana Harumi[1]; Matheus Prates Mori[1]; Nadja Cristhina de Souza Pinto[1]; Vanessa Morais Freitas[1]; Wilson Araújo Silva Junior[1]; Keiran Smalley[2]; Silvya S. Maria-Engler[1]
[1]*University of São Paulo, São Paulo, São Paulo, Brazil;* [2]*Moffitt Cancer Center, Tampa, Florida, United States*

Microenvironment and transcriptional plasticity propitiate distinct cell states within the tumor and the use of BRAFi contributes to the selection of resistant cells. One of the causes of therapy failure is tolerance to oxidative stress (OS), hydrogen peroxide (H_2O_2) is involved in all hallmarks of cancer and increases the expression of NRF2, a regulator of antioxidant response that suppresses the activity of MITF, the key of phenotype switching. The $MITF^{low}/AXL^{high}$ phenotype is linked to resistance and MITF controls PGC-1α, responsible for mitochondrial biogenesis and antioxidant proteins expression. BRAF mutation suppresses MITF, PGC-1α, OXPHOS and ROS, while BRAFi increases it. In this context, PRDXs are the main consumers of H_2O_2 acting as central players in redox signaling, and the role of PRDXs and OS in heterogeneity is poorly explored in melanoma. We stochastically isolated clonal subpopulations (C1, C2 and C3) from naïve melanoma (SK-MEL-28) and demonstrated that: C1 is less proliferative, more migratory and invasive, less sensitive to BRAFi, less dependent on OXPHOS and more sensitive to OS; C2 is more proliferative, less migratory and invasive, more sensitive to BRAFi and less sensitive to OS; and C3 is less proliferative, more migratory and invasive, less sensitive to BRAFi, more dependent on OXPHOS and more sensitive to OS. The intrinsically resistant clones C1 and C3 have lower MITF, Melan-A, PGC-1α, PRDX1 and C1 showed no pigmentation, higher NRF2, β-catenin and AXL, linking PRDX1 to clonal heterogeneity, intrinsic and acquired BRAFi resistance. PRDX2 expression is maintained among the clones, but acts as ROS sensor and is depleted in acquired BRAFi resistant cells. Besides, the PRDX mimetic gliotoxin was able to overcome BRAFi resistance. Our results illustrate new roles of PRDXs and redox sensitive reactions as contributors of heterogeneity and resistance.

Differential response of human melanocyte subpopulations and melanoma cells (eumelanic/pheomelanic/amelanotic) to UVB irradiation

Cristina Casalou[1]; Jay Mayatra[1]; Shosuke Ito[3]; Kazumasa Wakamatsu[2]; Desmond Tobin[1,3]
[1]*The Charles Institute of Dermatology, university College Dublin, Dublin, Dublin4, Ireland;* [2]*Institute for Melanin Chemistry, Fujita Health University, Aichi, Japan;* [3]*Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Dublin, Dublin4, Ireland*

Melanoma is a highly heterogeneous cancer, underpinned in part by its exceptional mutational burden, making this a very difficult tumor to treat. Thus, identification of earliest melanoma-genesis molecular alterations (beyond simply driver mutation) is needed.

This study investigated the differentiation and apoptosis status of primary human epidermal melanocytes (HEM; SPTI/II) and selected melanoma cell lines (FM3 [amelanotic], FM55P/FM55M, SK-MEL-23) after UVB irradiation (300 mJ/cm^2). Melanoma cell lines were included based on their melanin level and subtype (eu/pheomelanin), as determined by HPLC assay: SK-MEL-23: 12% eumelanin, 88% pheomelanin; FM55M: 89% eumelanin, 11% pheomelanin.

We confirmed the existence of multiple HEM subpopulations in adult human epidermis, by immunohistochemistry, using the transcription factors MITF and Sox10, as well as c-Kit and CD90. We speculate that HEM subpopulations, defined by variability in their expression level and subcellular localization of the above markers may indicate their differential susceptibility to oncogenic transformation. MITF, the master regulator of melanocyte survival, differentiation, and proliferation, is also a key determinant of melanoma behavior. Its highest baseline expression (by western blotting) was in pheomelanin-rich SK-MEL-23 cells, closely followed by amelanotic FM3. Eumelanic FM55P (primary) and FM55M (metastatic) melanoma cells showed low expression. UVB irradiation markedly downregulated total MITF protein expression in HEM and all melanoma cell lines tested. By contrast, UVB irradiation increased $p21^{WAF1}$ protein expression in primary HEM and primary melanoma (FM55P) cells, and concomitantly decreased expression of the anti-apoptotic marker Bcl_2, suggesting an attempt to limit cell cycle dysregulation in these pigment cells.

Dystroglycan receptor pathway in melanoma progression

Israel Castillo Gonzalez[1]; Gabriela Rapozo Guimarães[2]; Sophia Gandarillas[1]; Erasmo Perera[1]; Mariana Boroni[2]; Lidia Kos[1]
[1]*Biology, Florida International University, Miami, Florida, United States;* [2]*Laboratório de Bioinformática e Biologia Computacional, Instituto Nacional do Cancer, Sao Paulo, Brazil*

The interplay of tumor cells with the extracellular matrix in the basement membrane (BME) is pivotal in several steps of melanoma progression. Laminin in the BME binds to the alpha subunit of Dystroglycan (DG) receptor complex, which is highly glycosylated by a myriad of glycosyltransferases such as POMT1, FKTN, and Large1. Mutations of α-DG and its associated glycosyltransferases have been linked to muscular dystrophy, but little is known about their relevancy in melanoma progression. Bioinformatics analysis to inquire the implication of mutations in *DAG1* (DG receptor gene) and its associated genes in cutaneous melanoma patients indicated that patients bearing *POMT1* mutations had a significantly lower median month survival (27 months) compared to those with no *POMT1* mutations (61 months). In addition, high expression levels of *POMT1* and *FKTN* negatively impacted melanoma patient overall survival. Analysis of public single-cell gene expression data of melanoma and melanocyte samples showed that the malignant cell population had

Society for Melanoma Research - About

societymelanomaresearch.org/about

About the SMR

Overview

The Society for Melanoma Research's principal goal is bringing together members who vary widely in their professions—from basic researchers to translational researchers to clinicians. Each shares an abiding devotion to improving the lives of those suffering from melanoma through research.

In coming years, with the incorporation of new technologies into experimental and clinical research, we expect rapid advances in cutaneous melanoma prevention, diagnosis and therapy. To bring new technology-based discoveries from bench to bedside and back, we will need to attain a far greater level of cooperation between labs and clinics. In addition, we expect to develop specific inhibitors for metastatic melanoma, rendering it in the next few years a treatable disease.

However, we face major challenges in securing funding, building infrastructure, and gaining expertise in new technologies. To meet these challenges, we will need to form multidisciplinary collaborations. SMR, through yearly congresses, workshops, and its website, will play the role of a catalyst.

About the Society for Melanoma Research

The Society for Melanoma Research (SMR) is an all-volunteer group of scientists working to find the mechanisms responsible for melanoma and, consequently, new therapies for this cancer. SMR contributes to advances in melanoma research by bringing together researchers in a non-competitive way to unite the scientific community.

About Melanoma

Melanoma, the most serious type of skin cancer, is one of the fastest growing cancers in the U.S., and can strike people of all ages, all races and both sexes. Melanoma is the most common form of cancer for young adults 25 to 29-years-old and the second most common cancer in adolescents and young adults 15 to 29-years-old.

The Society for Melanoma Research

Advancement Through Collaboration

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Congress Home

SMR Congress

Abstracts

The 2019 Society for Melanoma Research International Congress provides the opportunity to present and/or view innovative data related to clinical and basic melanoma research and to network with an international audience representing a diverse spectrum of expertise.

This year's congress will host many proffered papers from a broad range of different topics, thereby increasing the opportunity to have your work in the spotlight and share it with the global clinical and research community.

Participants interested in presenting data should submit abstracts online. Abstract submission will begin spring, 2019. SMR will also notify everyone once the submission site is open. The Scientific Review Committee will select abstracts for oral and poster presentation. Once accepted, all abstracts will be published in Pigment Cell and Melanoma Research.

Click here to begin your abstract submission.

Click here to view all abstracts from the 2018 Congress.

Click here to view the late breaking abstracts from the 2018 Congress.

Key Dates:

- May 1st – Abstract Submissions Open
- August 9th – Abstract Submissions Close
- August 12th – Late Breaking Abstract Submissions Open
- September 17th – Abstract Notification
- November 1st – Late Breaking Abstract Submission Closes

FAQ's:

- Q: Are placeholders or abstract shells required for late-breaking abstracts?
  - A: No, shells are not able to be submitted in advance. The late breaking abstract site will open on July 30th.
- Q: Are trials in progress (TIP) abstracts accepted?
  - A: Yes
- Q: Are encore abstracts that have been previously presented/published accepted?
  - A: Yes, however it is not preferred.

If you have any questions or need assistance regarding your abstract, please contact Marissa Streun at 518-399-7181 or at Marissa@sswmeetings.com.

Abstract Guidelines

- Follow submission instructions provided at https://melanomacongress2019.abstractcentral.com/login Link needed
- Abstract submission is free of charge and must be completed online.
- Abstracts submitted by e-mail, post or fax will not be accepted.
- Abstracts must be written in English, the official language of the congress.
- List affiliations of author(s) as briefly as possible with city, state (if applicable), and country.
- Use standard and well-accepted abbreviations only.
- You will need to indicate the name of the presenting author at the congress (This is the person who will present the oral or poster presentation during scheduled times if accepted.)
- Upon acceptance, your abstract will be published in the PCMR journal the exact way it is entered into your submission. Please be sure all information is entered correctly (i.e. capitalizations, spelling, grammar, etc.)
- Abstract content should be single-spaced and typed in lower case. You have up to 2000 characters (counting spaces) that include the title and abstract body (does not include authors and affiliations). Please do NOT separate the section with sub headers (ie. Method, Results).
- The following list corresponds with the session topics slated to be presented at the SMR Congress. Abstracts are to be submitted based on the following categories:
  - Drug Resistance
  - Epigenetics and Metabolism
  - Genetics and Genomics
  - Immunotherapy
  - Melanoma and Melanocyte Biology
  - Targeted Therapy

- Tumor Microenvironment

Deadline for abstract submissions is August 9, 2019!
We look forward to receiving your submission!

Abstract Regulations and Instructions

1. Before submitting your abstract, please be sure to read the abstract submission guidelines.
2. Incomplete applications will not be considered.
3. The first / submitting author agrees to / undertakes to:
   a. Release full copyright to SMR and give full permission for the abstract, if accepted, to be published in the SMR 2019 Abstract book in printed and/or electronic format, as well as published online on at www.wiley.com.
   b. Warrant that he/she is an investigator with a substantial involvement in the study presented in the abstract.
   c. Confirm that all authors are aware of, and agree to, the content of the abstract and support the data therein; furthermore the presenting author will be responsible for obtaining, and, upon request, showing proof that all authors have given their permission for the submission of the abstract and content therein as well as for the potential inclusion of the abstract in the official SMR 2019 Congress Program.
   d. Upon acceptance, your abstract will be published in the PCMR journal the exact way it is entered into your submission. Please be sure all information is entered correctly (i.e. capitalizations, spelling, grammar, etc.)
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   f. Acknowledge any editorial assistance on the printed poster or oral presentation (not in the abstract body) in the writing of the abstract that was provided by a third party, providing the name of the writer and the company who provided this service.
   g. Ensure that for all studies involving human or animal subjects, permission has been obtained from the relevant regulatory authority and that properly informed consent, where appropriate, has been given.

Withdrawal of abstracts

If the submitting author wishes to withdraw his/her abstract from SMR 2019 after outcome notifications have been made available, he/she must submit a written request within 48 hours to Marissa@SSWMeetings.com. Any abstract withdrawal requests made after 48 hours from notification cannot be assured of removal from the 2019 SMR Congress Abstract Book.

Congress Registration

The designated presenting author of any abstracts accepted will be required to register for the Congress by the early registration deadline of Friday, October 18 and are responsible for their own travel arrangements.

Publication Schedule

Abstracts accepted for presentation (either Proffered Paper/Oral presentation or Poster Presentation) will be published in the Congress Abstract Book.

Accepted abstracts submitted by July 29, 2019 will be published in the Abstract Book section of "Pigment Cell & Melanoma Research", scheduled for an January 2020 release date. **Please note your abstract will be published exactly as it is submitted on-line.** Please ensure accuracy of your submitted abstract on or before the deadline of July 29, 2019 at 11:59 p.m. EST. Please do not create a new submission for abstracts requiring edits or completion.

Accepted Abstracts should not be published online or in print prior to the beginning of the official Congress session during in which they are presented.

Media & Embargo Policy

- Authors may disclose or announce that their abstract has been accepted for presentation at the congress, including announcements via press release.
- Abstracts will be available online. As soon as they are available, disclosure or announcement of the data published in the abstract is fully permissible.
- A press release describing the full details of the presentation can be timed for release on the day of presentation at the congress, not prior.
- If you have any further questions, please contact Marissa Streun Marissa@sswmeetings.com 518-399-7181.

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UNIVERSITY OF SÃO PAULO
SCHOOL OF PHARMACEUTICAL SCIENCES

SYMPOSIUM CELEBRATING THE
50TH ANNIVERSARY OF THE GRADUATE PROGRAM
IN PHARMACY – PATHOPHYSIOLOGY AND TOXICOLOGY
FCE/USP

CERTIFICATE – Honorable mention

We hereby certify that the author **Isabella Harumi Yonehara Noma**, with the poster titled "Targeting Peroxiredoxin-2 as a potential treatment for NRAS-mutated metastatic melanoma," under the supervision of Prof. Dr. Silvya Stuchi Maria Engler, received an award in the poster presentation category during the **Symposium celebrating the 50th anniversary of the Graduate Program in Pharmacy – Pathophysiology and Toxicology** of FCE/USP, held on 11/30/2022, receiving **Honorable Mention.**

-----// signature //-----

Prof. Dr. Sandra Helena Poliselli Farsky
Coordinator of the Graduate Program in Pharmacy
(Pathophysiology and Toxicology)

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I, Raphael Michels Fantinato de Moura, telephone number 415 425-2508, mailing address P.O. Box 90487, San Diego, CA 92169, certify that the professional translation of this document from Portuguese to English has been performed by myself, a qualified translator fluent in both languages, and that the following is an accurate and complete translation of the document.

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SIMPÓSIO EM COMEMORAÇÃO DOS 50 ANOS DO PROGRAMA DE PÓS-GRADUAÇÃO EM FARMÁCIA - FISIOPATOLOGIA E TOXICOLOGIA

FCF/USP

CERTIFICADO – menção honrosa

Certificamos que a autora **Isabella Harumi Yonehara Noma** com o pôster "*Targeting Peroxiredoxin-2 as a potential treatment for NRAS-mutated metastatic melanoma*", sob a orientação da Profa. Dra. Silvya Stuchi Maria Engler foi premiada na modalidade apresentação de pôster durante o **Simpósio em comemoração dos 50 anos do Programa de Pós-Graduação em Farmácia – Fisiopatologia e Toxicologia** da FCF/USP realizada em 30/11/2022, obtendo **menção honrosa.**

Profa. Dra. Sandra Helena Poliselli Farsky
Coordenadora do Programa de Pós-Graduação em Farmácia (Fisiopatologia e Toxicologia)

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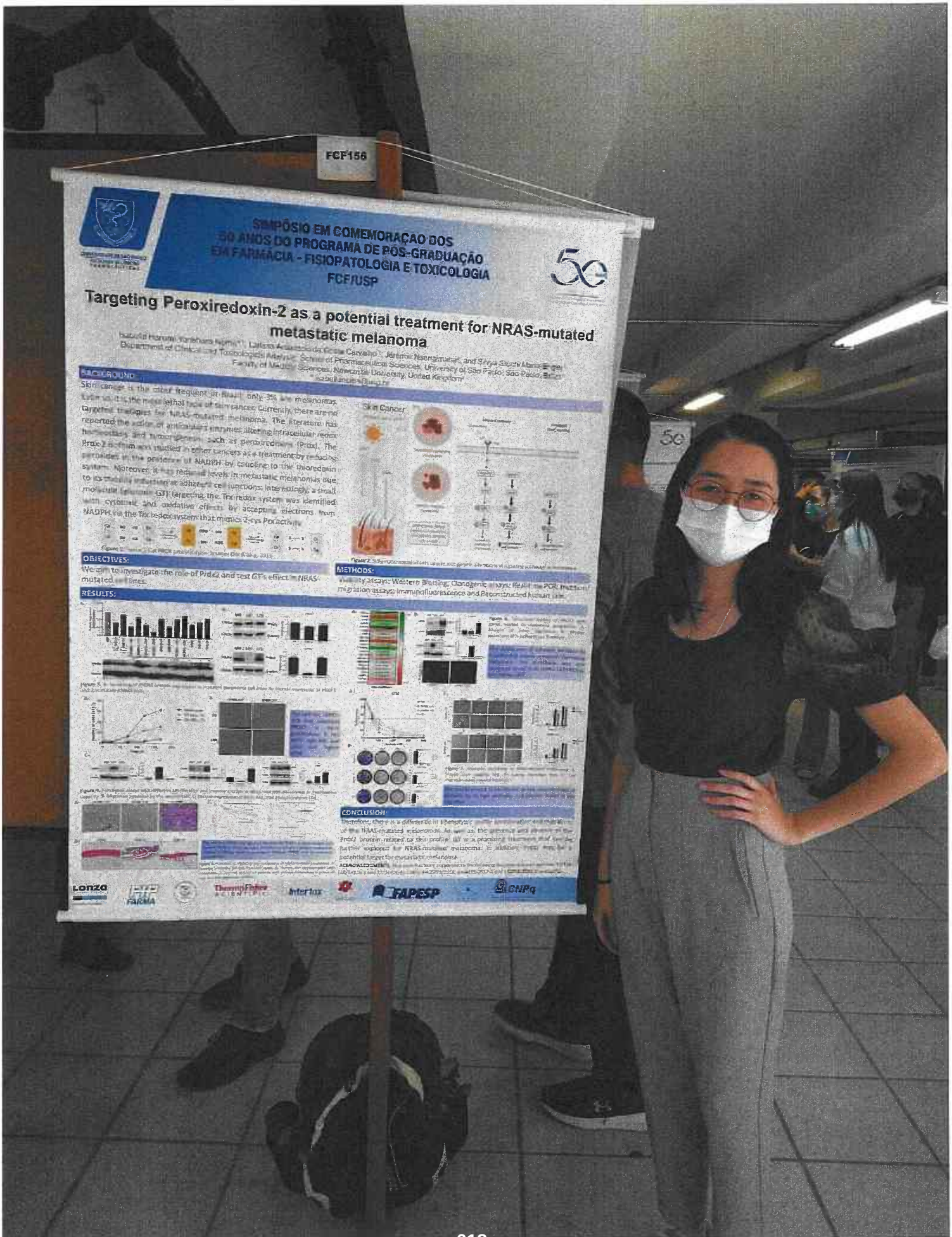
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CBTox 2022

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CERTIFICADO

We hereby certify that the abstract

Gliotoxin and its potential cytotoxicity against NRAS mutated-melanoma,

submitted by

Isabella Harumi Yonehara Noma, Larissa Anastacio da Costa Carvalho e Silvya Stuchi Maria-Engler

was presented in **Poster** form by **Isabella Harumi Yonehara Noma** in the XXII Brazilian Congress of Toxicology - CBTox 2022, held in Balneário Camboriú, from May 25th -28th, 2022.

ORGANIZAÇÃO: Sociedade Brasileira de Toxicologia

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Founded in August 1972, the **Brazilian Society of Toxicology (SBTox)** was established as a scientific society with the aim of bringing together teachers, professionals, companies and organizations interested in the development of Toxicology in Brazil. SBTox is dedicated to the continuous development of technical and scientific knowledge for the improvement and guarantee of human health, as well as for the safety of all living organisms and the protection of the environment.

The society actively participates in collaboration with other international organizations, being affiliated with the *International Union of Toxicology* (IUTOX), the Brazilian Society for the Advancement of Science (SBPC), the Federation of Experimental Biology Societies (FESBE), and the Brazilian Association of Toxicological Information and Assistance Centers (ABRACIT). Furthermore, SBTox has worked actively with regulatory bodies, contributing to the advancement of the field of Toxicology and to the establishment of public health and safety policies.

Throughout its history, SBTox has promoted scientific activities of excellence, most notably the organization of the Brazilian Congress of Toxicology (CBTox), an event that has become a landmark in the updating and exchange of knowledge in the field. The impact of the society's actions is widely recognized, especially in the training of several generations of toxicologists, who stand out as leading experts in Brazil and abroad.

III ISEP
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CHALLENGES TO HUMAN LEISHMANIASIS: DISCUSSION OF THE DISEASE BASED ON ITS BEHAVIOR

Cínthia Akemi Tanoshi[1] ; Isabella Harumi Yonehara Noma[1] ; Regivaldo Florentino Rodrigues[1]; Lincoln Luís Silva[1]; Diogo Francisco Rossoni[2]; Raíssa Bocchi Pedroso[1]; Sandra Mara Alessi Aristides[1].

[1]State University of Maringá, Program in Health Science, Maringá, PR, Brazil.
[2]State University of Maringá, Statistical Department, Maringá, PR, Brazil.

E-mail: cinthiaatanoshi@gmail.com

Introduction: Leishmaniasis is a disease caused by different *Leishmania* species. Brazil is one of seven countries with the majority of new cases of leishmaniasis in the world and Paraná state has concentrated about 90% of the illness of Southern region. Among the manifestations there are fever, weight loss and hepatosplenomegaly by visceral leishmaniasis (VL); deforming lesions by cutaneous leishmaniasis (CL); and when disseminate it can develop a severe form of mucocutaneous leishmaniasis (ML), with destruction of cartilage. Even death can occur in consequence of secondary infections, functional impairment and the toxicity of the treatment.

Objective: Analyse and describe the behavior of the *Leishmania*'s epidemiology and epidemic outbreaks in recent years.

Material and methods: The cross-sectional survey was performed using a secondary data of a public health database, DATASUS, with notifications sent to the Information System for Notifiable Diseases - SINAN [Sistema de Informação de Agravos de Notificação]. Through option Diseases and Notifiable diseases - from 2007, the variables related on Leishmaniasis in Paraná between 2007 to 2017 were selected and clustered in tables. The database was organized since January 2019 until March 2019 and after, the statistical analysis were conducted using the RStudio ™ program, while spatial modelling require QGIS ™ (3.4) and GeoDa™ softwares. The result was compared to numbers of leishmaniasis in Brazil. Ethical committee submission was not required by reason of DATASUS being a public domain without nominal identification.

Results: Over the years leishmaniasis kept constant despite of some abrupt increase numbers. A number of 4371 cases were diagnosticated only in Paraná between the period analysed representing 89.53% of southern region, almost 400 cases by year. According to the health region of residence - CIR, Cianorte (n=726), Maringá (n=672), Metropolitan region (n=439), Campo Mourão (n=374) and Londrina (n=345) are the focus of the disease in the state. The most reported cases (99.09%) were registered as American Tegumentary Leishmaniasis (ATL), that include mucocutaneous leishmaniasis (ML), cutaneous leishmaniasis (CL) and clinical aspects that are similar to them. These, 72.02% (n=3119) culminated on cure. On visceral leishmaniasis, 55.00% of 40 cases (n= 22) tend to heal and 15.00% (n=6) decease.

Conclusion: According the datas it was possible to comprehend the distribution of the parasite disease. Monitoring actions are fundamental for the purpose of taking intervention strategies in surveillance improving health care applications, as reducing the prevalence of the disease, focusing on specific and spatial areas.

Keywords: leishmania; leishmaniasis; epidemiology; health care; public health; spatial modeling; spatial analysis.

Grants: CAPES (Coordination for the Improvement of Higher Education Personnel) [Coordenação de Aperfeiçoamento de Pessoal de Nível Superior]

XXVII Brazilian Congress in Biomedical Engineering (CBEB 2020)
October 26-30 2020 Vitoria (Brazil)

CBEB2020
XXVII Congresso Brasileiro de Engenharia Biomédica

Official Certificate

ISABELLA HARUMI YONEHARA NOMA

presented the following work at the Brazilian Congress in Biomedical Engineering (CBEB2020), held in Vitoria, Brazil, on October 26-30, 2020:

GEOSPATIAL ANALYSIS OF DIAGNOSTIC IMAGING EQUIPMENT IN BRAZIL

On behalf of the Organizing Committee,

Teodiano Freire Bastos-Filho
CBEB2020 General Chair

SBEB 60th ANNIVERSARY IFMBE CNPq FAPES GOVERNO DO ESTADO DO ESPÍRITO SANTO FEST

UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOCIÊNCIAS E FISIOPATOLOGIA

PBF
Programa de Pós-Graduação em Biociências e Fisiopatologia

CERTIFICADO

Certificamos que, **ISABELLA HARUMI YONEHARA NOMA**

Atuou como revisora *ad hoc* de resumo simples no *VII International Meeting of Biosciences and Physiopathology* e XI Simpósio de Biociências Aplicadas à Farmácia (Processo nº 3352/2020), realizados no período de 16 a 18 de Setembro de 2020 pelo Programa de Pós-Graduação em Biociências e Fisiopatologia (PBF), do Centro de Ciências da Saúde (CCS), da Universidade Estadual de Maringá – PR.

Maringá, 30 de outubro de 2020.

Prof.ª Dra. Gessilda de Alcantara Nogueira de Melo
Coordenadora do Programa de Pós-Graduação em Biociências e Fisiopatologia &
Organizadora do *VII International Meeting of Biosciences and Physiopathology* e XI Simpósio de Biociências Aplicadas à Farmácia

VII *International Meeting of Biosciences and Physiopathology*
XI *Simpósio de Biociências Aplicadas à Farmácia*

Registrado no processo nº 3352/2020 - PRO/PBF/UEM

CERTIFICATE

We certify that Isabella Harumi Yonehara Noma served as ad roc reviewer of a simple abstract at the VII International Meeting of Biosciences and Physiopathology and XI Symposium of Biosciences Applied to Pharmacy (Process no. 3352/2020), held from September 16 to 18, 2020 by the Graduate Program in Biosciences and Physiopathology (PBF), of the Health Sciences Center (CCS), of the State University of Maringá - PR.

CERTIFICATE OF TRANSLATION

I hereby certify that I am familiar with both the English and Portuguese language and that the translation of the attached document is an accurate translation from the Portuguese language into English, and that the same is a true and complete translation to the best of my knowledge, ability and belief.

Signature: Isabella H. Y. Noma Date: 08-19-2024

Name: Isabella Harumi Yonehara Noma

VI International Meeting of Biosciences & Physiopathology

X Simpósio de Biociências Aplicadas à Farmácia

CERTIFICADO

Certificamos que Isabella Harumi Yonehara Noma atuou como revisor *ad hoc* de Resumos Simples do *VI International Meeting of Biosciences and Physiopathology - X Simpósio de Biociência Aplicadas à Farmácia*, realizado de 18 a 20 de setembro de 2019 em Ivatuba – PR.

Maringá, 21 de setembro de 2019

Registrado no Processo
nº 5172/2019-PRO/PBF/UEM

Prof.ª Dr.ª Débora de Mello Gonçales Sant'Ana
Coordenadora do Evento

Prof.ª Dr.ª Gessilda de Alcântara Nogueira de Melo
Coordenadora do Programa de Pós-Graduação em Biociências e Fisiopatologia

Realização: Programa de Pós-Graduação em Biociências e Fisiopatologia

Apoio: UEM Pró-Reitoria de Extensão e Cultura IAP Instituto Adventista Paranaense

CERTIFICATE

We hereby certify that Isabella Harumi Yonehara Noma served as ad hoc reviewer of Simple Abstracts for the VI International Meeting of Biosciences and Physiopathology - X Symposium of Biosciences Applied to Pharmacy, held from September 18 to 20, 2019 in Ivatuba - PR.

CERTIFICATE OF TRANSLATION

I hereby certify that I am familiar with both the English and Portuguese language and that the translation of the attached document is an accurate translation from the Portuguese language into English, and that the same is a true and complete translation to the best of my knowledge, ability and belief.

Signature: Isabella H. Y. Noma Date: 08-19-2024

Name: Isabella Harumi Yonehara Noma

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UNIVERSITY OF SÃO PAULO
SCHOOL OF PHARMACEUTICAL SCIENCES

SYMPOSIUM CELEBRATING THE
50TH ANNIVERSARY OF THE GRADUATE PROGRAM
IN PHARMACY – PATHOPHYSIOLOGY AND TOXICOLOGY
FCE/USP

CERTIFICATE – Honorable mention

We hereby certify that the author **Isabella Harumi Yonehara Noma**, with the poster titled "Targeting Peroxiredoxin-2 as a potential treatment for NRAS-mutated metastatic melanoma," under the supervision of Prof. Dr. Silvya Stuchi Maria Engler, received an award in the poster presentation category during the **Symposium celebrating the 50th anniversary of the Graduate Program in Pharmacy – Pathophysiology and Toxicology** of FCE/USP, held on 11/30/2022, receiving **Honorable Mention.**

-----// signature //-----

Prof. Dr. Sandra Helena Poliselli Farsky
Coordinator of the Graduate Program in Pharmacy
(Pathophysiology and Toxicology)

UNIVERSITY OF SÃO PAULO
SCHOOL OF PHARMACEUTICAL SCIENCES

I, Raphael Michels Fantinato de Moura, telephone number 415 425-2508, mailing address P.O. Box 90487, San Diego, CA 92169, certify that the professional translation of this document from Portuguese to English has been performed by myself, a qualified translator fluent in both languages, and that the following is an accurate and complete translation of the document.

Raphael Michels Fantinato de Moura **Date: January 13, 2026.**

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UNIVERSIDADE DE SÃO PAULO
FACULDADE DE CIÊNCIAS FARMACÊUTICAS

SIMPÓSIO EM COMEMORAÇÃO DOS 50 ANOS DO PROGRAMA DE PÓS-GRADUAÇÃO EM FARMÁCIA - FISIOPATOLOGIA E TOXICOLOGIA

FCF/USP

CERTIFICADO – menção honrosa

Certificamos que a autora **Isabella Harumi Yonehara Noma** com o pôster "*Targeting Peroxiredoxin-2 as a potential treatment for NRAS-mutated metastatic melanoma*", sob a orientação da Profa. Dra. Silvya Stuchi Maria Engler foi premiada na modalidade apresentação de pôster durante o **Simpósio em comemoração dos 50 anos do Programa de Pós-Graduação em Farmácia – Fisiopatologia e Toxicologia** da FCF/USP realizada em 30/11/2022, obtendo **menção honrosa.**

Profa. Dra. Sandra Helena Poliselli Farsky
Coordenadora do Programa de Pós-Graduação em Farmácia (Fisiopatologia e Toxicologia)

UNIVERSIDADE DE SÃO PAULO
FACULDADE DE CIÊNCIAS FARMACÊUTICAS

Exhibit G. Evidence that the Petitioner has performed in a critical role for an organization of distinguished reputation

Amended
April 28, 2025

Isabella Harumi Yonehara Noma, PhD
bellanoma@hotmail.com

Re: Letter Agreement for Postdoctoral Fellowship

Dear Isabella,

On behalf of Dr. Keiran Smalley and the H. Lee Moffitt Cancer Center and Research Institute, Inc. ("Moffitt") we are pleased to offer you a full-time, exempt Postdoctoral Fellow position in the Department of Tumor Microenvironment and Metastasis ("Department"). We are excited about the potential you bring to our organization in achieving our mission *"to contribute to the prevention and cure of cancer."* To follow are the terms and conditions of your appointment and employment.

Dena Gaeta, Manager of Basic Science can assist you with administrative questions about your appointment, employment, timekeeping, and other division details.

You will be involved in the Department's research activities and will be provided career development assistance and mentorship during this important step in your training.

1) Your proposed start date for your Postdoctoral appointment and employment by Moffitt shall be May 12, 2025 ("Start Date").

2) Along with this tremendous educational opportunity, you shall receive a salary of $31.01 per hour (annualized to $64,500.80). You will be eligible for annual performance evaluations and merit increases in accordance with Moffitt policy effective at any given time.

3) You will be eligible to participate in the comprehensive program of employee benefits provided to similarly situated team members. All benefits are subject to periodic review and may be amended from time to time by Moffitt in its sole and absolute discretion.

4) To assist in your transition to the Tampa Bay area, you will be awarded relocation assistance in the amount of $4,500 for a 24-month commitment and subject your agreement and execution of the Relocation Assistance Agreement that will be sent separately to you. This sum is considered taxable income and subject to all applicable withholdings. Payment of this sum is expected to be made within 30 days of your Start Date or execution of said agreement, whichever occurs later.

5) The term of the appointment is one year ("Initial Term") and, if appropriate, may be renewed on the one-year anniversary date ("Subsequent Term"). The Initial Term and Subsequent Terms are contingent upon satisfactory progress of mutually agreed goals, ongoing evaluations, available funding, and your compliance with applicable Moffitt policies and procedures. [

6) You will be subject to a 180-day introductory period during which you and Dr. Smalley will evaluate your suitability for and performance in the position.

7) You acknowledge and agree that your employment by Moffitt is on an at-will basis, and that this Letter Agreement does not bind you or Moffitt to any specific period of employment. Your employment is the will of both parties, and either party may terminate the employment relationship at any time, with or without notice, and for any reason not prohibited by law.

8) The health of our patients, visitors and team members is of utmost importance. As such, Moffitt team members are screened for tuberculosis prior to employment. Similarly, prior to flu season, team members are provided free flu vaccinations; team members who are unable or choose not to receive the vaccination are required to wear a mask during the defined flu season. Your employment with Moffitt is contingent upon your ability to present documentation to confirm your identity and your authorization to work in the United States, your successful completion of pre-employment screenings and requirements, and compliance with Moffitt's rules, regulations, policies and procedures, including all intellectual property-related policies and agreements. A human resources representative will contact you to initiate an appointment with Occupational Health which needs to be completed at least seven (7) days prior to your Start Date.

9) Upon successful completion of all pre-employment screenings and requirements, you will receive confirmation regarding your attendance at New Employee Orientation. This confirmation will provide you with the details about when and where to report. During New Employee Orientation, you will be provided with information about parking options and the corresponding costs. In addition to New Employee Orientation, you will be required to attend a separate Research Orientation which will be on Tuesday, May 12, 2025, from 9:00am – 12:30pm in the MRC Conference Room 3065. You will receive additional details upon starting.

10) You hereby represent and warrant that your execution and performance under this Letter Agreement does not conflict with, or result in, any violation of any other agreement, arrangement, or provision to which you are a party, including without limitation, any non-competition agreements with a third party.

11) In any action or dispute, at law or in equity, that may arise under or out of, or which otherwise relates to this Letter Agreement, the prevailing party shall recover its legal expenses, including reasonable attorneys' fees, legal assistants' fees, costs and expenses, from the non-prevailing party in addition to any other relief or remedy to which the prevailing party may be entitled.

12) This Letter Agreement shall be governed by and construed under the laws of the State of Florida. The venue of any dispute arising from this Letter Agreement shall be in the courts in Hillsborough County, Florida. The parties hereby knowingly and voluntarily waive any right they may have to a trial by jury with respect to any action or claim arising out of or related to this Letter Agreement.

We are truly excited at the prospect of you joining our team in contributing to the *prevention and cure of cancer.* We sincerely hope that you are interested in this position under the terms and conditions outlined above. If you are agreeable, please sign the Acknowledgement and Acceptance below and return to me via electronic mail at Maritza.Garcia-Saavedra@Moffitt.org.

Sincerely,

Maritza Saavedra
Senior Talent Advisor - Research

Acknowledgement and Acceptance:

I, _____, hereby accept appointment as a Postdoctoral Fellow and employment by Moffitt as set forth above.

Signature: _____ Date: _____
Isabella Noma, PhD

cc: Kieran Smalley, PhD
Senior Member

Dena Gaeta
Manager

November 06, 2025.

RE: Letter in Support of Isabella Yonehara Noma's EB-1 Petition

To Whom It May Concern,

I am writing to you to offer my strongest support for the visa application endeavors of Dr. Isabella Yonehara Noma. My name is Dr. Keiran Smalley, and I am currently the director of the Donald A. Adam Melanoma and Skin Cancer Center of Excellence and a Senior Member of the Department of Tumor Microenvironment and Metastasis at Moffit Cancer Center and Research Institute in Tampa, FL. My research focuses on the biology and therapeutic management of the melanoma, the most common type of skin cancer. I became personally familiar with Dr. Noma through my role as her postdoctoral mentor, and we have collaborated on several projects during the one year she spent in my laboratory at Moffitt Cancer Center. My time spent working with Dr. Noma leaves me uniquely qualified to testify to the merits of her work.

Dr. Noma holds a PhD in Pharmaceutical Sciences from the University of Sao Paulo, Brazil as well as an MSc. from the State University of Maringa (Brazil) and a BS in Health Sciences from the State University of Maringa. Upon earning her PhD in 2023, Dr. Noma accepted a role as a postdoctoral fellow in my laboratory at the Moffit Cancer Center in Tampa, Florida. It was in this role that I was able to work with Dr. Noma directly.

While in my lab, Dr. Noma worked on a challenging project that identified the role of a novel regulator of response to ultraviolet radiation in the skin that we believe leads to melanoma development. Her work involved advanced techniques including the manipulation of genetically engineered mice, irradiation and methods to study the effects of ultraviolet radiation on cell survival, intracellular signaling and immune escape pathways. This project required an extremely high level of specialized technical skills, that only a few researchers possess. Dr. Noma also had the opportunity to learn many other techniques while in my lab including analysis of high content single cell RNA-seq data, informatics and flow cytometry.

The study that Dr. Noma works on showed that overexpression of HDAC8 in UV-irradiated melanocytes and melanoma cells enhances their survival. This suggests that targeting HDAC8 might be a potential strategy for limiting the survival of these cells after UV damage, which is crucial since prolonged survival can lead to malignancy. The downregulation of p53 and acetylated p53 indicates a mechanism by which these cancer cells evade apoptosis. Since p53 is a critical tumor suppressor, understanding its inactivation provides insights into the progression of melanoma and highlights the need for interventions that can restore or mimic its function. We showed that HDAC8+ cells have reduced PD-L1 expression, which is important for immune evasion. This finding suggest the possibility of developing therapies that target HDAC8 to enhance immune recognition and destruction of melanoma cells, which will improve the effectiveness of immunotherapies.

It is testament to the high quality and practical applicability of Dr. Noma's research that she has already published her work in peer reviewed journals 19 times. These journals, including Antioxidants, Frontiers in Nutrition and Experimental Gerontology only select research of the highest quality that meets the rigorous standards of their peer reviewers and editorial boards. To date, Dr. Noma has been invited to present her work at many international and national conferences, the most notable of which being the Annual Society for Melanoma Research (SMR) conference in Edinburgh, Scotland in 2022. At this meeting, Dr. Noma presented her work to an international audience of melanoma and skin cancer experts and was selected to receive an award for her work. Only a limited number of awards were made at this conference, making this an impressive achievement that differentiated her from her peers.

In the United States, melanoma is the fifth most common form of cancer. Unlike many life-threatening afflictions, melanoma is highly prevalent among younger demographics, ranking as the most common form of cancer in adults ages 25-29. The latest data suggest that melanoma is the third most common form of cancer in men ages 15-29, and the fourth most common form of cancer in women ages 15-29. As such, the effective diagnosis and treatment of melanoma is a critical public health issue in the United States that affects people in a wide variety of age groups. Dr. Noma's research findings on the improvement of melanoma treatments offer important new insights into how melanoma can be prevented and then treated. Her research in this area spearheads national efforts to improve the treatment of melanoma patients by developing new strategies for the treatment of this terrible disease, saving the lives of many Americans.

In sum, Dr. Noma's impressive academic and professional background, her numerous research achievements, the impact her work has had on his field and her publication record all represent a unique set of skills. Dr. Noma's accomplishments make it clear that she is among the very best researchers in the field making her a real asset to the national interest of the United States.

If I can be of further assistance do not hesitate to contact me.

Yours sincerely,

Keiran Smalley, PhD.
Professor and Donald A. Adam Endowed Chair in Melanoma Research
Director, The Melanoma and Skin Cancers Center of Excellence
Moffitt Distinguished Scholar
The Moffitt Cancer Center
12902 Magnolia Drive
Tampa, FL,33612, USA

Curriculum Vitae for: Keiran Smalley, Ph.D.
Date: 7/24/24

Current Position:

Director of the Melanoma Center of Excellence,
Donald A. Adam Chair in Melanoma Research
Senior Member (with Tenure)
Departments of Tumor Biology, Cutaneous Oncology and
The Donald A. Adam Comprehensive Melanoma Research Center
Moffitt Cancer Center
12902 Magnolia Drive
Tampa, FL 33612
(813)745-8725
(813)449-8260
keiran.smalley@moffitt.org

Current Academic Appointments:

Professor
Department of Oncologic Sciences
University of South Florida
Tampa, FL

Education:

1992-1996	B.Sc. (Hons.)	University of Bath, UK.
1996-2001	Ph.D.	University of Cambridge, UK.

Postgraduate Training and Fellowship Appointments

2001-2001	Postdoctoral Fellowship in the laboratory of Prof. John Hartley. UCL Oncology Department, London, UK.
2001-2003	Postdoctoral Fellowship in the laboratory of Prof. Chris Marshall, Chester Beatty Laboratory, Institute of Cancer Research, London, UK.
2003-2005	Research Fellowship in the laboratory of Dr Meenhard Herlyn, The Wistar Institute, Philadelphia, USA.

Academic Appointments and Employment:

2005-2006	Staff Scientist, The Wistar Institute, Philadelphia, USA.
2006-2008	Senior Scientist, The Wistar Institute, Philadelphia, USA.
2008-2013	Assistant Member, Moffitt Cancer Center & Research Institute, Tampa, FL.
2008-2013	Assistant Professor, University of South Florida, Tampa, FL.
2013-2016	Associate Member (with tenure), Moffitt Cancer Center & Research Institute, Tampa, FL.
2013-2016	Associate Professor, University of South Florida, Tampa, FL.
2015-2016	Director, Melanoma and Skin Cancers Center of Excellence (Interim)
2016-present	Director, Melanoma and Skin Cancers Center of Excellence
2016-present	Senior Member, Moffitt Cancer Center.
2016-present	Professor, University of South Florida, Tampa, FL.
2016-present	Donald A. Adam Chair in Melanoma Research

Teaching Experience

University Courses:

2006-2008 **Teaching Assistant**: BIO600M - Cell & Molecular Biology, graduate level cancer biology course. University of Pennsylvania, Philadelphia, PA.

2008-2019	**Course Director and Lecturer:** PhD Cancer Biology program. Moffitt Cancer Center & Research Institute / USF, Tampa, FL.
2009-2013	**Course Director**: "Advances in Cancer Research". Meet with students weekly during fall semester providing guidance, supervision and feedback including their selection and presentation of pertinent journal articles.
	Lecturer: Teach course to students two times per year during Fall and Spring semesters. Also lecture to Oncology Fellows/Residents 2 times per year.

Teaching and Training Experience:

Graduate training:

2008	**Supervisor** for Masters Student Hao Wang, Biostatistics Program, University of Pennsylvania, PA.
2009-2014	**Thesis Committee** for Valentina Schneeberger, Kate Bailey (Moffitt Graduate Students), and Laura Hamel (USF Molecular Medicine Graduate Student).
2009-2014	**Major Professor** for Ph.D. Student, Inna Fedorenko*, Cancer Biology Program at Moffitt Cancer Center / USF *ARCS Foundation Scholar Award - 2011-2014 *Joanna M. Nicolay Melanoma Foundation Research Scholar Award - 2014
2009-2014	**Major Professor** for Ph.D. Student, Vito Rebecca, Cancer Biology Program at Moffitt Cancer Center / USF. *McKnight Foundation Scholar – 2013-2014
2011-2015	**Major Professor** for Ph.D., Student, Kim Paraiso*, Cancer Biology Program at Moffitt Cancer Center / USF *Joanna M. Nicolay Melanoma Foundation Research Scholar Award - 2012
2010	**Mentor** for the LINK-ets program student Buelah Joseph
2009-2010	**Mentor** for SPARK student Amy Iabarrino
2013-2015	**Mentor** for visiting Ph.D Student Sarah Sloot, MD University of Groningen.
2012-2013	**Mentor** for visiting Ph.D Student Renato Massaro, University of Sao Paulo.
2013-2014	**Mentor** for visiting Ph.D student Manoela Tiago, University of Sao Paulo.
2015	**External examiner** for Ph.D student Gholamreza Ardekani, University of British Columbia, Canada.
2015-2018	**Thesis Committee** member. Natalia Sumi
2015-2016	**Mentor.** Visiting Scientist. Dr Fernanda Flores, University of Sao Paulo.
2016	**External Examiner.** For PhD Student Andrew Miles, University of Melbourne, Australia.
2016-	**Thesis Committee** member. Christopher Letson, Defended 2023.
2016-2021	**Mentor** for PhD Student Chao Zhang. Defended 2021.
2017-	**Thesis Committee** member. Ella Bok
2017-	**Thesis Committee** member. Daniel Lester, Defended 2023.
2019-	**Thesis Committee** Member. Niveditha Nerlakanti
2019-	**Thesis Committee** Member. Neel Jasani
2021	**Qualifying Exam Committee** Member.
2021	**Thesis Committee** Member. Qian Liu, Defended 2024.
2022	**Thesis Committee** Member, Julia Billington
2022	**Thesis Committee** Member: Kaizhen Wang
2022	**External Examiner.** For MD/PhD Student Matthew Danker, McGill University, Canada
2024	**Major Professor** for Marvelous Adebowale. USF Program in Molecular Medicine

Honors and Awards

1995	Wellcome Trust Vacation Studentship.

2000	American Association for Cancer Research travel award.
2000	Skin Cancer Research Fund award.
2011	Invited Visiting Professor, University Sao Paulo, Sao Paulo, Brazil.
2012	Junior Faculty Researcher of the Year award, Moffitt Cancer Center.
2012	Invited Visiting Professor, University Sao Paulo, Sao Paulo, Brazil.
2015	Publication of the Year award. Moffitt Cancer Center.
2022	Team Publication of the Year Award. Moffitt Cancer Center.

Research Support

Current
External Grants:

R01 CA256193-01A1 (Smalley, Licht and Harbour - MPI). Smalley Contact
NIH/NCI 08/09/2021 – 07/31/2026
Effort: 20%
Characterization and targeting of the epigenetic state underlying uveal melanoma liver metastasis

NCI R01 CA262483-1A1 (Smalley) Contact PI
NIH/NCI 7/1/2022 - 6/30/2027
Effort 20%
Defining and targeting of the epigenetic-plasticity driven drug resistance in melanoma
The goals of this project are to determine if targeting HDAC8 can limit therapy escape in melanoma

R21 CA267141 (Smalley-PI)
Effort 8%
Defining and targeting the epigenetic programs involved in melanoma development.
The goals of this project are to determine how UV-induced HDAC upregulation allows damaged melanocytes to avoid immune detection and develop into melanoma.

George Edgecomb Society (Smalley-PI) 06/01/2023 – 05/31/2024
MCC
EFFORT 2%
Title: Defining the immune and signaling landscape of acral melanoma in African-American patients
Specific Aims: Aim 1: To define the immune microenvironment of acral melanoma from African-American patients. Aim 2: To define and target the unique cell signaling vulnerabilities of acral melanoma arising in African-American patients.

The Sullivan Gift. (Smalley-PI) 01/10/2013-01/10/2024
Philanthropic $1,500,000
Effort: 15%
Development of novel melanoma treatment biomarkers
To develop new methods to analyze signaling adaptation in circulating tumor cells and patient biopsies.

NCI R01 CA281153 (Zhang, Zheng, Smalley – MPI)
NCI/NIH 04/01/2024 – 03/31/2029
Effort: 15%
Title: Proteolysis targeting chimera against nuclear receptor NR4A1 for melanoma therapy
Major Goals for Smalley Lab: Dr. Smalley will add his unique expertise to Aim 2 to determine the role of NR4A1 in melanoma biology and progression. At the same time, his group will lead the therapy studies in Aim 3, in which the effects of NR4A1 inhibitors are studied in the single agent setting as well as in combination with various ICI combinations.

Bankhead-Coley Program (24B04) (Smalley -PI). 04/24/2024-03/31/2027

Florida Department of Health
Effort: 12%
Title: Optimizing immunotherapy strategies for melanoma brain metastases
Major Goals: To further optimize therapeutic strategies for patients whose brain metastases may have a non-T cell inflamed immune microenvironment. Aim 1: To determine how anti-LAG3+PD-1 and anti-CTLA-4+PD-1 reshape the immune microenvironment in patients with resectable MBM. Aim 2: To determine how CD4+ T cells orchestrate responses to LAG3-based ICI combinations in MBM. Aim 3: Defining and overcoming resistance to the LAG3+PD-1 combination in MBM.

Melanoma Research Alliance. (Smalley (contact)/Jaeger/Poulikakos MPIs) 06/01/2024-05/31/2027
Team Science Award
Effort: 5%
Title: **Optimizing the immune mediated-destruction of NRAS mutant melanoma**
Goal: The overarching goal is to 1. define the contribution of cell-intrinsic signaling mechanisms that drive RAS inhibitor sensitivity and immune responses to RAS inhibition and 2. Identify and evaluate novel vaccine-based approaches that boost immune responses in combination with RAS inhibition.

Completed

R21 CA256289 (Smalley-PI)
NIH/NCI 07/01/2021-06/30/2023
Effort 10%
Targeting the suppressive immune microenvironment in leptomeningeal melanoma metastases

9LA03 (Smalley-PI) 04/01/2019-03/31/2022
State of Florida: Live Like Bella
Effort: 5% $250,000
Defining and modeling pediatric melanoma development.

Melanoma Research Foundation (Smalley-PI) 10/01/2020 – 09/31/2021
Effort: 2% $50,000
To uncover the role of DC3 cells in modulating the melanoma immune environment.

8BC03 (Smalley) PI 04/02/2018 - 03/31/2021
State of Florida Bankhead Coley $733,174
Effort: 20%
Defining and therapeutically targeting HDAC8-driven reprogramming in melanoma brain metastasis development
To explore the role of HDAC8 in driving a melanoma phenotype that metastasizes to the brain

579030 MRA Team (Smalley) PI 06/01/2018 - 05/31/2021
Effort: 20% $900,000
Defining and targeting driver events in acral melanoma
To develop new animal models of acral melanoma and to define new therapeutic strategies

CA170653 (Smalley) PI 06/15/2018 - 06/14/2021
DoD Peer Reviewed Cancer Research Program (PRCRP) $400,000
Effort: 12.5%
Improving Therapy for Melanoma Brain Metastases
Goals: To define the signaling in the brain microenvironment that contributes to therapeutic escape in melanoma brain metastases

R21CA216756 (Smalley/Forsyth) Co-PIs 03/13/2017 - 02/28/2019
NIH/NCI $275,000
Effort: 12.5%
Using Single Cell Analysis to Identify Therapeutic vulnerabilities in leptomeningeal melanoma metastases
The goal of this project is to uncover new therapeutic targets in leptomeningeal melanoma metastases and to develop the rationale for future clinical trials.

7BC05 (Smalley) Contact PI 04/12/2017 - 02/29/2020
Effort: 20% $1,391,321
Defining and targeting epigenetic regulation in uveal melanoma.
This is a collaborative proposal with Dr Jonathan Licht (U Florida) and Dr Bill Harbour (U Miami) to investigate and target the epigenetic vulnerabilities in uveal melanoma.

Florida Academic Cancer Centers Alliance. (Smalley) co-P.I. 08/01/2019-07/31/2020
Epigenetic dysregulation in BRAF inhibitor escape
This is a pilot grant with the University of Miami to understand the epigenetic reprogramming that occurs in response to BRAF inhibition.

P50 SPORE in Human Cancer (Sondak) P.D. 04/01/2012-03/31/2018
NIH/NCI $1,497,000
Effort: 20%
Moffitt Skin Cancer SPORE
The long-range goal of the Moffitt Skin SPORE is to promote progress in the prevention and treatment of cutaneous malignancies based on developments in our laboratories that are applied to innovative clinical trials. Role: (Smalley) Co-Leader. Project 2, "Abrogation of therapeutic escape pathways in BRAF mutant melanoma". Responsible for the planning and execution of the preclinical aspects of this project and the supervision of the performance of the post-doctoral fellows and the graduate students.

R21 CA198550-01 (Smalley) P.I. 07/01/2015-06/30/2017
NIH/NCI $417,679
Effort: 20%
Modeling and Targeting Adaptive Signaling Responses in Single NRAS Mutant Melanoma Cells
The goal of this project is to study therapeutic adaptation of NRAS mutant melanoma cells at the single cell level and to design therapeutic strategies to abrogate escape.

1R01CA161107-01 (Smalley) P.I. 07/01/2011-06/31/2017
NIH/NCI Total Award: $1,732,625
Effort: 25% Total Direct Costs: $1,037,500
Microenvironment mediated drug resistance in melanoma
The major goals of this project are to determine how the interaction of the tumor and host contributes to intrinsic and acquired drug resistance in melanoma.

Florida Academic Cancer Centers Alliance. (Smalley) co-P.I. 08/01/2016-07/31/2017
HDAC inhibition in uveal melanoma.
This is a pilot grant with the University of Florida and the University of Miami to develop new therapies for the most deadly kind of eye cancer.

Personalized Medicine Institute (Smalley) P.I. 12/01/2015-11/30/2016
Effort 1% Total Award: $50,000
Defining the role of deleterious Eph receptor mutations in melanoma progression and drug resistance

Forma Therapeutics (Smalley) P.I. 04/01/2015-03/31/2016
Effort 1% Total Award: $50,000
Targeting epigenetic changes that occur on BRAF inhibitor therapy
The goal of this pilot project is to determine whether HDAC inhibition can reverse the epigenetic mechanisms of therapeutic escape.

Investigational (Smalley) P.I. 06/01/2012-01/31/2014
Sponsor: Boehringer-Ingleheim Total Award: $249,000
Effort: 10%
The anti-melanoma activity of BI-5

09BN-14 New Investigator Research(Smalley) P.I. 08/01/2009-06/30/2012
Bankhead-Coley Total Award: $375,000
Effort: 20% Total Direct Costs: $340,908
Defining the signaling network of NRAS mutated melanomas.
This proposal will build upon preliminary work showing that *NRAS*-mutated melanomas require distinct therapeutic approaches as they rely on different signaling pathways to those melanomas with *BRAF* mutations.

1U54CA143970-03 (Gatenby) P.D. 09/30/2009–08/31/2015
NIH/NCI Total Award: $11,308.462
Effort: 15% Total Direct Costs: $7,317,720
The Physical Microenvironment in Cancer biology and Therapy
The major goal of this project is to develop an integrative, multidisciplinary research program that applies mathematical methods to the investigation of problem in tumor biology and clinical oncology.
Role: Project 1, Co-Investigator: Responsible to lead the experimental part of project 1 of the U54, and supervises the work of the technician. This project is a close collaboration between Dr Smalley and Dr Anderson, who leads the theoretical/modeling part of the project.

New Investigator Research(Smalley) P.I. 06/01/2011-05/31/2012
Harry J. Lloyd Charitable Trust Total Award: $100,000
Effort: 20% Total Direct Costs: $100,000
The role of Axl signaling in melanoma survival and angiogenic response
This proposal will explore how constitutive Axl signaling mediates the survival of NRAS mutant melanoma cells and the role of paracrine Axl/Gas6 signaling in driving the angiogenic response.

New Investigator Research (Smalley) P.I. 08/01/2009-05/31/2011
Harry J. Lloyd Charitable Trust Total Direct Costs: $100,000
Organometallic protein kinase inhibitors as chemosensitizers in melanoma
This proposal will explore the hypothesis that GSK3β is a key node for the growth and survival of melanoma and that its inhibition will sensitize melanoma cells to chemotherapy treatment by altering the life-death balance, pushing the cells towards apoptosis.

Melanoma Pilot Research Award (Smalley) P.I. 01/01/2010-12/31/2011(NCE)
Institutional - Donald A. Adam Comprehensive Total Direct Costs: $75,000
Melanoma Research Center
Phospho-proteomic analysis of acquired PLX4032 resistance in melanoma
The aim of the current study is to investigate the mechanisms underlying acquired PLX4032 resistance. We will address whether a dual therapeutic strategy, targeting either BRAF/NFκB or BRAF/IGFR, will overcome acquired PLX4032 resistance.

Institutional - Donald A. Adam Comprehensive 02/01/2011–06/30/2011

Melanoma Research Center (Smalley) P.I. Total Direct Costs: $35,000
Axl: a novel therapeutic target in *NRAS*-mutated melanoma
The aim of the current study is to validate the expression of Axl in specimens of *NRAS* mutated melanoma by immunohistochemistry and phospho-proteomics.

Investigational (Smalley) P.I. 07/13/2009-06/30/2011(NCE)
Centocor, Inc. Total Direct Costs: $10,000
Effects of CENTOCOR's Anti-integrin Antibody, CNTO 95

Pilot Research Award (Smalley) P.I. 10/15/2009-10/14/2010
Bankhead-Coley Melanoma Pre-Spore Total Direct Costs: $75,000
Overcoming the intrinsic resistance of BRAF V600E mutated melanomas to PLX4032
The aim of this study is to investigate the mechanism of rebound PI3K activation by using genetic approaches to study the role of two genes known to regulate PI3K/AKT signaling; PTEN and SHIP2, and address whether a dual therapeutic strategy, targeting both BRAF and PI3K, is sufficient to prevent the onset of resistance to PLX4032.

Junior Faculty Career Development Award (Smalley) P.I. 01/01/2009-12/31/2009
Institutional - Donald A. Adam Comprehensive Total Direct Costs: $50,000
Melanoma Research Center
Defining the role of c-Kit signaling in sub-groups of melanoma
The aim of the study is to determine how c-KIT activity drives the growth and survival of melanoma cells with the expectation that this new information will allow for the better selection of melanoma patients who will respond to imatinib treatment.

Pilot Project Grant (Smalley) P.I. 01/01/2009-12/31/2009
American Cancer Society – Institutional Research Grant Total Direct Costs: $30,000
The mechanistic basis of imatinib response in sub-groups of melanoma
The aim of the study is to determine how the inhibition of c-KIT signaling drives the proliferation and survival of melanoma cells.

Career Award (Smalley) P.I. 01/01/2009-12/31/2010
Melanoma Research Foundation Total Direct Costs: $100,000
The Identification of Rational Drug Combinations for Melanoma Targeted Therapy
This project performs pre-clinical studies using melanoma cell lines with known genetic profiles to develop optimal targeted therapy regimens.

5 P50-CA093372 (Herlyn) P.D. 04/01/2006-04/01/2008
NIH Total Direct Costs: $150,000
SPORE in Skin Cancer
Career Development Award to Dr K.S.M. Smalley.
Targeting melanoma using novel organometallic PIM-1/GSK3β inhibitors.

Service

Moffitt Cancer Center

Committees:

2008-present	Member of the Graduate Education Committee, Moffitt Cancer Center
2010-2011	Member of the Bioinformatics Search Committee, Moffitt Cancer Center
2011-2012	Member of the Shared Facility Review Committee, Moffitt Cancer Center
2012-2014	Member of the Research Transformation Task Force, Moffitt Cancer Center
2013-2015	Member of the Faculty Research Incentive Task Force
2014-2016	Member of the Bioinformatics steering committee

2014-present	Member of the Clinical Genomics Action Committee
2015-2017	Junior Faculty Research Award Selection Committee
2016-2019	Chair, Proteomics Advisory Committee
2016-2018	Basic Science Division APT Committee
2016-present	Scientific Leadership Committee
2017-2018	Member of the Post-Tenure Review Task Force
2017-2021	Chair, Basic Science Division APT Committee
2018-2020	Member, Junior Researcher of the Year Award Committee
2018-present	Executive Sponsor for the Iovance Collaboration
2019-present	Member of the Molecular Genomics Advisory Committee
2020-2022	Member of the FACCA Annual Retreat Organizing Committee
2022-2023	Chair, Cancer Physiology Chair Search Committee
2023	Chair, Tumor Biology Chair Search Committee

Other Service

Conference organizer:

2011	Meeting Organizer for the Society of Melanoma Research Annual Meeting, Tampa, FL. This is the major yearly melanoma scientific meeting with 600-1000 participants.
2014	Organizer of the Annual SPORE meeting. This is the yearly meeting of the 5 Skin SPORE programs from around the US.
2018	Meeting Organizer for the Melanoma Research Foundation Brain Metastasis Meeting, Tampa, Fl. This was an invite only summit of leading brain metastasis researchers.
2018	Organizer of the Annual SPORE meeting. This is the yearly meeting of the Skin SPORE programs from around the US.
2023	Co-organized the CureOM (Cure Ocular Melanoma) meeting for patients and families, Tampa, FL.
2024	Organizer of the MRF Ras targeting in melanoma meeting, Washington, D.C.
2025	Organizer of the Pan-American Society for Pigment Cell Research (PASPCR) Annual Meeting, Tampa, FL.

Peer Review panels:

2007	Member of peer review panel for the State of Texas advanced research program.
2010	Member of Department of Defense (DoD) Skin Cancers peer review panel
2011	*Ad Hoc* Member of the NCI/CTEP– Investigational Drug Steering Committee.
2012	Member of the Department of Defense (DoD) Skin Cancers Discovery Panel.
2012	Member of the Department of Defense (DoD) *Ad Hoc* Skin Cancers peer review panel.
2012	Member of the Department of Defense (DoD) Melanoma and other Skin Cancers panel.
2013	*Ad Hoc* member of the NCI/NIH study section ZCA1 SRLB-C: Provocative Questions
2013	Member of the Department of Defense (DoD) Melanoma and other Skin Cancers panel.
2014	*Ad Hoc* member of the NCI/NIH study section: Cancer Biology 2
2014-2015	*Ad Hoc* Member of the NCI/NIH study section: BMCT
2014	Member of site visit team for the NCI Laboratory of Cancer Biology
2015	*Ad Hoc* Member of the NCI/NIH study section: Cancer Biology 2
2015	*Ad Hoc* Member of the NCI/NIH F09B study section (F training grants)
2015-2019	Standing Member of the NCI/NIH MCT1 Study Section.
2016	*Ad Hoc* Member of the P01 Study Section
2016	Member of the Department of Defense (DoD) Melanoma and other Skin Cancers panel.
2016	*Ad Hoc* NIH/NCI DP5 Directors Early Independence Award Study Section
2017	*Ad Hoc*, Member of the P01 Study Section
2020-	Scientific Research Officer (SRO) for the Melanoma Research Foundation.
2020-2021	Chair, MRP-MONC panel Department of Defense.
2021	*Ad Hoc* Member of the NCI/NIH MCT2 Study Section

2022	*Ad Hoc* Member CCB Study Section (declined due to SPORE commitments)
2022	Chair, MRP-PTE panel Department of Defense
2023	*Ad Hoc* Member of the MCT-B Study Section
2023	Chair, FP-2 Panel. Department of Defense.
2023-	Standing Member, Melanoma Research Alliance Grant Review Panel.
2024-26	Member of the AACR Ocular Melanoma Grant Review Panel.

Editor:

2010	Guest Editor for the Biochemical Pharmacology Special Issue on Targeted Cancer Therapy
2011-2015	Academic Editor for PLoS One.
2012	Editor: Advances in Pharmacology Volume 65:2 "Current challenges in personalized cancer therapy"
2015-present	Associate Editor: Pharmacological Research
2021	Guest Reviewing Editor: eLife.

Editorial Boards:

2009-2020	Editorial Board Member of the journal *Biochemical Pharmacology*
2009-present	Editorial Board Member of the journal *The American Journal of Clinical Dermatology*
2011-present	Editorial Board Member of the journal *Drugs*
2011-present	Editorial Board Member of the journal *Melanoma Research*
2012-present	Editorial Board Member for the journal *Clinical Cancer Research*
2013-present	Editorial Board Member for the journal *Melanoma Management*
2019-present	Editorial Board Member for the journal *Cancer Biology and Therapy*

Peer Reviews:
Typically 80-100 peer review requests every year for a range of journals including Nature, Cancer Cel, Cell, Nature Medicine, Nature Cancer, New England Journal of Medicine, The Proceedings of the National Academy of Sciences USA, Cancer Research, Clinical Cancer Research, Molecular Cancer Therapeutics, Oncogene, The Journal of Investigate Dermatology, Molecular Cancer Research, Journal of Biological Chemistry, Nature Reviews of Cancer, International Journal of Cancer, British Journal of Cancer, Pigment Cell & Melanoma Research, BMC Cancer, Biochemical Pharmacology, The British Journal of Pharmacology, Cancer, Neoplasia, Experimental Dermatology, Melanoma Research, Cancer Letters, Molecular and Cell Biology, and PLOS One.

Other grant reviews:

2002	BBSRC (UK) Biology I
2003	Catholic University of Leuven
2004-2010	Cancer Research UK
2005	Ohio Cancer Research
2005	The National University of Singapore
2006-2007	The Austrian Cancer Foundation
2008	The Genesis Oncology Trust, New Zealand
2008-2011	Osterreich National Bank, Austria
2009	The Health Review Board Ireland
2009-2011	The Melanoma Research Foundation
2009	The Medical Research Council (UK)
2010	The Israel Science Foundation
2010	Health Research Council of New Zealand
2010-2012	The Icelandic Research Council
2011	Dutch Cancer Society
2011	Consortium Québécois sur la découverte du médicament
2012	Fight for sight – UK.
2012	Austrian Academy of Sciences

2012	Medical Research Council, UK
2013	Institut Du Cancer, France
2015	Swiss Cancer League
2015	Czech Health Research Council
2015	Italian Ministry of Health
2016	Flemish Cancer Society, Belgium
2017	University Hospitals of Geneva, Switzerland
2018	Fonds Nationale de la Recherche Luxembourg
2020	State of Pennsylvania Department of Health
2020	Czech Science Foundation
2021	Sanford-Burnham Prebys. Cancer Center Pilot Grants.
2021	Swiss Cancer League
2021	Polish Science Foundation.
2021	State of Pennsylvania Department of Health
2021	INSERM (France)
2022	La Caixa Foundation
2022-present	The Melanoma Research Alliance.
2022	FAPESP (Brazil) Review of Research Center Grant
2022	The Czech Science Foundation
2023	La Caixa Foundation
2024	Foundation Against Cancer (Belgium)

Advocacy work:
Invited speaker at the American Cancer Society fundraiser for melanoma research, August 12th 2010, Brandon, FL.
Talks given at the Moffitt Leadership Series on behalf of the Moffitt Foundation in Aspen, CO August 2014 and Naples, FL, November 2014.
Talk given at a MRF fundraiser in Bonita Springs, FL Feb 2020.
Talk at MRF Fundraiser in Jacksonville, FL, October 2022.
Talk at the MRA Fundraiser in Naples, FL, March 2023.

Advisory roles:

2003	Novartis Oncology expert advisory board on BRAF Kinase Inhibitors. New York City, NY (Invited Member,).
2004	GlaxoSmithKline retreat on BRAF kinase inhibitors. Upper Providence, PA. (Invited member).
2005	GlaxoSmithKline Roundtable on BRAF inhibitors. Pennsylvania (Invited Member).
2009 - Present	Melanoma Molecular Map Project (Member of Scientific Advisory Board).
2009 – 2014	Advisor to the Penn/Wistar Targeted Therapies P01.
2011	Site visit panel member at the National University of Ireland, Galway.
2012-2013	Advisor to the University of Pittsburgh Immune/targeted therapies P01.
2012	Allovectin Advisory board, Los Angeles, CA.
2013-2017	Advisor to the Wistar Institute Melanoma Center
2014-2018	Advisor to the University of Pittsburgh P01 on immune targeting of EphA2.
2019	Melanoma Research Foundation Targeted therapies steering committee

Professional Association Memberships

2000–present	Member, American Association for Cancer Research
2006-present	Member, Society for Melanoma Research
2014-2018	Member of the Society for Melanoma Research Steering Committee
2018-present	Scientific Advisory Board: Melanoma Research Foundation
2023-present	Member of the Research Committee of the MRF Board

Peer-Reviewed Publications

1. **Smalley, K.S.M.**, Feniuk, W., Humphrey, P.P.A.: Differential agonist activity of somatostatin and L-362855 at human recombinant sst_4 receptors. Br.J. Pharmacol. 125:833-842, 1998.
2. **Smalley, K.S.M.**, Feniuk, W., Sellers, L.A., Humphrey, P.P.A.: The pivotal role of phosphoinositide-3 kinase in the human somatostatin sst_4 receptor mediated stimulation of p44/42 mitogen activated protein kinase and extracellular acidification. Biochem. Biophy. Res. Comm. 263:239-243, 1999.
3. **Smalley, K.**, Eisen, T.: The involvement of p38 Mitogen activated protein kinase in the α-melanocyte stimulating hormone (α-MSH)-induced melanogenic and anti-proliferative effects in B16 murine melanoma cells. FEBS Letters 476:198-202, 2000.
4. **Smalley, K.S.M**, Koenig, J.A., Feniuk, W., & Humphrey, P.P.A.: Ligand internalization and recycling by human recombinant somatostatin type 4 (h sst(4)) receptors expressed in CHO-K1 cells. Br. J. Pharmacol. 132: 1102-1110, 2001.
5. Stebbing, J., Benson, C., Eisen, T., Pyle, L., **Smalley, K.**, Bridle, H., Mak, I., Sapunar, F., Ahern, R., Gore, M.E.: The treatment of advanced renal cell cancer with high-dose oral thalidomide. Br. J. Cancer. 85:953-958, 2001.
6. **Smalley, K.S.M.**, Eisen, T.: Differentiation of human melanoma cells through p38 MAP kinase is associated with decreased retinoblastoma protein phosphorylation and cell cycle arrest. Melanoma Res. 12:187-192, 2002.
7. **Smalley, K.S.M.**, Eisen. T.: Farnesyl thiosalicylic acid inhibits the growth of melanoma cells through a combination of cytostatic and pro-apoptotic effects. Int. J. Cancer 98:514-522, 2002.
8. **Smalley, K.S.M.**: A pivotal role for ERK in the oncogenic behaviour of malignant melanoma? Int. J.Cancer. 104:527-532, 2003.
9. **Smalley, K.S.M.**, Eisen, T.G.: Farnesyl transferase inhibitor SCH66336 is cytostatic, pro-apoptotic and enhances chemosensitivity to cisplatin in melanoma cells. Int. J. Cancer. 105:165-175, 2003.
10. **Smalley, K.S.M.**, Brafford, P., Haass, N.K., Brander, J.M., Brown, E., Herlyn M.: Upregulated expression of Zonula Occludens (ZO) protein-1 in human melanoma associates with N-cadherin and facilitates invasion and adhesion. Am. J Pathol., 166:1541-1554, 2005.
11. Fang, D., Leishear, K., Nguyen, T.K., Finko, R., Cai, K., Fukunaga, M., Li, L., Brafford, P.A., Kulp, A.N., Xu, X, **Smalley, K.S.M.**, Herlyn, M.: Defining the conditions for the generation of melanocytes from human embryonic stem cells. Stem Cells 24:1668-1677, 2006.
12. Liu, Z-J., Xiao, M., Balint, K., **Smalley, K.S.M.**, Brafford, P., Qiu, R., Pinnix, C.C., Li, X., Herlyn, M.: Notch1 signaling promotes primary melanoma progression. Cancer Res. 66:4182-4190, 2006.
13. **Smalley, K.S.M.**, Haass, N.K., Brafford, P.A., Lioni, M., Flaherty, K.T., Herlyn, M.: Multiple signaling pathways must be targeted to overcome drug resistance in cell lines derived from melanoma metastases. Mol. Cancer Therap. 5:1136-1144, 2006.
14. King, A.J., Patrick, D.R., Batorsky, R.S., Lo, M.L., Do, H.T., Rusnak, D.W., Takle, A.K., Wilson, D.M., Wang, L., Karreth, F., Schaber, M.D., Voyick, J.J., Luo, L., Lakdawala, A.S., Adams, J.L., Contractor, R., **Smalley, K.S.M.**, Herlyn, M., Tuveson, D.A., Huang, P.S.: B-Raf V600E expression predicts the anti-tumor response to SB-590885, a potent and selective B-Raf inhibitor. Cancer Res. 66:11100-11105, 2006.
15. Lioni, M., Brafford, P., Andl, CD, Rustgi, A., El-Deiry, W., Herlyn, M., **Smalley, K.S.M.**: Loss of Claudin-7 regulates E-cadherin expression and increases invasion in Esophageal Squamous Cell Carcinoma. Am. J. Pathol. 170:709-721, 2007.
16. **Smalley, K.S.M.**, Contractor, R., Haass, N.K., Atilla, G.E., Williams, D.S., Bregman, H., Flaherty, K.T., Soengas, M.S., Meggers, E., Herlyn M: Novel organometallic protein kinase inhibitors pharmacologically activate p53 and induce apoptosis in human melanoma cells. Cancer Res. 67:209-217, 2007.
17. **Smalley, K.S.M.**, Contractor, R., Haass, N.K., Lee, J.T., Medina, C.A., Nathanson, K.L., Flaherty, K.T., Herlyn, M.: Ki67 expression levels are a better marker of reduced melanoma growth following MEK inhibitor treatment than phospho-ERK levels. Br. J. Cancer 96:445-449, 2007.
18. Lopez-Bergami, P., Huang, C., Goydos, J.S., Yip, D., Bar-Eli, M., Herlyn, M., **Smalley, K.S.M.**, Mahale, A., Eroshkin, A., Aaronson, S., Ronai, Z.: Rewired ERK-JNK Signaling Pathways in Melanoma. Cancer

Cell 11:447-460, 2007.

19. Haass, N.K., Sproesser, K., Nguyen, T.K., Contractor, R., Medina, C.A., Nathanson, K.L., Herlyn, M., **Smalley, K.S.M**.: The MEK inhibitor AZD6244 (ARRY-142886) induces growth arrest in melanoma cells and tumor regression when combined with docetaxel. Clin. Cancer Res. 14: 230-239, 2008.
20. Tsai, J., Lee, J.T., Wang, W., Zhang, Z., Cho, H., Mamo, S., Bremer, R., Gillette, S., Kong, J., Haass, N.K., Sproesser, K., Li, L., **Smalley, K.S.M.,** Fong, D., Zhu, Y-L., Marimuthu, A., Nguyen, H., Lam., B., Liu, J., Cheung, I., Rice, J., Suzuki, Y., Herlyn, M., Bollag, G.: Discovery of a novel selective inhibitor of oncogenic BRAF kinase with potent anti-melanoma activity. Proc. Natl. Acad. Sci. USA 105: 3041-3046, 2008.
21. Noma, K., **Smalley, K.S.M*.,** Lioni, M., Rustgi, A.K., El-Deiry, W., King, A.J., Herlyn, M*.: The essential role of stromal fibroblasts and the transforming growth factor (TGF)-β axis in esophageal squamous cell carcinoma-induced angiogenesis. Gastroenterology 134: 1981-1993, 2008. **co-corresponding authors*
22. Alfano, R.W., Leppla, S.H., Liu, S., Bugge, T.H., Herlyn, M., **Smalley, K.S.M.,** Duesbery, N.S., Houghton, A., Frankel, A.E.: Cytotoxicty of the engineered matrix metalloproteinase-activated anthrax lethal toxin is dependent on gelatinase expression and BRAF status in human melanoma cells. Mol. Cancer Therap. 7: 1218-1226, 2008.
23. Maksimoska, J., Williams, D.S., Attila-Gokcumen, E., **Smalley, K.S.M.**, Carroll, P.J., Webster, R.D., Debreczeni, J.E., Knapp, S., Herlyn, M., Meggers, E.: Isostructural Ruthenium and Osmium complexes display highly similar bioactivity. Chemistry 14: 4816-4822, 2008.
24. Lioni, M., Noma, K., Snyder, A., El-Deiry, W., Rustgi, A., Herlyn, M., **Smalley, K.S.M**.: Bortezomib induces apoptosis in esophageal squamous cell carcinoma cells through activation of the p38 MAPK mitogen-activated protein kinase pathway. Mol. Cancer Therap. 7: 2866-2875, 2008.
25. **Smalley, K.S.M.**, Contractor, R., Nguyen, T.K., Xiao, M., Medina, C.A., Edwards, R., King, A.J., Flaherty, K.T., Bosenberg, M., Herlyn, M., Nathanson, K.L.: Identification of a novel sub-group of melanomas with KIT/CDK4 overexpression Cancer Res. 68: 5743-5752, 2008.
26. Xie, P., Williams, D.S., Atilla-Gokcumen, G.E., Milk, L., Xiao, M., **Smalley, K.S.M.,** Herlyn, M., Meggers, E. & Marmostein, R.: Structure-based design of an organoruthenium Phosphatidly-Inositol-3 kinase inhibitor reveals a switch governing lipid kinase potency and selectivity. ACS Chem. Biol. 3: 305-316, 2008.
27. **Smalley, K.S.M.**, Lioni, M., Dalla Palma, M., Xiao, M., Desai, B., Hansson, J., Flaherty, K.T., Herlyn, M., Nathanson, K.L.: Increased cyclin D1 expression can mediate BRAF inhibitor resistance in BRAF V600E-mutated melanomas. Mol. Cancer Ther. 7: 2876-2883, 2008.
28. **Smalley, K.S.M.**, Xiao, M., Villanueva, J., Nguyen, T., Letrero, R., Wang, Y., Van Belle, P., Flaherty, K.T., Elder, D.E., Nathanson, K.L., Herlyn, M.: CRAF inhibition induces apoptosis in melanomas with non-BRAF V600E mutations. Oncogene 28: 85-94, 2009.
29. **Smalley, K.S.M.**, Nathanson, K.L., Flaherty, K.T.: Genetic subgrouping of melanoma reveals new opportunities for targeted therapy. Cancer Res. 69: 3241-3244, 2009.
30. **Smalley, K.S.M.**, Understanding signaling networks as the basis for molecularly targeted therapy. J. Invest. Derm. 130: 28-37, 2010.
31. Fang, B., Haura, E.B., **Smalley K.S.M,** Eschrich ,S.A, Koomen, J.M.: Methods for Investigation of Targeted Kinase Inhibitor Therapy using Chemical Proteomics and Phosphorylation Profiling. Biochem Pharmacol. 80: 739-747, 2010.
32. **Smalley K.S.M.**: PLX-4032, a small-molecule B-Raf inhibitor for the potential treatment of malignant melanoma. Curr Opin Investig Drugs. 11: 699-706, 2010.
33. Paraiso, K.H., Van Der Kooi, K., Messina, J.L., **Smalley, K.S.M:** Measuring constitutive ERK and AKT activity in cancer cell lines. Methods Enzymol. 484: 49-568, 2010.
34. Paraiso, K.H., Fedorenko, I.V., Cantini, L.P., Munko, A.C., Hall, M., Sondak, V.K., Messina, J.L., Flaherty, K.T., **Smalley, K.S.M.:** Recovery of pERK signaling allows melanoma cells to escape from BRAF inhibitor therapy. Br. J. Cancer. 102:1724-30, 2010.
35. **Smalley, K.S.M.,** Sondak, V.K.: Melanoma: the unlikely poster child for targeted therapy. New Engl. J. Med. 363: 876-878, 2010.

36. Lee, J.T., Li, L., Brafford, T., Einjden, M., Halloram, M.B., Sproesser, K., Haass, N.K., **Smalley, K.S.M.,** Tsai, J., Bollag, G., Herlyn, M.: PLX4032 a potent inhibitor of the BRAF V600E oncogene selectively eliminates V600E-positive melanomas. Pigment Cell Melanoma Res. 23:820-827, 2010.
37. Paraiso, K.H., Xang, Y., Rebecca, V., Abel, E.V., Fedorenko, I.V., Anderson, A.R., Sondak, V.K., Ribas, A., Koomen, J.K., Messina, J.L., **Smalley, K.S.M**.: PTEN loss confers BRAF inhibitor resistance to melanoma cells through suppression of BIM expression. Cancer Res. 71: 2750-2760, 2011. **chosen as a research highlight by Nature Reviews in Clinical Oncology. **one of the highest cited papers in Cancer Research in 2011/2012 – highlighted at the annual meeting.*
38. Koomen, J.M & **Smalley, K.S.M**.: Using quantitative proteomics to understand genotype specific intrinsic drug resistance in melanoma. Oncotarget 2: 329-345, 2011.
39. Sondak, V.K., **Smalley, K.S.M.,** Kudchadkar, R., Grippon, S., Kirkpatrick, P.: Ipilumumab. Nat. Rev. Drug Disc. 10:411-412, 2011.
40. Fedorenko, I.V., Paraiso, K.H.T., **Smalley, K.S.M.**: Intrinsic and acquired BRAF inhibitor resistance in BRAF V600E mutant melanoma. Biochem. Pharmacol. 82: 201-209, 2011.
41. Remily-Wood, E.R., Liu, R.Z., Xiang, Y., Chen, Y., Thomas, C.E., Rajyaguru, N., Kaufman, L.M., Ochoa, J.E., Hazlehurst, L., Pinilla-Ibarz, J., Lancet, J., Zhang, G., Haura, E., Shibata, D., Yeatman, T., **Smalley, K.S.M,** Dalton, W.S., Huang, E., Scott, E., Bloom, G.C., Eschrich, S.A., Koomen, J.M.: A database of reaction monitoring mass spectrometry assays for elucidating therapeutic response in cancer. Proteomics Clin App. 5: 383-396, 2011.
42. Flach, E.H*., Rebecca, V.W., Herlyn, M., **Smalley, K.S.M.,*** Anderson, A.R.: Fibroblasts contribute to melanoma growth and drug resistance. Mol. Pharm. 8: 2039-2049, 2011. **co-corresponding authors.*
43. Paraiso, K.H.T., Haarberg, H.E., Wood, E., Rebecca, V.W., Chen, Y., Xiang, Y., Ribas, A., Lo, R.S., Weber, J.S., Sondak, V.K., John, J.K., Sarnaik, A., Koomen, J.M., **Smalley, K.S.M.:** The heat shock protein-90 inhibitor XL888 overcomes BRAF inhibitor resistance mediated through diverse mechanisms. Clin. Cancer Res. 18: 2502-2514, 2012. **selected as a highlight of the issue with accompanying editorial.*
44. Kudchadkar, R., Paraiso, K.H.T., **Smalley, K.S.M.:** Targeting Mutant BRAF in Melanoma: Current Status and Future Development of Combination Therapy Strategies. Cancer J. 18: 124-131, 2012.
45. Rebecca, V.W., Sondak, V.K., **Smalley, K.S.M.:** From mummies to mutations: A brief history of melanoma. Melanoma Res. 22: 114-122, 2012.
46. **Smalley, K.S.M.,** McArthur, G.A.: Melanoma targeted therapy: This time it's personal. Semin. Oncol. 39: 204-214, 2012.
47. John, J.K. Cantini, L.P., Abel, E.V., Paganos, N., Meggers, E., Mathew, R., Messina, J.L., Herlyn, M., **Smalley, K.S.M.:** Inhibition of glycogen synthase kinase-3 blocks melanoma cell migration and melanoma/stromal interactions through downregulation of N-cadherin and FAK activity. J. Invest. Derm. Jul 19. doi: 10.1038/jid.2012.237, 2012.
48. Paraiso, K.H., **Smalley, K.S.M.:** Making sense of MEK1 mutations in intrinsic and acquired BRAF inhibitor resistance. Cancer Discovery. 2: 390-392, 2012.
49. Fedorenko, I.V., Gibney, G.T., **Smalley, K.S.M.:** *NRAS* mutant melanoma: biological behavior and future strategies for therapeutic management. Oncogene. Oct 15. Doi: 10.1038/onc.2012.453, 2012.
50. Beck D, Niessner H, **Smalley K.S.M.,** Flaherty K, Paraiso K. H.T., Busch C, Sinnberg T, Vasseur S, Iovanna J, Drießen S, Stork B, Wesselborg S, Schaller M, Biedermann T, Bauer J, Lasithiotakis K, Weide B, Eberle J, Schittek B, Schadendorf D, Garbe C, Kulms D and Meier F. Vemurafenib potently induces endoplasmic reticulum (ER) stress and ER stress-mediated apoptosis in BRAFV600E melanoma cells. Sci. Signaling. 6(260):ra7, 2013.
51. Haarberg, H.E., Paraiso, K.H.T., Rebecca, V.W., Wood, E., Sondak, V.K., Koomen, J.M., **Smalley, K.S.M.:** Inhibition of Wee1, AKT and CDK4 underlies the efficacy of the HSP90 inhibitor XL888 in an in vivo model of NRAS mutant melanoma. Mol. Cancer Ther. 12: 901-912, 2013.
52. Paraiso, K.H.T., **Smalley, K.S.M.:** Fibroblast mediated drug resistance in cancer. Biochem. Pharmacol. 85(8):1033-104, 2013.
53. Desai, B.M., Villanueva, J., Nguyen, T.T., Lioni, M., Xiao, M., Kong, J., Krepler, C., Vultur, A., Flaherty, K.T., Nathanson, K.L., **Smalley K.S.M,** Herlyn, M.: The Anti-Melanoma Activity of Dinaciclib, a Cyclin-Dependent Kinase Inhibitor, Is Dependent on p53 Signaling. PLoS One. 2013;8(3):e59588. doi: 10.1371/journal.pone.0059588.

54. Gibney, G.T., **Smalley, K.S.M.:** An unholy alliance: co-operation between BRAF and NF1 in melanoma development and drug resistance. Cancer Discovery. 3: 260-263, 2013.
55. Gibney, G.T., Messina, J.L., Fedorenko, I.V., Sondak, V.K., **Smalley K.S.M.:** Paradoxical oncogenesis – the long term consequences of BRAF inhibition in melanoma. Nat. Rev. Clin. Oncol. 10: 390-399, 2013.
56. Tafreshi, N.K., Silva, A., Estrella, V.C., McCardle, T.W., Chen, T., Jeune-Smith, Y., Lloyd, M.C., Enkemann, S.A., **Smalley, K.S.M.,** Sondak, V.K., Vagner, J., Morse, D.L: In vivo and in silico pharmacokinetics and biodistribution of a melanocortin receptor 1 targeted agent in preclinical models of melanoma. Mol. Pharm. 10: 3175-3185, 2013.
57. Pimiento, J.M., Larkin, E.M., **Smalley, K.S.M.,** Wiersma, G.L., Monks, N.R., Fedorenko, I.V., Peterson, C.A., Nickoloff, B.J.: Melanoma genotypes and phenotypes get personal. Lab Invest. 93:858-867, 2013.
58. Weber, J.L., **Smalley, K.S.M**., Sondak, V.K., Gibney, G.T.: Conjunctival melanomas harbor BRAF and NRAS mutations. Clin. Cancer Res. 19: 6329-6330, 2013.
59. Kim, E., Rebecca, V.W., Fedorenko, I.V., Mathew, R., Messina, J.L., **Smalley, K.S.M.**, Anderson, A.R.: Senescent fibroblasts in melanoma initiation and progression: an integrated theoretical, experimental, and clinical approach. Cancer Res. Epub September 30th 2013
60. Rebecca, V.W., Fedorenko, I.V., Kim, E., Anderson, A.R., Sondak, V.K., Amavaradi, R.K., Gibney, G.T., Kudchadkar, R.R., **Smalley, K.S.M.:** Inhibition of autophagy enhances the effects of the AKT inhibitor MK-2206 when combined with paclitaxel and carboplatin in *BRAF* wild-type melanoma. Pigment Cell Melanoma Res. 2014 27: 465-478
61. Tiago M, Oliveira EM, Brohem CA, Pennacchi PC, Paes RD, Haga RB, Campa A, Barros SB, **Smalley, K.S.M.,** Silvya ME. Tissue Eng Part A. 2014 Feb 18
62. Rebecca, V.W., Wood, E., Fedorenko, I.V., Paraiso, K.H.T., Haarberg, H.E., Chen, Y., Xiang, Y., Sarnaik, A., Gibney, G.T., Sondak, V.K., Koomen, J.M., **Smalley, K.S.M.:** Evaluating melanoma drug response and therapeutic escape with quantitative proteomics. Mol. Cell Proteomics 13: 1844-1854, 2014.
63. **Smalley, K.S.M.,** Weber, J.S.: Up close and personal: the challenges of precision medicine in melanoma. J. Natl. Cancer Inst. 2014 Feb 1;106(2):djt443. doi: 10.1093/jnci/djt443.
64. Johnson, D.B., **Smalley, K.S.M.,** Sosman, J.A.: Molecular Pathways: Targeting NRAS in Melanoma and Acute Myelogenous Leukemia. Clin Cancer Res. 15: 4186-4192, 2014.
65. Fedorenko, I.V., Fang, B., Koomen, J.M., Gibney, G.T., **Smalley, K.S.M.:** Amuvatinib has cytotoxic effects against NRAS-mutant melanoma but not BRAF-mutant melanoma. Melanoma Res. 24: 448-453, 2014.
66. Rebecca, V.W., **Smalley, K.S.M:.** Change or die: Targeting adaptive signaling in cancer cells. Biochem Pharmacol. 91: 417-25, 2014
67. Rebecca, V.W., Alicea, G.M., Paraiso, K.H., Lawrence, H., Gibney, G.T., **Smalley, K.S.M.:** Vertical inhibition of the MAPK pathway enhances therapeutic responses in NRAS-mutant melanoma. Pigment Cell Melanoma Res. 2014 27: 1154-1158, 2014.
68. Fedorenko IV, Gibney GT, Sondak VK, **Smalley, K.S.M.:** beyond BRAF: Where next for melanoma targeted therapy? Br J Cancer. 112: 217-226, 2015.
69. Fedorenko, I.V., Fang, B., Munko, A.C., Gibney, G.T., Koomen, J.M., **Smalley, K.S.M.:** Phosphoproteomic analysis of basal and therapy-induced adaptive signaling networks in *BRAF* and *NRAS* mutant melanoma. Proteomics. 15: 327-339, 2015.
70. Smyth, T., Paraiso, K.H.T., Hearn, K., Rodriguez-Lopez, A.M., Munck, J.M., Haarberg, H.E., Sondak, V.K., Thompson, N.T., Azab, M., Lyons, J.F., **Smalley, K.S.M.,*** Wallis, N.*: Inhibition of HSP90 by AT13387 Delays the Emergence of Resistance to BRAF Inhibitors and Overcomes Resistance to dual BRAF and MEK Inhibition in Melanoma Models. Mol Cancer Ther.13: 2793-2804, 2014. *co-corresponding authors.
71. Paraiso, K.H., Das Thakur, M., Fang, B., Koomen, J.M., Fedorenko, I.V., Tsao, H., Flaherty, K.T., Sondak, V.K., Messina, J.L., Pasquale, E., Villagra, A., Rao, U., Kirkwood, J.M., Meier, F., Sloot, S., Gibney, G.T., Stuart, D.T., Tawbi, H., **Smalley, K.S.M**.: Ligand independent EphA2 signaling drives the adoption of a targeted therapy metastatic phenotype. Cancer Discovery. 5: 264-273, 2015.
72. Phadke, M., Sini, P., **Smalley, K.S.M.:** The novel ATP-competitive MEK/Aurora kinase inhibitor BI-847325 overcomes acquired BRAF inhibitor resistance through suppression of Mcl-1 and MEK expression. Mol. Cancer Ther. 14: 1354-1366, 2015.

73. Fedorenko, I.V., Abel, E.A., Koomen, J.M., Fang, B., Wood, E., Chen, Y.A., Fisher, K.B., Iyegnar, S., Dahlman, K., Wargo, J., Flaherty. K.T., Sosman, J.A., Sondak, V.K., Messina, J.L., Gibney, G., **Smalley, K.S.M.:** Fibronectin induction abrogates the BRAF inhibitor response of *BRAF* V600E/PTEN-null melanoma cells. Oncogene. *35:* 1225-35, 2016.
74. **Smalley, K.S.M.,** Sondak, V.K.: BRAF/MEK inhibition is the standard of care for *BRAF* mutant melanoma. Lancet. 386: 410-412, 2015.
75. Woan, K.V., Lienlaf, M., Perez-Villaroel, P., Lee, C., Cheng, F., Knox, T., Woods, D.M., Barrios, K., Powers, J., Sahakian, E., Wang, H.W., Canales, J., Marante, D., **Smalley, K.S.M.**, Bergman, J., Seto, E., Kozikowski, A., Pinilla-Ibarz, J., Sarnaik, A., Celis, E., Weber, J., Sotomayor, E.M., Villagra A.: Targeting histone deacetylase 6 mediates a dual anti-melanoma effect: Enhanced antitumor immunity and impaired cell proliferation. Mol Oncol. 9: 1447-1457, 2015.
76. Phadke, M., Gibney, G., Rich, C., Fedorenko, I.V., Chen A., Kudchadkar, R., Sondak, V.K., Weber, J., Messina, J., **Smalley, K.S.M.:** XL888 limits vemurafenib-induced proliferative skin events by suppressing paradoxical MAPK activation. J Invest Derm. 135: 2542-2544, 2015.
77. Fedorenko, I.V., Wargo, J., Flaherty, K.T., Messina, J.L., **Smalley, K.S.M.:** BRAF inhibition generates a host-tumor niche that mediates therapeutic escape. J Invest Derm.135: 3115-3124, 2015.
78. Sloot, S., Zager, J.S., Kudchadkar, R.R., Messina, J.L., Benedict, J.J., Gonzalez, R.J., DeConti, R., Turner, L.M., McCardle, T., **Smalley, K.S.M**, Weber, J.S., Sondak, V.K., Gibney, G.T.: BRAF inhibition for advanced locoregional BRAF V600E mutant melanoma: a potential neoadjuvant strategy. Melanoma. Res 26: 83-77, 2016.
79. Lienlaf, M., Perez-Villarroel, P., Knox, T., Pabon, M., Sahakian, E., Powers, J., Woan, K.V., Lee, C., Cheng, F., Deng, S., **Smalley, K.S.M**, Montecino, M., Kozikowski, A., Pinilla-Ibarz, J., Sarnaik, A., Seto, E., Weber, J., Sotomayor, E.M., Villagra, A.: Essential role of HDAC8 in the regulation of PD-L1 in melanoma. Mol Oncol. 10: 735-750, 2016.
80. **Smalley, K.S.M.,** Eroglu, Z., Sondak, V.K.: Combination therapies for melanoma: a new standard of care? Am J. Clin Derm. 17: 99-105, 2016.
81. Klionsky, D.J., (~1500 authors), **Smalley, K.S.M**. et al.: Guidelines for the use and interpretation of assays for monitoring autophagy. Autophagy, 12: 1-222, 2016.
82. **Smalley, K.S.M.**, Fedorenko, I.V., Kenchappa, R., Sahebjam, S., Forsyth, P.A.: Managing leptomeningeal melanoma metastases in the era of targeted and immune therapy. Int. J Cancer. *In Press*
83. Merlino, G., Herlyn, M., Fisher, D.E., Bastian, B.C., Flaherty, K.T., Davies, M.A., Wargom J.A., Curiel-Lewandrowski, C., Weber, M.J., Leachman, S.A., Soengas, M.S., McMahon, M., Harbour, J.W., Swetter SM, Aplin AE, Atkins MB, Bosenberg MW, Dummer R, Gershenwald JE, Halpern AC, Herlyn D, Karakousis GC, Kirkwood JM, Krauthammer M, Lo RS, Long GV, McArthur G, Ribas A, Schuchter L, Sosman, J.A., **Smalley K.S.M**, Steeg, P, Thomas, N.E., Tsao, H., Tueting, T., Weeraratna, A., Xu, G., Lomax, R., Martin, A., Silverstein, S., Turnham, T., Ronai, Z.A.: The State of Melanoma: Challenges and Opportunities. Pigment Cell Melanoma Res. 29: 404-416, 2016
84. **Smalley, K.S.M**., Fedorenko, I.V., Kenchappa, R., Sahebjam, S., Forysth, P.A.: Managing leptomeningeal melanoma metastases in the era of immune and targeted therapy. Int. J. Cancer. 139: 1195-1201, 2016
85. Fedorenko, I.V., Evernden, B., Kenchappa, R.S., Sahebjam, S., Ryzhova, E., Puskas, J., McIntosh, L., Caceres, G., Magliocco, A., Etame, A., Harbour, J.W., **Smalley, K.S.M.*,** Forsyth, P.A.: A rare case of leptomeningeal carcinomatosis in a patient with uveal melanoma: case report and review of literature. Melanoma Res. 26: 481-486, 2016. *Corresponding author
86. Emmons, M.F., Faião-Flores, F., **Smalley, K.S.M**: The role of phenotypic plasticity in the escape of cancer cells from targeted therapy. Biochem Pharmacol. 122: 1-9, 2016
87. Kim, E., Rebecca, V.W., **Smalley, K.S.**, Anderson, A.R.: Phase I trials in melanoma: A framework to translate preclinical findings into the clinic. Eur. J. Cancer. 67: 213-222, 2016.
88. Faião-Flores, F., Alves-Fernandes, D.K., Pennacchi, P.C., Sandri, S., Vicente, A.L., Scapulatempo-Neto, C., Vazquez, V.L., Reis, R.M., Chauhan, J., Goding, C.R., **Smalley, K.S.M.**, Maria-Engler, S.S.: Targeting the hedgehog transciprtion factors FLI1 and GLI2 restores sensitivity to vemurafenib resistant human melanoma cells. Oncogene Epub *Oct 17th 2016.*

89. Sharma, R., Fedorenko, I.V., Spence, P., Sondak, V.K., **Smalley, K.S.M.***, Koomen, J.M.*: Activity-Based Protein Profiling Shows Heterogeneous Signaling Adaptations to BRAF Inhibition. J. Proteome Res. 15: 4476-4489, 2016. *Co-corresponding authors
90. Massaro, R.R., Faião-Flores, F., Rebecca, V.W., Sandri, S., Alves-Fernandes, D.K., Pennacchi, P.C., **Smalley, K.S.M.**, Maria-Engler, S.S.: Inhibition of proliferation and invasion in 2D and 3D models by 2-methoxyestradiol in human melanoma cells. Pharmacol Res. 119: 242-250, 2017.
91. Li, J., Smalley, I., Schell, M.J., **Smalley, K.S.M.,** Chen, Y.A.: SinCHet: a MATLAB toolbox for single cell heterogeneity analysis in cancer. Bioinformatics. 2017 Sep 15;33(18):2951-2953. doi: 10.1093/bioinformatics/btx297.
92. Forsyth, P.A., Smalley, **K.S.M.,** Sondak, V.K.: BRAF-MEK inhibition in melanoma brain metastases: a new hope. Lancet Oncol. 2017 Jul;18(7):836-837. doi: 10.1016/S1470-2045(17)30449-7.
93. Niessner, H., Sinnberg, T., Kosnopfel, C., **Smalley, K.S.M.,** Beck, D., Praetorius, C., Mai, M., Beissert, S., Kulms, D., Schaller, M., Garbe, C., Flaherty, K.T., Westphal, D., Wanke, I., Meier, F. : BRAF Inhibitors Amplify the Proapoptotic Activity of MEK Inhibitors by Inducing ER Stress in NRAS-Mutant Melanoma. Clin Cancer Res. 2017 Oct 15;23(20):6203-6214. doi: 10.1158/1078-0432.CCR-17-0098. Epub 2017 Jul 19.
94. Sloot, S., Chen, Y.A., Zhao, X., Weber, J.L., Benedict, J.J., Mulé, J.J., **Smalley, K.S.M**, Weber, J.S., Zager, J.S., Forsyth, P.A., Sondak, V.K., Gibney, G.T.: Improved survival of patients with melanoma brain metastases in the era of targeted BRAF and immune checkpoint therapies. Cancer. 2018 Jan 15;124(2):297-305. doi: 10.1002/cncr.30946.
95. Robinson, J.P., Rebecca, V.W., Kircher, D.A., Silvis, M.R., Smalley, I., Gibney, G.T., Lastwika, K.J., Chen, G., Davies, M.A., Grossman, D., **Smalley, K.S.M.,** Holmen, S.L., VanBrocklin, M.W.: Resistance mechanisms to genetic suppression of mutant NRAS in melanoma. Melanoma Res. 2017 Dec;27(6):545-557. doi: 10.1097/CMR.0000000000000403.
96. Phadke, M., Remsing, Rix, L.L., Smalley, I., Bryant, A.T., Luo, Y., Lawrence, H.R., Schaible, B.J., Chen, Y.A., Rix, U., **Smalley, K.S.M.**: Dabrafenib inhibits the growth of BRAF-WT cancers through CDK16 and NEK9 inhibition. Mol Oncol. 2018 Jan;12(1):74-88. doi: 10.1002/1878-0261.12152.
97. Verduzco, D., Kuenzi, B.M., Kinose, F., Sondak, V.K., Eroglu, Z., Rix, U., **Smalley, K.S.M.**: Ceritinib Enhances the Efficacy of Trametinib in *BRAF/NRAS*-Wild-Type Melanoma Cell Lines. Mol Cancer Ther. 2018 Jan;17(1):73-83. doi: 10.1158/1535-7163.MCT-17-0196.
98. Smalley, I., **Smalley, K.S.M.:** ERK Inhibition: A New Front in the War against MAPK Pathway-Driven Cancers? Cancer Discov. 2018 Feb;8(2):140-142. doi: 10.1158/2159-8290.CD-17-1355.
99. Sloot, S., Chen, Y,A., Zhao, X., Weber, J.L., Benedict, J.J., Mulé, J.J., **Smalley, K.S.M**, Weber, J.S., Zager, J.S., Forsyth, P.A., Sondak, V.K., Gibney, G.T.: Reply to Improving the survival of patients with American Joint Committee on Cancer stage III and IV melanoma. Cancer. 2018 Mar 15. doi: 10.1002/cncr.31261. [Epub ahead of print]
100. Eroglu, Z. Chen,Y.A. Gibney, G.T., Weber, J.S., Kudchadkar, R., Khushalani, N.I., Markowitz, J., Brohl, A.S. Tetteh, L.F., Li, J., Zhao, X., Sharma, R., Darville, L.F., Fang, B., Smalley, I., Messina, J.L., Koomen, J.M., Sondak, V.K., **Smalley, K.S.M.:** Combined BRAF and HSP90 inhibition in patients with unresectable *BRAF* V600E mutant melanoma. Clin. Cancer Res. 2018 24: 5516-5524
101. Eroglu, Z., Holmen, S.L., Chen, Q., Khushalani, N.I., Amaravadi, R., Thomas, R., Ahmed, K.A., Tawbi, H., Chandra, S., Markowitz, J., Smalley, I., Liu, J.K.C, Chen, Y.A., Najjar, Y.G., Karreth, F.A., Abate-Daga, D., Glitza, I.C., Sosman, J.A., Sondak, V.K., Bosenberg, M., Herlyn, M., Atkins, M.B., Kluger, H., Margolin. K, Forsyth, P.A., Davies, M.A., **Smalley, K.S.M.**: Melanoma central nervous system metastases: an update to approaches challenges and opportunities. Pigment Cell Melanoma Res. Epub 2019 Feb 3
102. Mancera, N., **Smalley, K.S.M.,** Margo, C.A.: Melanoma of the eyelid and periocular skin: Histopathologic classification and molecular pathology. Surv. Opthalmol. Epub Dec 20th 2018.
103. Alves-Fernandes, D.K., Oliveira, É.A., Faião-Flores, F., Alicea-Rebecca, G., Weeraratna, A.T., **Smalley, K.S.M.,** Barros, S.B.M., Maria-Engler SS.: ER stress promotes antitumor effects in BRAFi/MEKi resistant human melanoma induced by natural compound 4-nerolidylcathecol (4-NC). Pharmacol Res. 2019 Mar;141:63-72

104. Emmons, M.F., Flores, F., Sharma, R., Thapa, R., Messina, J.L., Becker, J.C., Schadendorf, D., Seto, E., Sondak, V.K., Koomen, J.M., Chen, Y.A., Lau, E., Wan, L., Licht, J.D., **Smalley, K.S.M**.: HDAC8 regulates a stress response pathway in melanoma that mediates escape from BRAF inhibitor therapy. Cancer Res. 2019 Jun 1;79(11):2947-2961
105. Yin, Q., Han, H., Fang, B., Zhang, G., Zhange, C., Roberts, E., Izumi, V., Zheng, M., Jiang, S., Yin, X., Kim, M., Cai, J., Haura, E., Koomen, J.M., **Smalley, K.S.M**., Wan, L. : K27-linked Ubiquitination of BRAF by ITCH Engages Cytokine Response to Maintain MEK-ERK Signaling. Nature Comm. 2019 Apr 23;10(1):1870.
106. Xie, M., Zheng, H., Madan-Lala, R., Dai, W., Gimbrone, N.T., Chen, Z., Kinose, F., Blackstone, S.A., **Smalley, K.S.M.,** Cress, W.D., Haura, E.B., Rix, U., Beg, A.A.: MEK inhibition modulates cytokine response to mediate therapeutic efficacy in lung cancer. Cancer Res. 2019 Jul 30.
107. Smalley, I., Kim, E., Li, J., Spence, P., Wyatt, C.J., Eroglu, Z., Sondak, V.K., Messina. J.L., Babacan, N.A., Maria-Engler, S.S., De Armas, L., Williams, S.L., Gatenby, R.A., Chen, Y.A., Anderson, A.R.A., **Smalley, K.S.M.:** Leveraging transcriptional dynamics to improve therapeutic responses in melanoma. EBioMedicine. 2019 Oct 5. pii: S2352-3964(19)30627-9
108. Faião-Flores, F., Emmons, M.F., Durante, M.A., Kinose, F., Saha, B., Fang, B., Koomen, J.M., Chellappan, S.P., Maria-Engler, S.S., Rix, U., Licht, J.D., Harbour, J.W., **Smalley, K.S.M.:** HDAC inhibition enhances the in vivo efficacy of MEK inhibitor therapy in uveal melanoma Clin. Cancer Res. 2019 Sep 15;25(18):5686-5701
109. Goncalves, J., Flores, F.F., Emmons, M.F., Aplin, A.E., Harbour, J.W., Licht, J.D., Wink, M., **Smalley, K.S.M.:** Decitabine limits escape from MEK inhibition in uveal melanoma Pigment Cell Melanoma Res. *Epub* Nov 23rd 2019.
110. Faião-Flores F, **Smalley KS**. Histone deacetylase inhibitors: a promising partner for MEK inhibitors in uveal melanoma? Melanoma Manag. 2019 Dec 16;6(4): MMT29
111. Smalley, I, Law, V., Wyatt, C., Evernden, B., Fang, B., Koomen, J.M., Welsh, E.A., Macaulay, R., Forsyth, P.A., **Smalley, K.S.M.:** Proteomic analysis of CSF from patients with leptomeningeal melanoma metastases identifies signatures associated with disease progression and therapeutic resistance. Clin. Cancer Res. *Epub* Jan 10th 2020.
112. Piña, Y., Fusco, M.J., Macaulay, R.J., Walko, C.M., Peguero, E., Evernden, B.R., **Smalley, K.S.M.**, Forsyth, P. Using personalized medicine in gliomas: a genomic approach to diagnosis and overcoming treatment resistance in a case with pleomorphic xanthoastrocytoma. J Neurol. 2020 Mar;267(3):783-790.
113. Glitza, I.C., **Smalley, K.S.M,** Brastianos, P.K., Davies, M.A., McCutcheon, I., Liu, J.K.C, Ahmed, K.A., Arrington, J.A., Evernden, B.R., Smalley, I., Eroglu, Z., Khushalani, N., Margolin, K., Kluger, H., Atkins, M.B., Tawbi, H., Boire, A., Forsyth, P.: Leptomeningeal disease in melanoma patients: An update to treatment, challenges, and future directions. Pigment Cell Melanoma Res. 2020 Jan 8. doi: 10.1111/pcmr.12861.
114. **Smalley, K.S.M.** Two Worlds Collide: Unraveling the Role of the Immune System in BRAF-MEK Inhibitor Responses. Cancer Discovery 2020 Feb;10(2):176-178.
115. Yin, Q., Wyatt, C.J., Han, T., **Smalley, K.S.M.,** Wan, L.: ITCH as a potential therapeutic target in human cancers. Semin Cancer Biol. 2020 Mar 10.
116. Chen, Y.A., Teer, J.K., Eroglu, Z., Wu, J.Y., Koomen, J.M., Karreth, F.A., Messina, J.L., **Smalley, K.S.M.:** Translational pathology, genomics and the development of systemic therapies for acral melanoma. Semin Cancer Biol. 2020 Apr;61:149-157.
117. Wang, X., Yu, X., Krauthammer, M., Hugo, W., Duan, C., Kanetsky, P.A., Teer, J.K., Thompson, Z.J., Kalos, D., Tsai, K.Y., **Smalley, K.S.M.**, Sondak, V.K., Chen, Y.A., Conejo-Garcia, J.R.: The Association of *MUC16* Mutation with Tumor Mutation Burden and Its Prognostic Implications in Cutaneous Melanoma Cancer Epidemiol Biomarkers Prev. 2020 Sep;29(9):1792-1799.
118. Zhang, C., Smalley, I., Emmons, M.F., Sharma, R., Izumi, V., Messina, J., Koomen, J.M., Pasquale, E.B., Forsyth, P.A., **Smalley, K.S.M.:** Noncanonical EphA2 Signaling Is a Driver of Tumor-Endothelial Cell Interactions and Metastatic Dissemination in BRAF Inhibitor–Resistant Melanoma. J Invest Dermatol. 2020 Sep 2:S0022-202X(20)32047-9. doi: 10.1016/j.jid.2020.08.012. Online ahead of print.
119. Taylor, N.J,, Gaynanova, I., Eschrich, S.A., Welsh, E.A., Garrett, T.J., Beecher, C., Sharma, R., Koomen, J.M., **Smalley, K.S.M.**, Messina, J.L., Kanetsky, P.A: . Metabolomics of primary cutaneous

melanoma and matched adjacent extratumoral microenvironment PLoS One. 2020 Oct 27;15(10):e0240849

120. Phadke, M., Ozgun, A., Eroglu, Z., **Smalley, K.S.M.:** Melanoma brain metastases: Biological basis and novel therapeutic strategies. Exp Dermatol. 2021 Jan 17. doi: 10.1111/exd.14286. Epub ahead of print. PMID: 33455008.
121. Law, V., Baldwin, M., Ramamoorthi, G., Kodumudi, K., Tran, N., Smalley, I., Duckett, D., Kalinski, P., Czerniecki, B., **Smalley. K.S.M,** Forsyth, P.A.: A Murine Ommaya Xenograft Model to Study Direct-Targeted Therapy of Leptomeningeal Disease. J Vis Exp. 2021 Jan 29;(167). doi: 10.3791/62033. PMID: 33586709.
122. Neelature Sriramareddy, S., **Smalley, K.S.M**.: MEK-ing the Most of It: Strategies to Co-target Gαq and MAPK in Uveal Melanoma. Clin Cancer Res. 2021 Mar 1;27(5):1217-1219. doi: 10.1158/1078-0432.CCR-20-4530. Epub 2020 Dec 22. PMID: 33355300; PMCID: PMC7925419.
123. Phadke, M.S., Chen, Z., Li, J., Mohamed, E., Davies, M.A., Smalley, I., Duckett, D., Palve, V., Czernieck,i B.J., Forsyth, P.A., Noyes, D., Adeegbe, D.O., Eroglu, Z., Nguyen, K.T., Tsai, K.Y., Rix, U., Burd, C.E., Chen, Y.A., Rodriguez, P.C., **Smalley, K.S.M.:** Targeted therapy given after anti-PD-1 leads to prolonged responses in mouse melanoma models through sustained antitumor immunity. Cancer Immunol. Res. 9: 554-567, 2021. PMID: 33653716. * *highlighted on the front cover.*
124. Smalley, I., Chen, Z., Phadke, M.S., Li, J., Yu, X., Wyatt, C., Evernden, B., Messina, J.L., Sarnaik, A., Sondak, V.K., Zhang, C., Law, V., Tran, N., Etame, A., Macaulay, RJB, Eroglu, Z., Forsyth, P.A., Rodriguez, P.C., Chen, Y.A., **Smalley, K.S.M.:** Single cell characterization of the immune microenvironment of melanoma brain and leptomeningeal metastases. Clin. Cancer Res. 27: 4109-4125, 2021. PMID: 34035069.
125. **Smalley, K.S.M.,** Teer, J.K., Chen, Y.A., Wu, J.Y., Yao, J., Koomen, J.M., Chen, W.S., Rodriguez-Waitkus, P., Karreth, F.A., Messina, J.L.L A Mutational Survey of Acral Nevi. JAMA Dermatol. 157: 831-835, 2021. PMID: 33978681.
126. Basu, A., Albert, G.K., Awshah, S., Datta, J., Kodumudi, K.N., Gallen, C., Beyer, A., **Smalley, K.S.M.**, Rodriguez, P.C., Duckett, D.R., Forsyth, P.A., Soyano, A., Koski, G.K., Lima, Barros Costa R., Han, H., Soliman, H., Lee, M.C., Kalinski, P., Czerniecki, B.J.: Identification of Immunogenic MHC Class II Human HER3 Peptides that Mediate Anti-HER3 $CD4^+$ Th1 Responses and Potential Use as a Cancer Vaccine. Cancer Immunol Res. 2022 Jan;10(1):108-125. doi: 10.1158/2326-6066.CIR-21-0454. Epub 2021 Nov 16. PMID: 34785506.
127. Law, V., Chen, Z., Vena, F., Smalley, I., Macaulay, R., Evernden, B.R., Tran, N., Pina, Y., Puskas, J., Caceres, G., Bayle, S., Johnson, J., Liu, J.K.C, Etame, A., Vogelbaum, M., Rodriguez, P., Duckett, D., Czerniecki, B., Chen, A., **Smalley, K.S.M,** Forsyth, P.A.: A preclinical model of patient-derived cerebrospinal fluid circulating tumor cells for experimental therapeutics in leptomeningeal disease from melanoma. Neuro Oncol. 2022 Feb 25:noac054. doi: 10.1093/neuonc/noac054. Epub ahead of print. PMID: 35213727.
128. Li, J., Smalley, I., Chen, Z., Wu, J.Y., Phadke, M.S., Teer, J.K., Nguyen, T., Karreth, F.A., Koomen, J.M., Sarnaik, A.A., Zager, J.S., Khushalani, N.I., Tarhini, A.A., Sondak, V.K., Rodriguez, P.C., Messina, J.L., Chen, Y.A., **Smalley, K.S.M.:** Single cell characterization of the cellular landscape of acral melanoma identifies novel targets for immunotherapy. Clin Cancer Res. 2022 Mar 4:clincanres.3145.2021. doi: 10.1158/1078-0432.CCR-21-3145. Epub ahead of print. PMID: 35247927.
129. Sriramareddy, S.N., Faião-Flores, F., Emmons, M.F., Saha, B., Chellappan, S., Wyatt, C., Smalley, I., Licht, J.D., Durante, M.A., Harbour, J.W., **Smalley, K.S.M.:** HDAC11 activity contributes to MEK inhibitor escape in uveal melanoma. Cancer Gene Ther. 2022 Mar 24. doi: 10.1038/s41417-022-00452-7. Epub ahead of print. PMID: 35332245.
130. Karz A., Dimitrova, M., Kleffman, K., Alvarez-Breckenridge, C., Atkins, M.B., Boire, A., Bosenberg, M., Brastianos, P., Cahill, D.P., Chen, Q., Ferguson, S., Forsyth, P., Glitza Oliva, I.C., Goldberg, S.B., Holmen, S.L., Knisely, J.P.S., Merlino, G., Nguyen, D.X., Pacold, M.E., Perez-Guijarro, E., **Smalley, K.S.M.**, Tawbi, H.A., Wen, P.Y., Davies, M.A., Kluger, H.M., Mehnert, J.M., Hernando, E.: Melanoma central nervous system metastases: An update to approaches, challenges, and opportunities. Pigment Cell Melanoma Res. 2022 Nov;35(6):554-572. doi: 10.1111/pcmr.13059. Epub 2022 Sep 1. PMID: 35912544.

131. Kaler, C.J., Dollar, J.J., Cruz, A.M., Kuznetsoff, J.N., Sanchez, M.I., Decatur, C.L., Licht, J.D., **Smalley, K.S.M.**, Correa, Z.M., Kurtenbach, S., Harbour, J.W.: BAP1 Loss Promotes Suppressive Tumor Immune Microenvironment via Upregulation of PROS1 in Class 2 Uveal Melanomas. Cancers (Basel). 2022 Jul 28;14(15):3678. doi: 10.3390/cancers14153678. PMID: 35954340; PMCID: PMC9367253.
132. Thompson, Z.J., Teer, J.K., Li, J., Chen, Z., Welsh, E.A., Zhang, Y., Ayoubi, N., Eroglu, Z., Tan, A.C., **Smalley, K.S.M.**, Chen, Y.A.: Drepmel-A Multi-Omics Melanoma Drug Repurposing Resource for Prioritizing Drug Combinations and Understanding Tumor Microenvironment. Cells. 2022 Sep 16;11(18):2894. doi: 10.3390/cells11182894. PMID: 36139469; PMCID: PMC9497118.
133. Mandula, J.K., Chang, S., Mohamed, E., Jimenez, R., Sierra-Mondragon, R.A., Chang, D.C., Obermayer, A.N., Moran-Segura, C.M., Das, S., Vazquez-Martinez, J.A., Prieto, K., Chen, A., **Smalley, K.S.M.**, Czerniecki, B., Forsyth, P., Koya, R.C., Ruffell, B., Cubillos-Ruiz, J.R., Munn, D.H., Shaw, T.I., Conejo-Garcia, J.R., Rodriguez, P.C.: Ablation of the endoplasmic reticulum stress kinase PERK induces paraptosis and type I interferon to promote anti-tumor T cell responses. Cancer Cell. 2022 Oct 10;40(10):1145-1160.e9. doi: 10.1016/j.ccell.2022.08.016. Epub 2022 Sep 22. PMID: 36150390; PMCID: PMC9561067.
134. Velayutham, S., Seal, T., Danthurthy, S., Zaias, J., **Smalley, K.S.M.,** Minond, D.: In Vivo Acute Toxicity Studies of Novel Anti-Melanoma Compounds Downregulators of hnRNPH1/H2. Biomolecules. 2023 Feb 10;13(2):349. doi: 10.3390/biom13020349. PMID: 36830718; PMCID: PMC9953269.
135. Phadke, M.S. & **Smalley, K.S.M.** : Targeting mutant NRAS in melanoma. J Clin Oncol. 2023 Mar 22:JCO2300205. doi: 10.1200/JCO.23.00205. Epub ahead of print. PMID: 36947724.
136. Mooradian, M.J., Cleary, J.M., Giobbie-Hurder, A., Darville, L.N.F, Parikh, A., Buchbinder, E.I., Cohen, J.V., Lawrence, D.P., Shapiro, G.I., Keer, H., Chen, H.X., Ivy, S.P., **Smalley, K.S.M.**, Koomen, J.M., Sullivan, R.J.: Dose-escalation trial of combination dabrafenib, trametinib, and AT13387 in patients with BRAF-mutant solid tumors. Cancer. 2023 Jun 15;129(12):1904-1918. doi: 10.1002/cncr.34730. Epub 2023 Apr 11. PMID: 37042037.
137. Smalley, I., Boire, A., Brastianos, P., Kluger, H.M., Hernando-Monge, E., Forsyth, P.A., Ahmed, K.A., **Smalley, K.S.M,** Ferguson, S., Davies, M.A., Glitza, Oliva I.C.: Leptomeningeal disease in melanoma: An update on the developments in pathophysiology and clinical care. Pigment Cell Melanoma Res. 2023 Aug 25. doi: 10.1111/pcmr.13116. Epub ahead of print. PMID: 37622466.
138. Eroglu, Z., Chen, Y.A., Smalley, I., Li, J., Markowitz, J.K., Brohl, A.S., Tetteh, L., Taylor, H., Sondak, V.K., Khushalani, N.I., **Smalley, K.S.M**.: Combined BRAF, MEK, and heat-shock protein 90 (HSP90) inhibition in advanced BRAF V600-mutant melanoma. Cancer. 2023 Sep 30. doi: 10.1002/cncr.35029. Epub ahead of print. PMID: 37776537.
139. Emmons, M.F., Bennett, R., Riva, A., Zhang, C., Macaulay, R., Dupere-Richer, D., Carvalho, L., Gupta, K., Fang, B., Seto, E., Koomen, J.M., Li, J., Chen, Y.A., Licht, J.D., **Smalley, K.S.M**.: HDAC8-mediated inhibition of EP300 drives a transcriptional state that increases melanoma brain metastasis. Nature Comms., 2023 Nov 29;14(1):7759. doi: 10.1038/s41467-023-43519-1. PMID: 38030596; PMCID: PMC10686983.
140. Carvalho, L., Aguiar, F., **Smalley, K.S.M.,** Possik, P.A.: Acral melanoma: new insights into the immune and genomic landscape. Neoplasia. 2023 Dec;46:100947. doi: 10.1016/j.neo.2023.100947. Epub 2023 Oct 31. PMID: 37913653; PMCID: PMC10637990.
141. Phadke, M.S., Li, J., Chen, Z., Mandula,, J.K., Karapetyan, L., Forsyth, P.A., Chen, Y.A., **Smalley, K.S.M.:** Differential requirements for CD4+ T cells in the efficacy of the anti-PD-1+LAG-3 and anti-PD-1+CTLA-4 combinations in melanoma flank and brain metastasis models Journal for ImmunoTherapy of Cancer 2023;**11**:e007239. doi: 10.1136/jitc-2023-007239
142. Wang, L., Xiao, Y., Luo, Y., Master, R.P., Mo, J., Kim, M.C., Liu, Y., Maharjan, C.K., Patel, U.M., De, U., Carelock, M.E., Tithi, T.I., Li, X., Shaffer, D.R., Guertin, K.R., Zhuang, H., Moser, E., **Smalley, K.S.M**., Lv, D., Zhou, D., Zheng, G., Zhang, W.: PROTAC-mediated NR4A1 degradation as a novel strategy for cancer immunotherapy. J Exp Med. 2024 Mar 4;221(3):e20231519. doi: 10.1084/jem.20231519. Epub 2024 Feb 9. PMID: 38334978; PMCID: PMC10857906.

Invited Publications

1. **Smalley, K.S.M.**, Herlyn, M.: The great escape: another way for melanoma to leave physiological control? J. Invest. Dermatol. 121:xi-xii, 2003.
2. Haass, N.K*., **Smalley, K.S.M***., Herlyn M.: The role of altered cell–cell communication in melanoma progression. J. Mol. Histol. 35:309-318, 2004 (*equal contribution).
3. **Smalley, K.S.M.**, Herlyn, M.: Loitering with intent: New of evidence for the role of BRAF mutations in the proliferation of melanocytic lesions. J. Invest. Dermatol. 123: xvi-xvii, 2004.
4. Haass, N.K., **Smalley, K.S.M.**, Li, L. Herlyn, M.: Adhesion, migration and communication in melanocytes and melanoma. Pigment Cell Res. 18:150-159, 2005.
5. **Smalley, K.S.M.**, Herlyn M.: Targeting intracellular signaling pathways as a novel strategy in melanoma therapy. Ann. N.Y. Acad. Sci. 1059:16-25, 2005.
6. Meier, F., Schittek, B., Busch, S., Garbe, C., **Smalley, K.**, Satyamoorthy, K., Li, G., Herlyn, M.: The RAS/RAF/MEK/ERK and PI3K/AKT signaling pathways present molecular targets for the effective treatment of advanced melanoma. Front. Biosci. 10:2986-3001, 2005.
7. **Smalley, K.S.M.**, Brafford, P.A, Herlyn, M.: Selective evolutionary pressure from the tissue microenvironment drives tumor progression. Semin. Cancer Biol. 15:451-459, 2005.
8. **Smalley, K.S.M.**, Lioni, M, Herlyn, M.: Targeting the stromal fibroblasts: a novel approach to melanoma therapy. Expert Rev. Anticancer Ther. 5: 1069-1078, 2005.
9. **Smalley, K.S.M**. Herlyn, M.: Towards the targeted therapy of melanoma. Mini Rev. Med. Chem. 6:387-393, 2006.
10. **Smalley, K.S.M.**, Lioni, M. Herlyn, M.: Life isn't flat: Taking Cancer Biology to the next dimension. In Vitro Cell. Develop. Biol. 42:242-247, 2006.
11. **Smalley, K.S.M.** & Herlyn, M.: Is knowledge about the genetic and epigenetic alterations in melanoma a basis for targeted therapy? Exp. Derm. 15:844-845, 2006.
12. **Smalley, K.S.M.**: Meeting report from the Rene Touraine Foundation Symposium, November 10th 2006. Exp. Rev. Derm. 2:7-8, 2007.
13. **Smalley, K.S.M.**, Flaherty, K.T.: Is ERK activation a good biomarker for estradiol and tamoxifen effects? Cancer Biol. Ther. 6: 119-120, 2007.
14. **Smalley, K.S.M.**, Herlyn, M., Flaherty, K.T.: BRAF/MEK inhibitors in melanoma therapy: New hope or another false dawn. Exp. Rev. Derm. 2: 179-190, 2007.
15. **Smalley, K.S.M.**, Lioni, M., Noma, K., Haass, N.K., Herlyn, M.: 3D in vitro cell culture models for anti-cancer drug discovery. Exp. Opin. Drug Disc. 3: 1-10, 2008.
16. Flaherty, K.T. **Smalley, K.S.M.:** Somatic genetics and targeted therapies for cutaneous melanoma. Update Cancer Therap. *In Press*
17. **Smalley, K.S.M.**, Herlyn, M.: Integrating tumor initiating cells into the paradigm for melanoma targeted therapy. Int. J. Cancer 126: 1245-1250, 2009
18. **Smalley, K.S.M.**, Flaherty, K.T.: Integrating BRAF/MEK inhibitors into combination therapy for melanoma. Br. J Cancer 100: 431-435, 2009.
19. **Smalley, K.S.M.**, Sondak, V.K., Weber, J.S.: c-KIT as the driving oncogenic event in sub-types of melanoma. Histol. Histopath. 24: 643-650, 2009.
20. Sondak, V.K., **Smalley, K.S.M.**: Targeting BRAF and KIT in metastatic melanoma: ASCO 2009 meeting report. Pigment Cell Melanoma Res. 22: 386-387, 2009.
21. Haass, N.K., **Smalley, K.S.M.**: Melanoma biomarkers: Current status and utility in diagnosis, prognosis and response to therapy. Mol. Diag. Ther. 13: 283-296, 2009.
22. **Smalley, K.S.M.**, Flaherty, K.T.: Preclinical & clinical development of targeted therapy in melanoma: attention to detail. Pigment Cell Melanoma Res. 22: 529-531, 2009.
23. **Smalley, K.S.M.**, Flaherty, K.T.: development of a new chemical class of BRAF inhibitors offers hope for melanoma treatment. Future Oncol. 6: 775-778, 2009.
24. **Smalley, K.S.M.:** Introduction to the Biochemical Pharmacology Special Issue on Targeted Cancer Therapy. Biochem. Pharmacol. 80: 549, 2010.
25. Hersey, P., **Smalley, K.S.M.**, Weeraratna, A., Bosenberg, M., Zhang, X.D., Haass, N.K., Paton, E., Mann, G., Scolyer, R.A. Meeting report from the 7th International Melanoma Congress, Sydney, November, 2010. Pigment Cell Melanoma Res. 24: e1-e15, 2011.

26. Rebecca, V.W, **Smalley, K.S.M:** Tumor heterogeneity and strategies to overcome kinase inhibitor resistance in cancer: lessons from melanoma. Expert Opin Investig. Drugs. 20:137-40, 2011.
27. John JK, **Smalley K.S.M:** Identification of BRAF mutations in eruptive melanocytic nevi: new insights into melanomagenesis? Expert Rev. Anticancer Ther. 11: 711-714, 2011.
28. **Smalley, K.S.M.,** Aplin, A., Holler, C., Bosserhoff, A., Bosenberg, M., Ribas, A., Barnhill, R., Kudchadkar, R., Messina, J.L.: Meeting report from the 8th International Melanoma Congress, Tampa, FL. Pigment Cell Melanoma Res. 25:e1-e11, 2012.
29. Lo, R.S., Ribas, A., Long, G.V., Ballotti, R., Berger, M., Hugo, W., Gibney, G.T., Bosenberg, M., Bernstein, E., Villanueva, J., **Smalley, K.S.M.:** Pigment Cell Melanoma Res. 2013 Apr 4. doi: 10.1111/pcmr.12103
30. **Smalley, K.S.M.:** Mutant BRAF: A novel mediator of environmental escape in melanoma? J. Invest. Dermatol. 2013 May;133(5):1135-7. doi: 10.1038/jid.2012.474.
31. Kenchappa, R.S., Tran, N., Rao, N.G., **Smalley, K.S.M.,** Gibney, G.T., Sondak, V.K., Forsyth, P.A.: Novel treatments for melanoma brain metastases. Cancer Control. 20: 298-306, 2013.
32. **Smalley, K.S.M.** Overcoming melanoma drug resistance through metabolic targeting. Pigment Cell Melanoma Res. 26: 793-795, 2013.
33. Sloot, S., Fedorenko, I.V., **Smalley, K.S.M.**, Gibney GT.: Long-term effects of BRAF inhibitors in melanoma treatment: friend or foe? Exp. Opin. Pharmacother. 15: 589-592, 2014.
34. Haarberg, E.H., **Smalley, K.S.M**.: Resistance to Raf inhibition in Cancer. Drug Disc. Today 11: 27-32, 2014.
35. Verduzco, D., Flaherty, K.T., **Smalley, K.S.M.:** Feeling energetic? Targeting metabolism in melanoma. Exp Derm. 24: 657-658, 2015.
36. Fedorenko, I.V., **Smalley, K.S.M.:** The complexity of microenvironment mediated drug resistance. Genes Cancer. 6: 367-368, 2015.
37. Sahebjam, S., Forsyth, P.A., **Smalley, K.S.M.,** Tran, N.D.: Experimental Treatments for Leptomeningeal Metastases From Solid Malignancies. Cancer Control. 24: 42-46, 2017.
38. Smalley, **K.S.M.**, Forsyth PA.: The Blood Brain Barrier and BRAF inhibitors: Implications for patients with melanoma brain metastases. Pharmacol Res. 2017 Nov 13. pii: S1043-6618(17)31451-2. doi: 10.1016/j.phrs.2017.11.013.
39. Faião-Flores, F., **Smalley, K.S.M.:** Get with the Program! Stemness and Reprogramming in Melanoma Metastasis. J Invest Dermatol. 2018 Jan;138(1):10-13. doi: 10.1016/j.jid.2017.07.001.
40. Abate-Daga, D., Ramello, M.C., Smalley, I., Forsyth, P.A., **Smalley, K.S.M.:** The biology and therapeutic management of melanoma brain metastases. Biochem Pharmacol. 2017 Dec 24. pii: S0006-2952(17)30731-1. doi: 10.1016/j.bcp.2017.12.019.
41. **Smalley, K.S.M**.: Why do women with melanoma do better than men? Elife. 2018 Jan 16;7. pii: e33511. doi: 10.7554/eLife.33511.
42. Emmons, M.F., **Smalley, K.S.M**.: Ironing-Out the Details: New Strategies for Combining Ferroptosis Inhibitors with Immunotherapy in Melanoma. J Invest Dermatol. 2021 Sep 23:S0022-202X(21)01436-6. doi: 10.1016/j.jid.2021.06.014. Epub ahead of print. PMID: 34565560.
43. Smalley, I., **Smalley, K.S.M.:** Space Is the Place: Mapping the Cell-Cell Interactions That Predict Immunotherapy Responses in Melanoma. Cancer Res. 2022 Sep 16;82(18):3198-3200. doi: 10.1158/0008-5472.CAN-22-2192. PMID: 36111402.
44. **Smalley, K.S.M**.: Too Much Fuel on the Fire? Linking Obesity, Metabolism, and Melanoma Outcomes. Clin Cancer Res. 2023 Jan 4;29(1):5-7. doi: 10.1158/1078-0432.CCR-22-3028. PMID: 36315002.

Book Chapters

1. **Smalley, K.** review of Melanoma: Techniques and Protocols by B. Nickoloff. Molecular Biotechnology 21:293-294, 2002.
2. **Smalley, K.S.M**., Herlyn, M.: Zonula-occludens (ZO-1) protein-1. The Encyclopedia of Cancer. Springer, Heidelberg, 2007.

3. **Smalley, K.S.M.**, Desai, B., Herlyn, M.: Targeting the melanoma stem cell population. Melanoma: Translational research and emerging therapies. Editors Agarwala and Sondak. Informa Press, 2008
4. **Smalley, K.S.M.**, Flaherty, K.T.: Targeting BRAF as a novel paradigm for melanoma therapy. Melanoma: Translational research and emerging therapies. Editors Agarwala and Sondak. Informa Press, 2008
5. Vultur, A., **Smalley, K.S.M.**, Herlyn M.: Melanoma, causes and targets for therapy. Molecular Oncology. Editors Sawyers and Rauscher. Oxford University Press, 2010.
6. **Smalley, K.S.M**, Flaherty, K.T.: NRAS. Encyclopedia of Cancer Targets. Editor Marshall. Springer, 2010.
7. Han, D. **Smalley, K.S.M.:** Targeting the cell cycle and the p53 pathway in combination with BRAF inhibitors. BRAF Targets in Melanoma. Editor Sullivan. Springer, 2014.

Invited Seminars

July 2003	The American Society for Photobiology, Baltimore, MD."A new model of UV-induced malignant melanoma"
Nov. 2004	Melanoma SPORE Meeting, Bryn Mawr, PA. "3D approaches to melanoma therapeutics"
Nov. 2004	2nd International Melanoma Research Congress,Phoenix, AZ. "Upregulated expression of ZO-1 in human melanoma facilitates invasion via interaction with N-cadherin and the actin cytoskeleton"
Apr. 2005	Penn State University, Hershey, PA. "Taking melanoma therapy to the third dimension."
Nov. 2005	GlaxoSmithKline, Collegeville, PA. "New 3D In vitro models for melanoma"
Jun. 2006	Society for In Vitro Biology Meeting, Minneapolis, MN. "Novel 3D cell culture models reveal the drug resistant phenotype of melanoma"
Jul. 2006	National Cancer Institute, SPORE meeting, Baltimore, MD. "GSK3b as a therapeutic target in melanoma"
Nov. 2006	Rene Touraine Foundation. Paris, France. "The genetics and epigenetics of melanoma"
Jul. 2007	National Cancer Institute, SPORE meeting, Baltimore, MD. "c-Kit and CDK4 overexpression drive the oncogenic transformation of melanomas that are wild-type for BRAF and N-Ras"
Aug. 2007	CELLutions Summit meeting, Boston, MA. "Taking cancer biology to the 3rd dimension"
Feb. 2008	Timberline Symposium on Epithelial Biology, Mount Hood, OR. "Modeling melanoma progression using organotypic cultures"
Mar. 2008	Society for Surgical Oncology Satellite Meeting, Chicago, IL. "Cutaneous cancer stem cells"
Nov. 2008	2nd International meeting of melanoma centers, Clearwater, FL. "New models for melanoma treatment"
May 2009	Cancer Research UK, Cambridge Research Institute, Cambridge, UK. "Personalizing melanoma therapy"
Aug. 2009	The Wistar Institute, Philadelphia, PA. "New insights into BRAF inhibitor therapy in melanoma"
Aug. 2009	Thomas Jefferson University, Philadelphia, PA. "Understanding melanoma drug resistance"
Oct. 2010	iSBTc Annual Meeting, Washington D.C. Plenary Speaker,"Deciphering BRAF inhibitor resistance"
Nov. 2010	Society for Melanoma Research Meeting, Sydney Australia. Plenary Speaker, "Understanding intrinsic and acquired resistance to BRAF inhibitors"
Jun. 2011	University of Sao Paulo, Sao Paulo, Brazil. Keynote Speaker, "Skin lesions induced by ultraviolet radiation workshop"
Nov. 2011	Society for Melanoma Research Meeting, Tampa, FL. Plenary Speaker, "A broad based strategy for overcoming BRAF inhibitor resistance"
Mar. 2012	University of Miami, Miller School of Medicine, Miami, FL. "Targeted therapy for melanoma: from response to resistance"
Apr. 2012	AACR Annual Meeting, Chicago, IL. "Beyond BRAF: targeting NRAS mutant melanoma"
May 2012	Annual Meeting of the Dermatological Nursing Association, "New therapies for metastatic melanoma"

Jun. 2012	Ponce School of Medicine, Puerto Rico. "Using proteomics to personalize melanoma therapy"
Jul. 2012	Invited seminar speaker Georgetown University, Washington DC.
Jul. 2012	Invited Speaker, University of Sao Paulo, Brazil.
Sept. 2012	Invited Plenary Speaker, Pan-American Society for Pigment Cell Research Meeting, Utah. "Advances in melanoma targeted therapy".
Oct. 2012	Invited speaker Thomas Jefferson University, Philadelphia, PA.
Nov. 2012	Society for Melanoma Research Meeting, Hollywood, CA. Co-Chair, "Melanoma Biology".
Mar. 2013	Invited Grand Rounds Speaker, University of Pittsburgh Medical School.
Apr. 2013	Session Chair AACR annual meeting, Washington, DC.
Apr. 2013	Invited Speaker AACR annual meeting, Washington, DC.
May 2013	Keynote Speaker, Penn State Melanoma Symposium, Hershey, Pa.
July 2013	Invited Speaker, Boehringer-Ingleheim, Vienna, Austria
July 2013	Invited Speaker, World Melanoma Congress, Hamburg, Germany
Sept. 2013	Invited Speaker, Skin SPORE meeting, MD Anderson, Houston, Tx.
April 2014	Invited Speaker, the Van Andel Institute, Grand Rapids MI.
June 2014	Plenary Speaker, AACR Special conference on drug resistance. Orlando, FL.
August 2014	Invited Speaker, the Huntsman Cancer Center, Salt Lake City, UT.
Nov 2014	Invited Speaker, Society of Melanoma Research Meeting, Zurich, Switzerland
Nov 2014	Invited Speaker, Melanoma Biology & Patient Management Conference, Washington, DC
Jan 2015	Invited Speaker, Melanoma Seminars. The Wistar Institute, Philadelphia, PA
Apr 2015	Invited Speaker, Grand Rounds, NYU School of Medicine, New York City, NY.
Oct 2015	Invited Speaker, MRF special workshop: The State of Melanoma. San Francisco, CA.
Nov 2015	Invited Speaker, The Elkin Lecture. Emory University, Atlanta, Ga.
Dec 2015	Invited Speaker, Inter-Spore meeting. Philadelphia, PA.
Mar 2016	Invited Speaker, Florida International University
May 2016	Invited Speaker, University of Colorado, Denver, Co.
Aug 2016	Invited Speaker, World Melanoma Congress, Vienna, Austria
Oct 2016	Invited Speaker, University of Queensland, Brisbane, Australia.
Oct 2016	Invited Speaker, The Peter Macallum Cancer Center, Melbourne, Australia
Oct 2016	Invited Speaker, The Australasian Melanoma Conference, Sydney, Australia
Feb 2017	Invited Speaker, 3rd Annual Moffitt Pathology Symposium, Sarasota, FL.
Mar 2017	Grand Rounds, MGH, Harvard Medical School.
Aug 2017	Invited Plenary Speaker and Chair, IPCCC Annual Meeting, Denver, Co.
Feb 2018	Chair and Speaker, The 2nd MRF-sponsored melanoma brain metastasis workshop, Tampa.
April 2018	Invited Grand Rounds Speaker, Roswell Park Cancer Institute, NY
Nov 2018	Invited Plenary Speaker, The Annual Montagna Symposium on Melanocyte Biology, Gleneden Beach, OR.
April 2019	Invited Speaker, Mt. Sinai School of Medicine, NYC.
Oct 2019	Grand Rounds Speaker, Wake Forest School of Medicine
Nov 2019	Invited Speaker, Cure OM meeting, Salt Lake City, Utah.
Dec 2019	Grand Rounds Speaker, University of Florida Cancer Center
Jun 2020	Keynote Speaker, Barretos Cancer Hospital, Barretos, Brazil. (postponed)
Jun 2020	Invited Speaker, MRF brain metastasis meeting. NYC. (postponed)
Aug 2020	Invited Speaker Society for Neurooncology Brain Met Meeting, Toronto, Canada (postponed).
Oct 2020	Invited Speaker, Annual FACCA meeting (online).
Feb 2021	Invited Speaker, Melanoma Research Alliance. Annual Meeting.
April 2021	Invited Speaker, Grand Rounds. Drexel University. (online)
April 2021	Invited Speaker, Grand Round. University of Virginia. (online)
Aug 2021	Invited Speaker Society for Neurooncology Brain Met Meeting, Toronto, Canada (online)
Sept 2021	Invited Speaker, Pan-American Society for Pigment Cell Research Annual Meeting.
Oct 2021	Invited Speaker, Annual Skin Carcinogenesis Meeting (online)

Nov 2021	Invited Speaker, MRF brain metastasis meeting, Washington DC.
Dec 2021	Grand Rounds Speaker, Roswell Park Cancer Center
May 2022	Invited Speaker, Barretos Hospital Brazil, (virtual)
June 2022	Invited Speaker, The Inter-SPORE Annual Meeting, New York University, NY.
Aug 2022	Grand Rounds Speaker, MD Anderson Cancer Center, Houston TX.
Sept 2022	Invited Speaker, Melanoma Program, University of California San Francisco, CA.
Oct 2022	Invited Speaker, Society for Melanoma Research Meeting, Edinburgh, Scotland.
June 2023	Invited Speaker, Annual Skin SPORE meeting, Pittsburgh, PA.
Nov 2023	Invited Speaker, Society for Melanoma Research Meeting, Philadelphia, PA.
July 2024	Keynote Speaker, Brazilian Society for Cell Biology, Sao Paulo
Aug 2024	Invited Speaker, Wistar Institute Melanoma Symposium, Phildelpahia, PA.

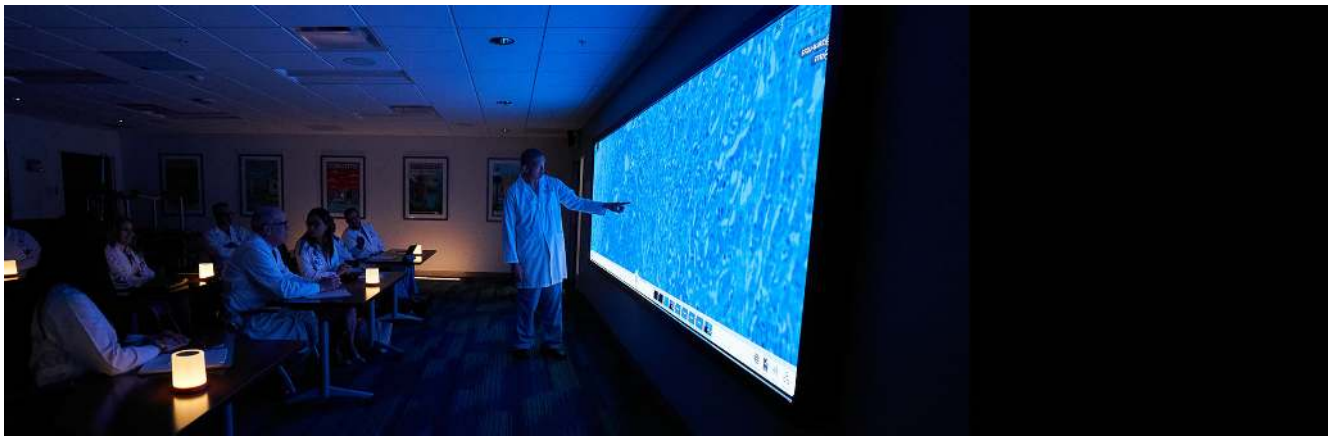
Patents

Koomen, J.M., **Smalley, K.S.M.,** Xiang, Y., Wood, E.: Methods and materials for monitoring and treating medical conditions. US patent 61/583,059.

Smalley, K. : Drug combination to treat melanoma. PCT/US2015/055568.

Media Coverage & Interviews

1. Brower, V.: BRAF inhibitors: research accelerates in wake of positive findings. J. Natl. Cancer Inst. 102:214-215, 2010.
2. Seppa, N.: New drug fights metastatic melanoma. Science News. 178: 12, 2010. (this story was reprinted in Wired, U.S. World & News Report, The Boston Globe and numerous other places).
3. Szabo, L.: Promising new melanoma drug, questions and answers. USA Today, 25th August, 2010.
4. Willyard, C.: Companies compete over BRAF mutation specific drugs. Nature Med. 2010 17: 268, 2011.
5. Martz, L.: New players in melanoma. Nature SciBX, (15); doi: 10.1038/scibx.2011.415
6. Kirk, R.: In melanoma, resistance is futile? Nature Rev Clin. Oncol. 8: 253, 2011.
7. Lucchetti, C., Rizzolio, F., Castronovo, M., Toffoli, G.: Research highlights. Overcoming BRAF resistance to PLX4032 by AKT inhibition in PTEN-deficient melanoma cells. Pharmacogenomics. 12:1381, 2011.
8. Krutoholow, E.: Roche's Zelboraf steadily adopted in advanced melanoma. The Financial Times, April 3rd 2012.
9. Allison, M.: MEK inhibitor nears approval. Nature Biotech. 31, 4 (2013) doi:10.1038/nbt0113-4
10. Haas, M.J.: MERTK upstream of BRAF. Nature SciBX, (2013) 16; 5-7
11. Appearance on ABC Action News Tampa, FL discussing the FDA-approved of BRAF/MEK inhibitors.
12. Interview on WUSF (the local NPR station) health news discussing new treatments for melanoma.



The first National Cancer Institute-designated Comprehensive Cancer Center based in Florida

About Moffitt

Equipped with top research and care experts working side-by-side, Moffitt is uniquely positioned to revolutionize cancer treatment, elevate care and save more lives.

Effective treatment starts with the right diagnosis. As with all types of cancer, an early diagnosis can increase the likelihood of a positive outcome. Our multispecialty medical professionals are dedicated to the treatment and research of cancer, and cancer only.

As Florida's first **National Cancer Institute**-designated Comprehensive Cancer Center and one of only 30 leading cancer centers in the U.S. participating in the **National Comprehensive Cancer Network**, Moffitt is at the **very forefront of cancer centers** worldwide.

Equipped with top research and care experts working side-by-side, Moffitt is uniquely positioned to revolutionize cancer treatment, elevate care and save more lives.

Your Best Chance for Beating Cancer ™

MISSION:
To contribute to the prevention and cure of cancer

VISION:
Create revolutionary breakthroughs and innovations that rapidly impact and save more lives.

Frequently Asked Questions

Outcomes

Contact Us

Awards & Recognition

National Comprehensive Cancer Network (NCCN)

NCI Designation

Moffitt is proud to have earned the highest accolades in numerous fields.

Moffitt Boards

We invite you to take a look at the individuals who lead Moffitt in its mission to contribute to the prevention and cure of cancer.

Corporate Compliance

The Corporate Compliance Program supports the center's mission and works to ensure the institution adheres to all applicable laws, rules and guidelines.

Education

Education and training is a fundamental part of our core mission. Moffitt provides numerous education opportunities.

Executive Leadership

Meet Moffitt's executive leadership, who identify and define the institution's objectives and priorities.

Foundation

When you donate to cancer research, your gift will go directly to helping researchers and scientists advance cancer treatments and help cure patients.

Government Relations

Government Relations has played a critical role in the growth and success of Moffitt by advocating for increased funding for research and education.

Moffitt Medical Group (MMG)

The Moffitt Medical Group (MMG) is Florida's largest multi-disciplinary medical group practice that is dedicated to cancer care.

Nursing

Moffitt is proud to have earned the prestigious Magnet® designation in recognition of its nursing excellence.

Outcomes

Moffitt's survival rates are higher than the national average. Learn more about the survival rates for certain cancer types.

Partnerships

We are proud to partner with other organizations that support our mission, help increase access to superior patient care and participate

Podcasts

Moffitt Cancer Center Podcast Network strives to educate, engage, and inspire patients, their families, healthcare professionals and the community.

Research

Moffitt's impact on cancer research spans basic science, prevention and clinical research with a focus on translating discoveries into better care.

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With survival rates higher than the national averages, Moffitt can be your best chance for beating cancer. Our multispecialty medical professionals are dedicated to the treatment and research of cancer.

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America’s Best Cancer Hospitals 2024 by Newsweek

oncodaily.com/insight/120766

Emma Ter-Azaryan August 8, 2024

Insight

Newsweek announced the list of **America’s Best Cancer Hospitals 2024.** After analysis, the top 200 leading hospitals in oncology were ranked.

The Top 10 Best Hospitals for Cancer in the US, are as follows:

1. Moffitt Cancer Center
2. Memorial Sloan Kettering Cancer Center
3. Mayo Clinic – Rochester
4. The Johns Hopkins Hospital
5. Cleveland Clinic
6. Dana-Farber Brigham Cancer Center
7. Massachusetts General Hospital
8. UCSF Medical Center
9. Stanford Health Care – Stanford Hospital
10. Barnes-Jewish Hospital

US Oncologists and Hospitals shared their opinion on it:

Moffitt Cancer Center:

“We are thrilled to announce that *Moffitt Cancer Center* has once again been named the #1 Cancer Hospital in Florida and the Southeast by Newsweek. Moffitt has also been recognized on Newsweek’s list of the World’s Best

Oncology Hospitals.

These incredible recognitions are a testament to the quality of research, care and exceptional cancer outcomes provided by our nationally recognized team and the invaluable feedback from our patients and oncology professionals alike.

Congratulations to everyone at Moffitt for this outstanding achievement! Together, we are advancing our mission to contribute to the prevention and cure of cancer."

Patrick Hwu, the President and CEO of Moffitt Cancer Center:

"Thrilled Moffitt Cancer Center has once again been named the #1 Cancer Hospital in Florida and the Southeast by Newsweek.
Moffitt Cancer Center has also been recognized on Newsweek's list of the World's Best Oncology Hospitals. These recognitions are a testament to the quality of research, care and exceptional outcomes provided. Congratulations, team!"

Carolina East Medical Center:

"The SECU Comprehensive Cancer Center at Carolina East Medical Center has been recognized as one of Newsweek's America's Best Cancer Hospitals! This is the second year for this great recognition, and we are extremely proud to be on the list.

Congratulations to our incredible team!"

Memorial Sloan Kettering Cancer Center:

"Additional MSK Awards and Appointment honorees include: Dr. *Andrea Cercek* being awarded a 2024 A Grant of Her Own by
The V Foundation, MSK being named one of *America's Best Cancer Hospitals by Newsweek*."

To read the **2024-2025 Best Hospitals by the U.S. News and World Report,** visit Oncodaily.com

- Insight

Barnes-Jewish Hospital cancer Carolina East Medical Center Cleveland Clinic Dana-Farber Brigham Cancer Center Mayo Clinic - Rochester Memorial Sloan Kettering Cancer Center Moffitt Cancer Center OncoDaily Oncology Stanford Health Care - Stanford Hospital The Johns Hopkins Hospital The V Foundation

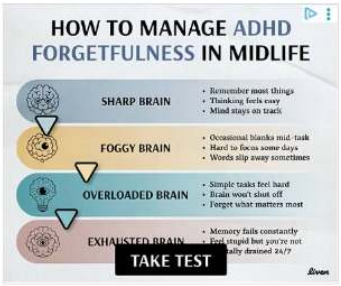
H. Lee Moffitt Cancer Center and Research Institute Cancer

Tampa, FL

#23 in **Cancer**

Cancer Scorecard

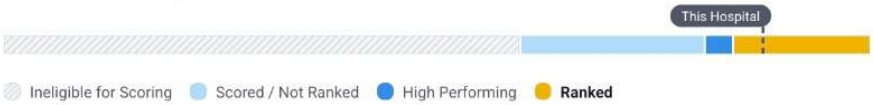
Cancer ratings are based on analysis of factors such as patient outcomes in the medical and surgical treatment of cancer, volume of high-risk patients, patient experience, nurse staffing, and advanced clinical technologies. Each hospital is then given a score and the 50 top-scoring hospitals are nationally ranked. The top 10% within the specialty are considered high performing, and the rest are unranked. Use our National Score Distribution to view how well this hospital scored for cancer among all the other hospitals being evaluated. See the full U.S. News national rankings in Cancer or hospital ratings in leukemia, lymphoma & myeloma, colon cancer surgery, gynecological cancer surgery, lung cancer surgery and prostate cancer surgery.



Overall Cancer Rating

National Rank	#23
Overall Score	69.1/100

National Score Distribution



BEST CANCER HOSPITALS

1. #1 University of Texas MD Anderson Cancer Center
2. #2 Memorial Sloan Kettering Cancer Center
3. #3 Dana-Farber Brigham Cancer Center
4. #4 Mayo Clinic
5. #5 Massachusetts General Hospital

SEE FULL RANKINGS LIST »

SEE NEARBY HOSPITAL RANKINGS IN CANCER »

Outcomes and Experience (57.0% of score)

Higher numbers suggest better survival odds, fewer complications and more patients treated.

Survival

Survival 30 days after being admitted relative to other hospitals treating similarly complex conditions.

Excellent

How often patients go directly home from this hospital rather than being discharged to another facility, an indication that the patient was well enough to do home-based self care.

Excellent

Patient experience
Results of a standardized government-endorsed survey of hospital inpatients about the overall quality of their stay.

Excellent

Number of patients
Relative number of high-risk patients treated for cancer-related illness. More experience is associated with better outcomes.

Very High

Key Programs, Services and Staff (17.5% of score)

Care of challenging patients demands hands-on attention and highly specialized expertise.

Nurse staffing
More nursing care per patient is associated with better outcomes and better patient experience.

Average

Intensivists
Whether hospital has at least one adult intensive-care unit staffed by a doctor specifically certified or trained to care for ICU patients.

✔

Advanced technologies
Such as image-guided radiation therapy, intensity-modulated radiation therapy and shaped-beam radiation for irregularly-shaped tumors.

Excellent

Patient services
Such as programs for hospice, pain management and palliative care.

Excellent

Professional Recognition (25.5% of score)

Endorsements by respected national organizations demonstrate high standards.

Recognized as Nurse Magnet hospital
Recognized by the American Nurses Credential Center. Indicates the hospital meets high nursing standards.

✔

NCI-designated cancer center
Recognized by the National Cancer Institute as a comprehensive or clinical cancer center.

✔

Accredited by FACT
Certified by Foundation for the Accreditation of Cellular Therapy (FACT) for bone marrow and tissue transplants.

Fully Accredited

Expert opinion
Percentage of surveyed cancer specialists who named this hospital as among the best for complex care.

Good

Most hospitals are named by a few

RANKINGS AND RATINGS AT THIS HOSPITAL

Adult Specialties −

Cancer
Ear, Nose & Throat
Gastroenterology & GI Surgery
Urology

Procedures and Conditions +

specialists or not at all.

Conditions/Procedures Applicable to Cancer

Leukemia, Lymphoma & Myeloma — High Performing
SEE PERFORMANCE DETAILS »

Colon Cancer Surgery — High Performing
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Gynecological Cancer Surgery — High Performing
SEE PERFORMANCE DETAILS »

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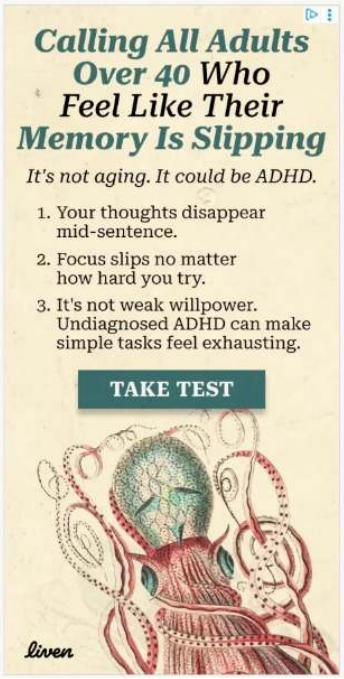

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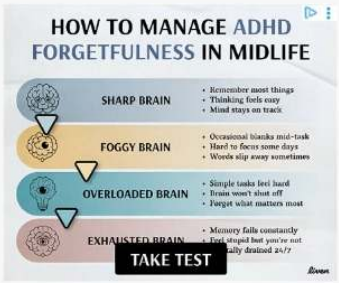

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Awards & Recognition

At Moffitt Cancer Center, we've earned our share of accolades over the years, with members of our staff receiving some of the highest physician and cancer awards within the medical field. As an organization, we've consistently been recognized by U.S. News & World Report as one of the best hospitals for the treatment of cancer in the country.

While we appreciate the recognition, we remain focused on the real prize: a cure. Only then can we sit back and reflect on what we've accomplished.

Additional acknowledgments include:

- Moffitt is the top-ranked cancer hospital in Florida and the Southeast in Newsweek's 2025 list of "**America's Best Cancer Hospitals.**"
- The **National Association for Business Resources** recognized Moffitt as one of the Top 101 Best and Brightest Companies to Work For® in the nation for 2024. This is the ninth year in a row Moffitt was recognized.
- Awarded platinum-level designation by the **Family Friendly Workplace Tampa Bay**, which recognizes workplace practices that directly support employee families, as well as provide funding to family-supporting programs.
- Moffitt was named one of **Foundry's Computerworld's 2025 Best Places to Work in IT,** a prestigious annual ranking recognizing organizations that excel in fostering outstanding work environments for technology professionals.
- Moffitt is accredited by the American College of Surgeons **Commission on Cancer** (CoC). Moffitt has been awarded accreditation continuously since 1989 for our commitment to providing comprehensive, high-quality and multispecialty patient-centered care. To maintain accreditation, facilities must undergo an on-site review every three years.
- Moffitt achieved exemplar status for its **Nurses Improving Care for Healthsystem Elders (NICHE)** program, the highest designation in an international nurse-driven initiative designed to help hospitals improve the care of older adults. Moffitt is one of only 38 medical centers in the nation to have achieved this status.
- Moffitt once again made the **Tampa Bay Times Top Workplaces ranking for the 14th time**. The anonymous survey uniquely measures culture drivers that are critical to the success of any organization, including alignment, execution and connection.
- Magnet® designation in recognition of our nursing excellence. **Magnet recognition** is granted by the American Nurses Credentialing Center (ANCC), the credentialing body of the American Nurses Association, to honor outstanding health care organizations for nursing professionalism, teamwork, quality patient care and innovations in nursing practices.
- Recognized by the **Institute for Patient- and Family-Centered Care**
- Named a Fertile Hope Center of Excellence, an award presented to "cancer centers for proactively addressing cancer-related fertility."

Call Today
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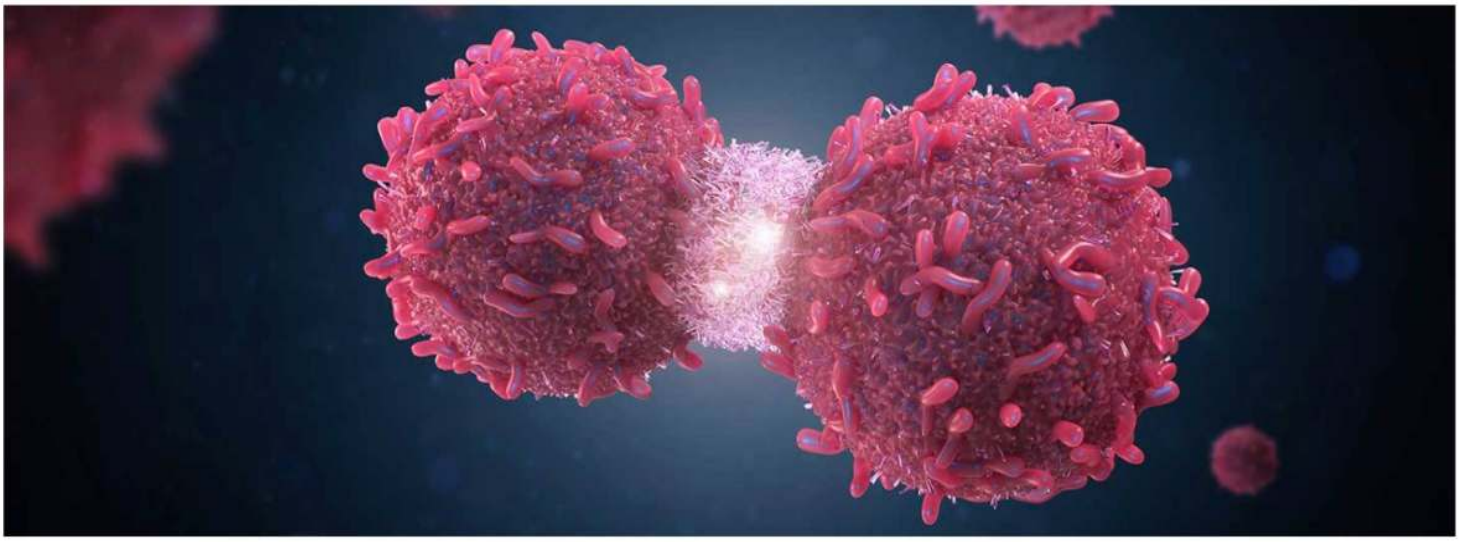
Discover More Florida's First and the Region's Only... About Moffitt Cancers We Treat

Tumor Microenvironment and Metastasis Department

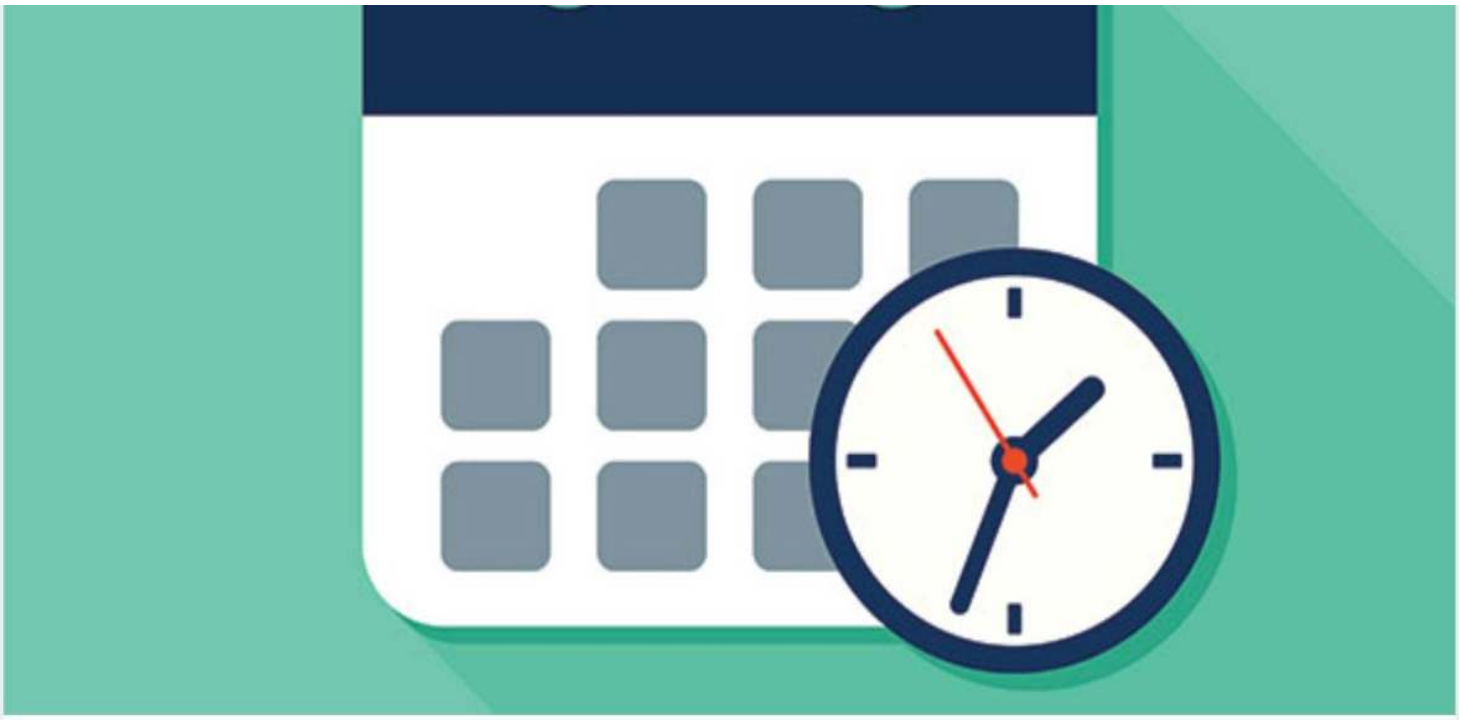


The Department of Tumor Microenvironment and Metastasis conducts cutting-edge research on the molecular mechanisms underlying the growth and metastasis of tumors with an emphasis on identifying novel therapeutic strategies to combat human cancer. Specifically, investigators in the department focus on mechanisms underlying tumor metastasis, role of metabolic regulators in promoting tumor growth, signaling pathways that confer resistance to therapy, protein modifications that promote tumor growth and suppress immunotherapy, role of cancer stem cells in tumor growth and metastasis, and methods for early detection of tumors using circulating nucleic acids. These studies on basic cancer biology are complemented by the involvement of 11 clinician-scientists who have secondary appointments in the department.

The department provides a highly collegial and conducive atmosphere for conducting high-quality research and also in training the next generation of scientists. The faculty has trained highly successful graduate students and post-doctoral fellows who have embarked on successful independent careers in science. We welcome you to further explore the activities of the department and encourage you to become a part of our research team.

Employment Opportunities

Search employment opportunities in the Tumor Microenvironment and Metastasis Department.

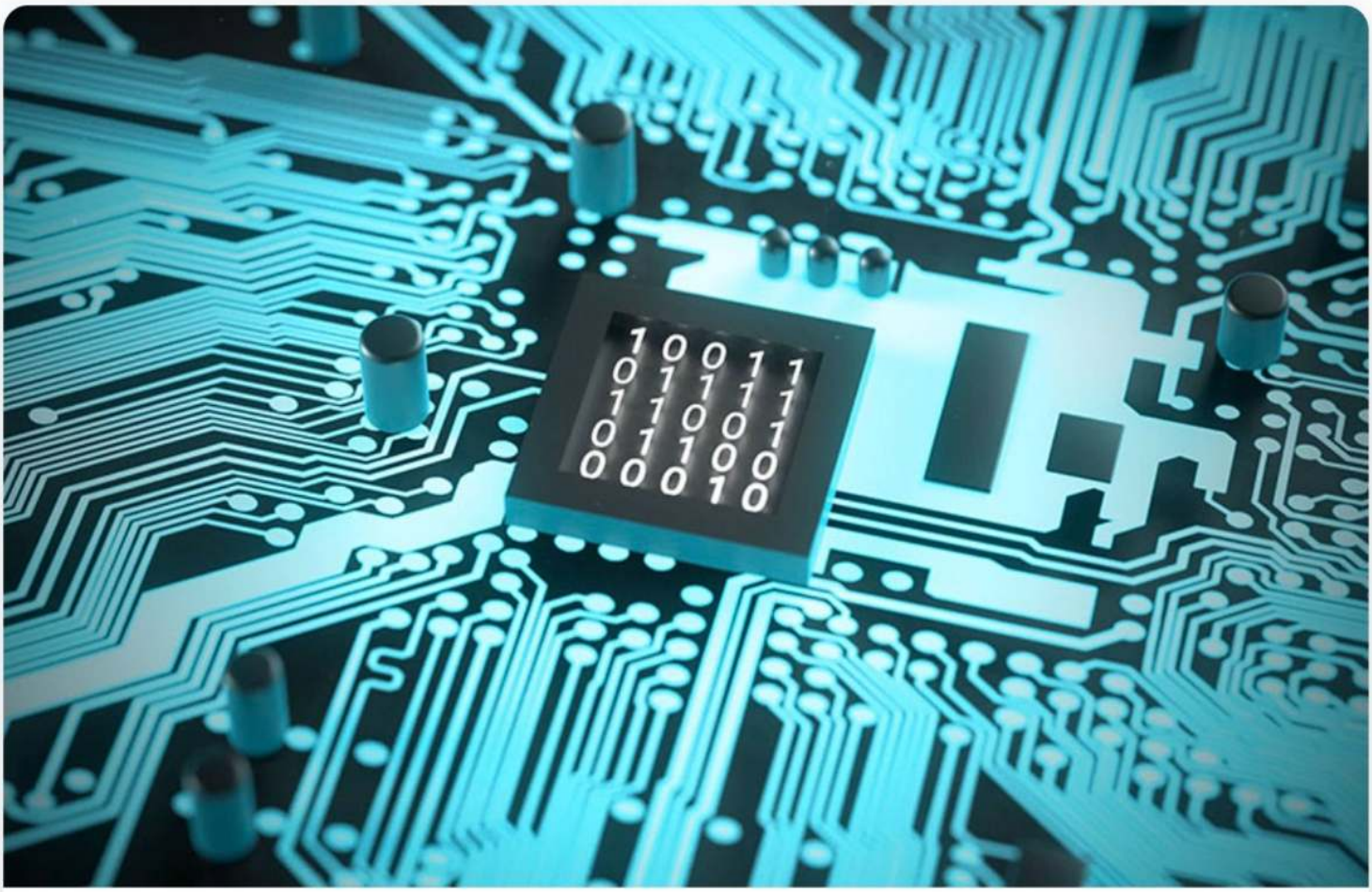


Science In Progress Schedule

The Tumor Microenvironment and Metastasis Department Science In Progress seminars are held internally on Fridays. See the schedule.

Meet our Faculty

Meet the faculty of the Tumor Microenvironment and Metastasis Department.



Department Metrics

View the grants, clinical trials and publications from the Tumor Microenvironment and Metastasis Department.

Our Mission

Our Mission

To contribute to the prevention and cure of cancer.

Connect with a Cancer Expert

NCI Designated Comprehensive Cancer Center

CANCER CENTER of EXCELLENCE Florida HEALTH

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NCCN National Comprehensive Cancer Network®

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Discover More

Molecular Oncology Department

Metastatic Breast Cancer Treatment in Florida

Breast Cancer Treatment in Florida

Moffitt Cancer Center Announces Strategic Collaboration with AstraZeneca to Accelerate Oncology Cell Therapies

September 13, 2024

TAMPA, Fla. – Moffitt Cancer Center announces today a strategic collaboration with AstraZeneca that aims to accelerate the development of cell therapies, specifically chimeric antigen receptor T cell (CAR T) and T cell receptor (TCR T) therapies.

Under this collaboration, AstraZeneca will have priority access to Moffitt's leading clinical environment and forge strong connections between physician-scientists to accelerate the investigation of novel cell therapies.

Advances in cell therapy, including CAR Ts and TCR Ts, are reshaping cancer care by offering potential new treatments for cancers with limited treatment options. The field has grown exponentially since the first CAR T therapies were approved in 2017. Moffitt was pivotal in the clinical trials that led to several FDA approvals for cell therapies and continues to grow its capabilities in this field, with an extensive network of cancer centers across the United States.

The collaboration aims to address cell therapy development challenges and expand cell therapies' reach to more patients in the United States and beyond. A key focus will be on advancing clinical studies to investigate cell therapies in solid tumors and further optimizing clinical operations to streamline and expedite the delivery of autologous cell therapies to patients.

“We are excited to collaborate with AstraZeneca to push the boundaries of what's possible in cancer treatment. By combining our clinical expertise with AstraZeneca's innovative pipeline of investigational cell therapies, global footprint and leadership in oncology, we aim to bring potential new cell therapies to patients faster and more efficiently,” said **Patrick Hwu, M.D.**, president and CEO of Moffitt.

“This collaboration will strengthen our connections with the Moffitt Cancer Center to accelerate the development of our autologous cell therapy pipeline as we strive to redefine cancer treatment for more people living with hematological and solid cancers,” said Carsten Linnemann, Head of Oncology Cell Therapy Clinical Development, AstraZeneca.

About Moffitt Cancer Center

Moffitt is dedicated to one lifesaving mission: to contribute to the prevention and cure of cancer. The Tampa-based facility is one of only 57 National Cancer Institute-designated Comprehensive Cancer Centers, a distinction that recognizes Moffitt’s scientific excellence, multidisciplinary research, and robust training and education. Moffitt’s expert nursing staff is recognized by the American Nurses Credentialing Center with Magnet® status, its highest distinction. For more information, call 1-888-MOFFITT (1-877-812-3798), visit MOFFITT.org, and follow the momentum on Facebook, Twitter, Instagram and YouTube.

###

“

We are excited to collaborate with AstraZeneca to push the boundaries of what's possible in cancer treatment.

Patrick Hwu, M.D.
President and CEO

Moffitt Media Relations

Kim Polacek, APR, CPRC
National Media Manager
813-456-3342
Kim.Polacek@Moffitt.org

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Moffitt Cancer Center and CMIC Group Announce...

Moffitt Cancer Center and Cryoport Announce...

Moffitt Cancer Center Announces Dr. Josh...

Moffitt Cancer Center and CMIC Group Announce Strategic Partnership to Accelerate Global Cancer Clinical Trials

September 17, 2024

TAMPA, Fla. and TOKYO – **Moffitt Cancer Center**, a world-renowned cancer treatment and research leader, and **CMIC Group**, a leading contract research organization (CRO) with comprehensive clinical and pharmaceutical services in the Asia-Pacific (APAC) region, announced today a strategic partnership to accelerate cancer clinical trials and improve patient outcomes globally.

Moffitt will serve as a go-to U.S. clinical trial site for studies managed by CMIC, leveraging its expertise to identify and enroll eligible patients for innovative cancer therapies. CMIC will be the go-to partner for managing Moffitt and its partners' clinical trials within Japan and major APAC countries. The partnership is intended to facilitate and accelerate the execution of cross-border clinical trials for promising therapies. By increasing the diversity of trial participants and expediting the development of breakthrough therapies, this partnership underscores both organizations' dedication to broadening access to innovative treatments and contributing to global cancer research advancements.

CMIC is Japan's first and largest clinical CRO, operating in twelve APAC countries, including Australia, China, South Korea, Taiwan, etc. With over 1,000 CRAs and more than 150 regulatory consultants, CMIC can support global clinical trials in the Asia Pacific arm in various therapeutic areas. CMIC provides comprehensive services in drug development, clinical to commercial GMP manufacturing, clinical site management, regulatory consulting and contract sales.

"This partnership allows us to significantly expand the portfolio of cutting-edge clinical trials available to our patients while providing easy and rapid access for CMIC customers who need a U.S. site for clinical trials," said **Xavier Avat**, Chief Business Officer at Moffitt.

"U.S.-based clinical programs preparing Pharmaceuticals and Medical Devices Agency plans in Japan will also be able to quickly engage and leverage CMIC Group's expertise in regulatory and trial management in Japan and more broadly in the Asia Pacific region."

Moffitt is one of the leading cancer treatment centers in the U.S., recognized for its pioneering research and dedication to advancing cancer care through clinical trials and innovative therapies. As a National Cancer Institute-designated Comprehensive Cancer Center, Moffitt is committed to transforming the future of cancer treatment by translating scientific discoveries into treatments that improve patient outcomes. With an extensive portfolio of clinical trials and a focus on personalized medicine, Moffitt continues to make significant strides in immunotherapy, cell and gene therapy, and precision oncology. Through this collaboration with CMIC, Moffitt will continue to expand its global impact, fostering partnerships that accelerate the pace of cancer research and advance patient care across continents.

"We are excited to join forces with Moffitt Cancer Center. This strategic collaboration will enhance our ability to leverage Moffitt's expertise in cancer research and cell and gene therapy for our Asia-Pacific customers," said Teruyoshi Okuda, co-head of CMIC CRO business." It underscores the shared vision of Moffitt and CMIC to advance global healthcare through collaborative efforts that bridge geographical boundaries and bring cutting-edge research and clinical solutions to a broader audience."

About Moffitt Cancer Center

Moffitt *is dedicated to one lifesaving mission: to contribute to the prevention and cure of cancer. The Tampa-based facility is one of only 57* ***National Cancer Institute-designated Comprehensive Cancer Centers****, a distinction that recognizes Moffitt's scientific excellence, multidisciplinary research, and robust training and education. Moffitt's expert nursing staff is recognized by the American Nurses Credentialing Center with Magnet® status, its highest distinction. For more information, call 1-888-MOFFITT (1-877-812-3798), visit* ***MOFFITT.org****, and follow the momentum on* ***Facebook****,* ***Twitter****,* ***Instagram*** *and* ***YouTube****.*

About CMIC Group

CMIC was the first company in Japan to start a CRO (Clinical Research Organization) business in 1992, and now provides comprehensive pharmaceutical support services from development to manufacturing, sales and marketing. We also support overseas pharmaceutical, biotechnology and medical device companies entering the Japanese market, conducting clinical trials in Asia, and supporting pharmaceutical development and manufacturing in the United States and Japan. CMIC also provides new healthcare solutions

*to support individuals and local governments and aims to develop a business model called PHVC ("Personal Health Value Creator") that "maximizes the health value of each individual" based on our extensive experience and track record in fully supporting the value chain of pharmaceutical companies. The CMIC Group has more than 7,500 employees and 28 group companies worldwide. For more information, please visit our website: **https://www.cmicgroup.com/**.*

###

“

This partnership allows us to significantly expand the portfolio of cutting-edge clinical trials available to our patients while providing easy and rapid access for CMIC customers who need a U.S. site for clinical trials.

Xavier Avat
Chief Businses Officer

Moffitt Media Relations

697

Kim Polacek, APR, CPRC
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813-456-3342
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Individualized Cancer Treatment in Florida

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Cancer Healthcare Professionals in Florida

MOFFITT CANCER CENTER

< Research Education and Training

Postdoctoral Training

Join the fight against cancer while building your career as a Moffitt Postdoctoral Fellow

Moffitt Cancer Center supports a faculty of nearly 200 basic science and clinical researchers, and as an NCI-designated Comprehensive Cancer Center, our research

enterprise offers training in numerous key areas in the fight against cancer. By combining cutting-edge research facilities with a collaborative, interdisciplinary environment, we provide fellows with the skills and support they need to gain independence and pursue a career path of their choosing, whether in academics, industry, government, or others.

Areas of investigation

Areas of investigation at Moffitt are focused on highly translational science and are organized within **five programs**:

- Cancer Biology and Evolution
- Cancer Epidemiology
- Health Outcomes and Behavior
- Immuno-Oncology
- Molecular Medicine

Commitment to Training

MCC attracts postdoctoral fellows from all over the world and trains them to become

outstanding scientific leaders. We currently have over 100 postdoctoral fellows representing more than 25 nations and a wide range of disciplines within the five Moffitt Research Programs. Our rich group of diverse scientific disciplines and backgrounds facilitates a robust collegial experience for our trainees.

Moffitt researchers and postdoctoral fellows have access to our 14 Cancer Center Support Grant-funded **Shared Research Core Facilities**. The Cores are designed to provide a centralized, standardized research platform with a collaborative and integrative approach to serving the research needs of our investigators. Each Core has a dedicated scientific director to provide oversight and guidance to the scientific direction and technical resources of the facility. In addition to providing technical expertise and collaborative consultations, the Cores also provide educational and training opportunities for interested postdoctoral fellows.

All Moffitt postdoctoral fellows receive **dedicated career support** from the Office of Postdoctoral Affairs (OPA).

It is the mission of the OPA to provide an environment where fellows from all over the world feel comfortable, understood and involved, making their postdoctoral experience more personally and professionally gratifying. Please contact us at **opa@moffitt.org** for more information.

Office of Postdoctoral Affairs

Integrated Program in Cancer and Data Science (ICADS) +

T32 in Translational Behavioral Oncology +

Postdoctoral Training and Support

Postdoctoral Association

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Our Mission

To contribute to the prevention and cure of cancer.

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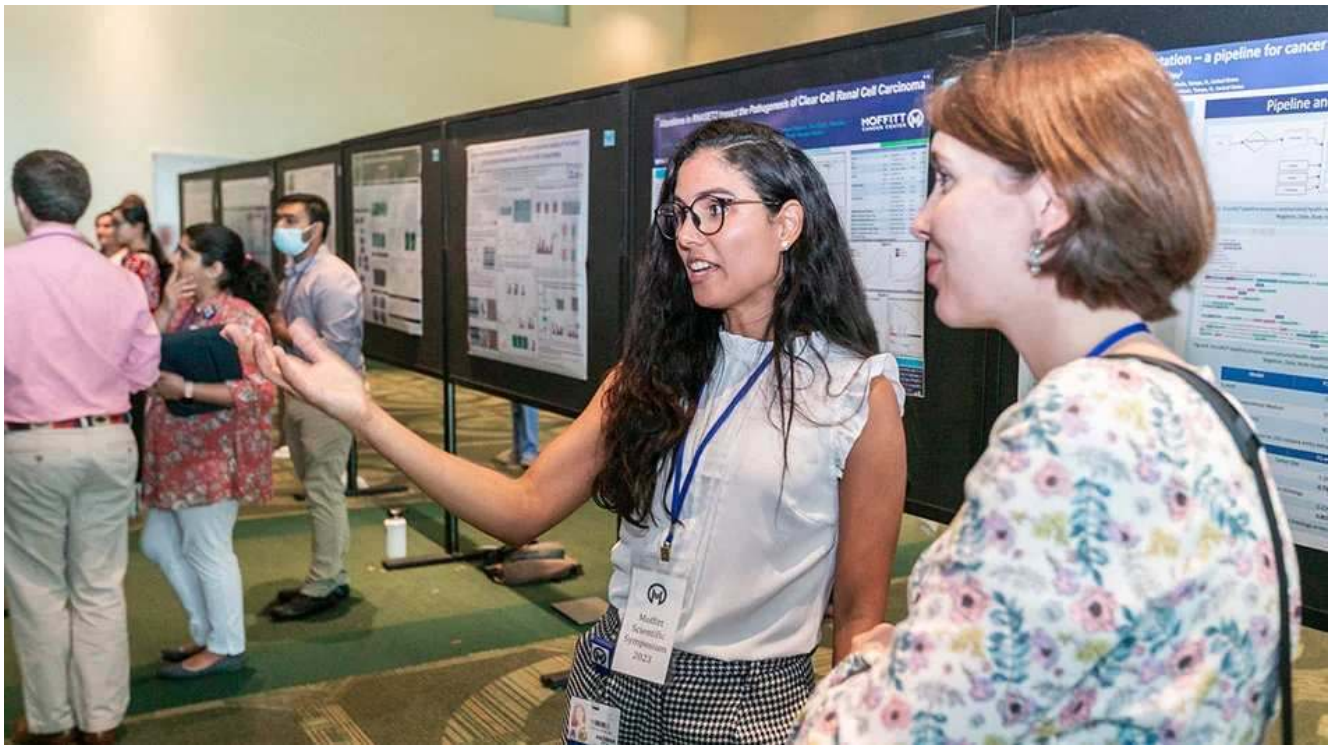
Training and Education

Become a Moffitt Postdoc

Research Education and Training (RET)

Become a Moffitt Postdoc



Are you interested in a career in cancer research? If you have completed your PhD (or equivalent terminal degree in your field), then postdoctoral training at Moffitt Cancer Center may be for you. **Find current postdoc job openings.**

There is also the opportunity to participate in NCI T32 postdoctoral training programs at Moffitt. These formal programs are specifically designed to advance key fields in cancer research. Please check individual program descriptions for eligibility and application information:

- **Behavioral Oncology T32**
- **Integrated Program in Cancer and Data Science T32**

Why Choose Moffitt?

- Located within the coastal community of Tampa Bay, one of the fastest-growing metropolitan areas in the U.S., Moffitt is the only **NCI Comprehensive Cancer Center** based in the state of Florida. **Learn more about Moffitt**.
- Nearly 200 Moffitt faculty members represent a diverse group of scientists and physicians, all dedicated to cancer research. **Learn more about the faculty.**
- Moffitt is ranked No. 10 in the nation and is the top-ranked cancer hospital in Florida and the Southeast. **Learn more about Moffitt's achievements.**

Moffitt Innovators of Tomorrow Symposium

Apply to the Moffitt Innovators of Tomorrow Symposium for the opportunity to visit our campus, present your research, meet our faculty and current postdocs, and see how you can advance your career by becoming a Moffitt postdoc.

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Discover More Health Professional Education Postdoctoral Training Postdoc Stories

Exhibit H. Final Merits Determination

THE UNITED STATES OF AMERICA

I-797B | NOTICE OF ACTION | DEPARTMENT OF HOMELAND SECURITY U.S. CITIZENSHIP AND IMMIGRATION SERVICES

Receipt Number		Case Type
IOE8530195667		I129 - PETITION FOR A NONIMMIGRANT WORKER
Received Date	**Priority Date**	**Petitioner**
11/01/2024		H LEE MOFFITT CANCER CENTER AND RESEARCH INSTITUTE INC
Notice Date	**Page**	**Beneficiary**
02/27/2025	1 of 2	YONEHARA NOMA, ISABELLA HARUMI

H LEE MOFFITT CANCER CENTER AND RESEARCH
c/o PATEL, KHUSHBU PARESH
BUCHANAN INGERSOLL AND ROONEY PC
401 E JACKSON STREET STE. 2400
TAMPA FL 33602

Notice Type: Approval Notice
Class: O1A
Valid from 02/27/2025 to 01/05/2028

The above petition has been approved, and notification has been sent to the listed consulate. You may also send the tear-off bottom part of this notice to the worker(s) to show the approval. Please contact the consulate with any questions about visa issuance. **THIS FORM IS NOT A VISA AND MAY NOT BE USED IN PLACE OF A VISA.**

Petition approval does not authorize employment or training. When the workers are granted status upon admission to the United States, they can then work for the petitioner, but only as detailed in the petition and for the period authorized. When seeking admission to the United States, the following classifications may be eligible for a grace period of up to 10 days before, and up to 10 days after the petition validity period: CW-1, E-1, E-2, E-3, H-1B, H-2B, H-3, L-1A, L-1B, O-1, O-2, P-1, P-2, P-3, TN-1, and TN-2. H-2A nonimmigrants may be eligible for a grace period of up to one week before and 30 days after the petition validity period. If provided at admission, this grace period will be annotated on the beneficiary's I-94 by Customs and Border Protection (CBP). The grace period is a period of authorized stay but does not provide the beneficiary authorization to work beyond the petition validity period. Please contact the IRS with any questions about tax withholding.

If circumstances change, the petitioner can file Form I-824 to have us notify another consulate of this approval. If any of the workers are already in the U.S. the petitioner can file a new Form I-129 to seek to change or extend their status based on this petition. Changes in employment or training may also require a new petition. Include a copy of this notice with any other required documentation.

The approval of this visa petition does not in itself grant any immigration status and does not guarantee that the alien beneficiary will subsequently be found to be eligible for a visa, for admission to the United States, or for an extension, change, or adjustment of status.

Number of workers: 1

Please see the additional information on the back. You will be notified separately about any other cases you filed.

USCIS encourages you to sign up for a USCIS online account. To learn more about creating an account and the benefits, go to https://www.uscis.gov/file-online.

California Service Center
U.S. CITIZENSHIP & IMMIGRATION SVC
PO Box 30113
Tustin CA 92781
USCIS Contact Center: www.uscis.gov/contactcenter

Please tear off portion below and forward it to the alien worker.

The alien may use this portion when applying for a visa at an American consulate abroad, or if no visa is required, when applying for admission to the U.S.

Receipt#: IOE8530195667 Case Type: I129
Notice Date: February 27, 2025 Petitioner: H LEE MOFFITT CANCER CENTER AND RESEARCH INSTITUTE INC,
Petitioner Validity Dates: Valid from 02/27/2025 to 01/05/2028 Number of Workers: 1

Name	DOB	COB	Class	Consulate/POE	OCC
YONEHARA NOMA, ISABELLA HARUMI	09/03/1996	BRAZIL	O1A	SAO PAULO	070

THE UNITED STATES OF AMERICA

I-797B | NOTICE OF ACTION | DEPARTMENT OF HOMELAND SECURITY U.S. CITIZENSHIP AND IMMIGRATION SERVICES

Receipt Number		Case Type
IOE8530195667		I129 - PETITION FOR A NONIMMIGRANT WORKER
Received Date 11/01/2024	**Priority Date**	**Petitioner** H LEE MOFFITT CANCER CENTER AND RESEARCH INSTITUTE INC
Notice Date 02/27/2025	**Page** 2 of 2	**Beneficiary** YONEHARA NOMA, ISABELLA HARUMI

Name	DOB	COB	Class	Consulate/POE	OCC
YONEHARA NOMA, ISABELLA HARUMI	09/03/1996	BRAZIL	O1A	SAO PAULO	070

The Small Business Regulatory Enforcement and Fairness Act established the Office of the National Ombudsman (ONO) at the Small Business Administration. The ONO assists small businesses with issues related to federal regulations. If you are a small business with a comment or complaint about regulatory enforcement, you may contact the ONO at www.sba.gov/ombudsman or phone 202-205-2417 or fax 202-481-5719.

NOTICE: Although this application or petition has been approved, USCIS and the U.S. Department of Homeland Security reserve the right to verify this information before and/or after making a decision on your case so we can ensure that you have complied with applicable laws, rules, regulations, and other legal authorities. We may review public information and records, contact others by mail, the internet or phone, conduct site inspections of businesses and residences, or use other methods of verification. We will use the information obtained to determine whether you are eligible for the benefit you seek. If we find any derogatory information, we will follow the law in determining whether to provide you (and the legal representative listed on your Form G-28, if you submitted one) an opportunity to address that information before we make a formal decision on your case or start proceedings.

Please see the additional information on the back. You will be notified separately about any other cases you filed.

USCIS encourages you to sign up for a USCIS online account. To learn more about creating an account and the benefits, go to https://www.uscis.gov/file-online.

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USCIS Contact Center: www.uscis.gov/contactcenter

Please tear off portion below and forward it to the alien worker.

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Exhibit I. Personal Documents

1ST CIVIL REGISTRY OFFICE OF NATURAL PERSONS
Births, Marriages, and Deaths

Fernando César Menezes Nagib Neme
REGISTRAR

Sandra C. C. Nagib Neme — SWORN CLERK
Claudio Teixeira Flóes — SWORN CLERK

REGISTRY OFFICE
NAGIB NEME

Avenida XV de Novembro Nº 630. Fone/Fax: (044) 222-7166. P.O. Box 795. Maringá - Paraná

BOOK A-239 **PAGES 208** **ENTRY 077845**

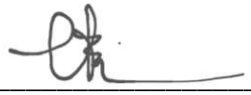
BIRTH CERTIFICATE

I **CERTIFY** that, in the book, page, and entry referenced, from the Birth Records of this Office, it is recorded that on September 4, 1996, the birth record of .-.-.-.-.-.-.-.

ISABELLA HARUMI YONEHARA NOMA was registered.

Female, born on the third of September, nineteen ninety-six (09/03/1996), at eight thirty-five a.m. (08:35h), at São Marcos Hospital and Maternity, in Maringá, Paraná. Daughter of **EDSON NOMA** .-.-.-.-.-.-.-.

I, Carolina Favero da Silva, telephone number 415 425-2508, mailing address P.O. Box 90487, San Diego, CA 92169, certify that the professional translation of this document from Portuguese to English has been performed by myself, a qualified translator fluent in both languages, and that the following is an accurate and complete translation of the document.


_____ Date: August 28, 2025.

CARTÓRIO
NAGIB NEME

1º OFÍCIO DO REGISTRO CIVIL DAS PESSOAS NATURAIS
Nascimentos, Casamentos e Óbitos
Fernando César Menezes Nagib Neme
OFICIAL REGISTRADOR
Sandra C. C. Nagib Neme
ESCREVENTE JURAMENTADA
Cláudio Teixeira Flóes
ESCREVENTE JURAMENTADO
Avenida XV de Novembro Nº 630 • Fone/Fax: (044) 222-7166 • Caixa Postal 795 • Maringá - Paraná

LIVRO A-239 **FOLHAS 208** **TERMO 077845**

CERTIDÃO DE NASCIMENTO

CERTIFICO que, do livro, folha e termo citados, de ASSENTO DE NASCIMENTOS deste Ofício, consta que, foi lavrado no dia 04 de setembro de 1996, o assento de Nascimento de.-.-.-.-.-.-.-.-.

ISABELLA HARUMI YONEHARA NOMA

do sexo feminino, nascida no dia três de setembro de um mil, novecentos e noventa e seis (03/09/1996), às oito horas e trinta e cinco minutos (08:35h), no Hospital e Maternidade São Marcos, em Maringá-PR.-.-.-.-.-.-.-.-

GF001610

Isabella H. Y. Noma

Assinatura do titular / Signature du titulaire
Bearer's signature / Firma del titular

Este passaporte deve ser assinado pelo titular, salvo em caso de incapacidade.

Ce passeport doit être signé par le titulaire, sauf en cas d'incapacité.

This passport must be signed, except where the bearer is unable to do so.

Este pasaporte debe ser firmado por el titular, salvo en caso de incapacidad.

REPÚBLICA FEDERATIVA DO BRASIL

PASSAPORTE
PASSPORT

TIPO / TYPE: P
PAÍS EMISSOR / ISSUING COUNTRY: BRA
PASSAPORTE Nº / PASSPORT No: GF001610

SOBRENOME / SURNAME
YONEHARA NOMA

NOME / GIVEN NAMES
ISABELLA HARUMI

NACIONALIDADE / NATIONALITY
BRASILEIRO(A)

DATA DO NASCIMENTO / DATE OF BIRTH
03 SET/SEP 1996

IDENTIDADE Nº / PERSONAL No

SEXO / SEX
F

NATURALIDADE / PLACE OF BIRTH
MARINGÁ/PR

FILIAÇÃO / FILIATION
MAGDA YURIKO YONEHARA

EDSON NOMA

DATA DE EXPEDIÇÃO / DATE OF ISSUE
18 AGO/AUG 2022

AUTORIDADE / AUTHORITY
NUPAS/SR/SP

VÁLIDO ATÉ / DATE OF EXPIRY
17 AGO/AUG 2032

P<BRAYONEHARA<NOMA<<ISABELLA<HARUMI<<<<<<<<<
GF001610<3BRA9609037F3208173<<<<<<<<<<<<<02

UNITED STATES OF AMERICA

VISA

Issuing Post Name
SAO PAULO

Control Number
20251015550023

Surname
YONEHARA NOMA

Given Name
ISABELLA HARUMI

Visa Type /Class
R 01

Passport Number	Sex	Birth Date	Nationality
GF001610	F	03SEP1996	BRZL

Entries	Issue Date	Expiration Date
M	15APR2025	13APR2026

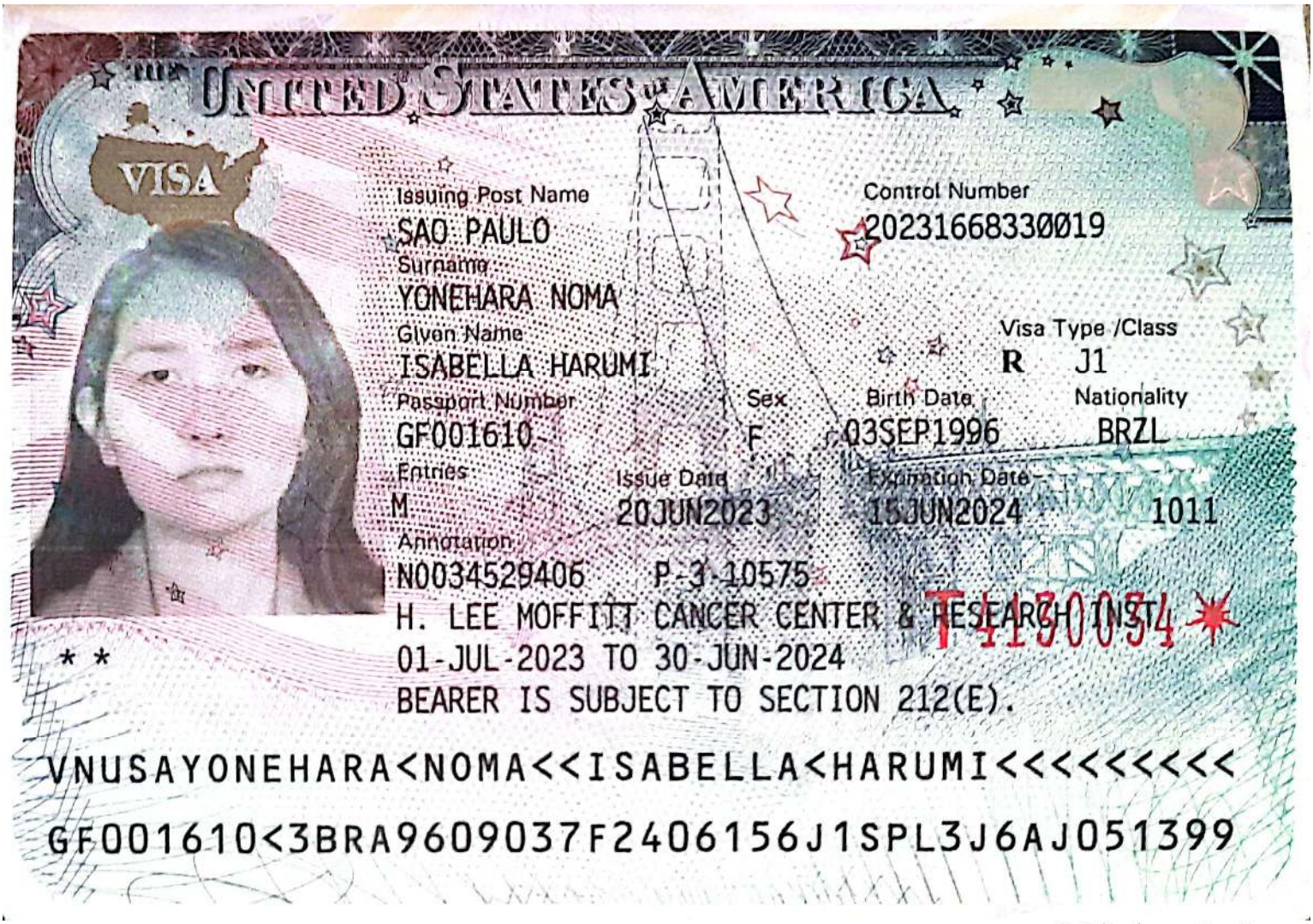
Annotation
NOT VALID UNTIL TEN DAYS PRIOR TO THE
PETITION VALIDITY DATE
PN-H LEE MOFFITT CANCER CENTER AND RESEARCH
P#-IOE8530195667 PED-05JAN2028

V03740G5

VNUSAYONEHARA<NOMA<<ISABELLA<HARUMI<<<<<<<<<<

GF001610<3BRA9609037F2604136O1SPL456IF051393

713



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View Travel History

Travel history includes up to 100 arrivals and departures spanning the last ten years

Travel History Results

Document Number: **GF001610**

Document Country of Issuance: **Brazil**

Row	DATE	TYPE	LOCATION
1	2025-05-03	Arrival	CHI
2	2024-07-24	Departure	ATL
3	2023-07-29	Arrival	WAS

OMB No. 1651-0111 Expiration Date: 08/31/2025

For: **ISABELLA HARUMI YONEHARA NOMA**

U.S. Customs and Border Protection
Securing America's Borders

Most Recent I-94

Note to employers, local, state or federal agency granting benefits:
Please visit the CBP I-94/I-95 Website and click on the tab for "Get Most Recent I-94/I-95" to perform a search for the applicant to confirm that the biographic and travel information displayed on this I-94/I-95 printout matches the "Get Most Recent I-94/I-95" returned results for this applicant. Reference the CBP I-94/I-95 Website FAQs.

Admission I-94 Record Number: 517915696A4

Arrival/Issued Date: 2025 May 03

Class of Admission: O1

Admit Until Date: 2028 January 10

Details provided on the I-94 Information form:

Last/Surname: YONEHARA NOMA

First (Given) Name: ISABELLA HARUMI

Birth Date: 1996 September 03

Document Number: GF001610

Country of Citizenship: Brazil

- Effective April 26, 2013, DHS began automating the admission process. An alien lawfully admitted or paroled into the U.S. is no longer required to be in possession of a preprinted Form I-94/I-95. A record of admission printed from the CBP website constitutes a lawful record of admission. See 8 CFR § 1.4(d).
- What to do if someone requests your admission info: If an employer, local, state or federal agency requests admission information, present your admission (I-94/I-95) number along with any additional required documents requested by that employer or agency.
- For security, close your browser after retrieving your I-94/I-95 number.

OMB No. 1651-0111
Expiration Date: 08/31/2025

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http://selo.funarpen.com.br

FEDERATIVE REPUBLIC OF BRAZIL
CIVIL REGISTRY OF NATURAL PERSONS

MARRIAGE CERTIFICATE

Name	CPF:
PEDRO HENRIQUE CAETANO DA SILVA **	087.762.369-43 **
Name	CPF:
ISABELLA HARUMI YONEHARA NOMA **	076.624.799-60 **

Registration
080275 01 55 2024 2 00149 224 0042721 34

Full maiden names, dates and places of birth, nationality, and filiation of the spouses

PEDRO HENRIQUE CAETANO DA SILVA, born on February 28, 1996, in Ivatuba-Paraná, Brazilian, single, self-employed painter, son of VALDEVINO JUSTINO DA SILVA and ZENAIDE DE FATIMA CAETANO, residing at Rua Professor Itamar Orlando Soares, 155, apartment 102, Zona 07, Maringá, Paraná, ZIP Code: 87.020-270 **

ISABELLA HARUMI YONEHARA NOMA, born on September 3, 1996, in Maringá-Paraná, Brazilian, single, biomedical, daughter of EDSON NOMA and MAGDA YURIKO YONEHARA, residing at Rua Professor Itamar Orlando Soares, 155, apartment 102, Zona 07, Maringá-Paraná, ZIP code: 87.020-270. **

Date of marriage registration (in full):	Day	Month	Year
June twenty-ninth, two thousand twenty-four **	29	06	2024

Matrimonial property regime
Partial Community of Property **

Name each spouse adopted (if changed)
---------- **

Notes/Annotations to be added
Marriage celebrated at this Office, before the Justice of the Peace, Mrs. Maria Renata Diogo Pereira **

Registration notes

1st Spouse:

Document type	Number	Date of issue	Issuing authority	Expiration date

ZIP Code	87.020-270		Blood Type	---

2nd Spouse:

Document type	Number	Date of issue	Issuing authority	Expiration date

ZIP Code	87.020-270		Blood Type	---

*The registration notes above do not exempt the interested party from presenting the original document when required by the requesting agency or when necessary for the identification of the holder.

Office Name
2ND CIVIL REGISTRY AND 6TH NOTARY OFFICE
Registrar
Maria Regina Pereira Boeira
City, District / State
City and Judicial District of Maringá - State of Paraná
Address
Avenida Prudente de Moraes, No. 228, Zone 07
ZIP Code: 87.020-010 - Phone: (44) 3032-0101

The content of this certificate is true. I certify.

Maringá, Paraná, June 29, 2024.

----//signature//----
Maria Andrea Gonçalves *[unreadable]*
Sworn Clerk

MARIA REGINA REGISTRY OFFICE
2ND CIVIL REGISTRY AND
6TH NOTARY OFFICE
Maria Regina Pereira Boeira
Registrar

FUNARPEN BC 005972928 BRP

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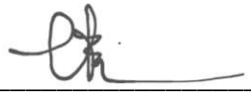
I, Carolina Favero da Silva, telephone number 415 425-2508, mailing address P.O. Box 90487, San Diego, CA 92169, certify that the professional translation of this document from Portuguese to English has been performed by myself, a qualified translator fluent in both languages, and that the following is an accurate and complete translation of the document.


_____ Date: August 28, 2025.

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SELO DE FISCALIZAÇÃO
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https://selo.funarpen.com.br

REPÚBLICA FEDERATIVA DO BRASIL
REGISTRO CIVIL DAS PESSOAS NATURAIS

CERTIDÃO DE CASAMENTO

Nome	CPF
PEDRO HENRIQUE CAETANO DA SILVA ••	087.762.369-43 ••
ISABELLA HARUMI YONEHARA NOMA ••	076.624.799-60 ••

Matrícula
080275 01 55 2024 2 00149 224 0042721 34

Nomes completos de solteiro, datas e locais de nascimento, nacionalidade e filiações dos cônjuges

PEDRO HENRIQUE CAETANO DA SILVA, nascido aos 28 de fevereiro de 1996, natural de Ivatuba-PR, de nacionalidade brasileira, solteiro, pintor autônomo, filho de VALDEVINO JUSTINO DA SILVA e de ZENAIDE DE FATIMA CAETANO, residente e domiciliado na Rua Professor Itamar Orlando Soares, 155, apto. 102, Zona 07, em Maringá-PR, CEP: 87.020-270 ••

ISABELLA HARUMI YONEHARA NOMA, nascida aos 03 de setembro de 1996, natural de Maringá-PR, de nacionalidade brasileira, solteira, biomédica, filha de EDSON NOMA e de MAGDA YURIKO YONEHARA, residente e domiciliada na Rua Professor Itamar Orlando Soares, 155, apto. 102, Zona 07, em Maringá-PR, CEP: 87.020-270 ••

Data do registro do casamento (por extenso)	Dia	Mês	Ano
Vinte e nove de junho de dois mil e vinte e quatro ••	29	06	2024

Regime de bens do casamento
Comunhão Parcial de Bens ••

Nome que cada um dos cônjuges passou a utilizar (quando houver alteração)
---------- ••

Averbações/Anotações à acrescer
Casamento celebrado neste Serviço, perante a Juíza de Paz Sra. Maria Renata Diogo Pereira. ••

Anotações de cadastro

1º Cônjuge

Tipo documento	Número	Data expedição	Orgão expedidor	Data de validade

CEP residencial	87.020-270	Grupo Sanguíneo	---

2º Cônjuge

Tipo documento	Número	Data expedição	Orgão expedidor	Data de validade

CEP residencial	87.020-270	Grupo Sanguíneo	---

* As anotações de cadastro acima não dispensam a parte interessada da apresentação do documento original, quando exigido pelo órgão solicitante ou quando necessário para identificação de seu portador.

Nome do Ofício
2º REGISTRO CIVIL E 6º TABELIONATO DE NOTAS

Oficial Registrador
Maria Regina Pereira Boeira

Município e Comarca / UF
Município e Comarca de Maringá - Estado do Paraná

Endereço
Avenida Prudente de Moraes, nº 228, Zona 07
CEP: 87.020-010 - Fone: (44) 3032-0101

O conteúdo da certidão é verdadeiro. Dou fé.

Maringá-PR, 29 de junho de 2024.

CARTÓRIO MARIA REGINA
2º REGISTRO CIVIL E 6º TABELIONATO DE NOTAS
Maria Regina Pereira Boeira
Titular

Maria Andrea Gonçalves Graziozzi
Escrevente Juramentada

Indústria Gráfica Brasileira Rota

BC 00597928 BRP

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CITY OF DOUTOR CAMARGO

JUDICIAL DISTRICT OF MARINGÁ – STATE OF PARANÁ

NOCCHI PEACE COURT AND ANNEX REGISTRY OFFICE

José Deuslene Jardim Mocchi

Registrar – CPF 208.208.619-91

Dulcineide Aparecida Ferreira Nocchi
Sworn Clerk - CPF 810.484.599-91

Wagner Alan Nocchi
Sworn Clerk - CPF 240.259.689-91

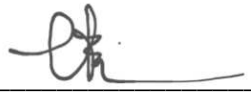
Address: Avenida Ivaí, 498 – Phone: (44) 238-1210 – Doutor Camargo – Paraná

Birth Record No. 2,528

I CERTIFY that on page 214 :-:-:-:-:- of Book No. A - 9 - of the Birth Registry, the birth record of **"PEDRO HENRIQUE CAETANO DA SILVA"** was registered today. :-:-:-:-:-:-:-:-:-:-:-:-:-:

:-:-:-:-:-:-:-:-:-:-:-:-

I, Carolina Favero da Silva, telephone number 415 425-2508, mailing address P.O. Box 90487, San Diego, CA 92169, certify that the professional translation of this document from Portuguese to English has been performed by myself, a qualified translator fluent in both languages, and that the following is an accurate and complete translation of the document.


_____ Date: August 28, 2025.

MUNICÍPIO DE DOUTOR CAMARGO

COMARCA DE MARINGÁ — ESTADO DO PARANÁ

CARTÓRIO DE PAZ E ANEXOS NOCCHI

José Deuslene Jardim Nocchi
TITULAR - CPF 206 208 619-91

Dulcineide Aparecida Ferreira Nocchi
Escrev. Juramentada - CPF 810 484 599-91

Wagner Alan Nocchi
Escrev. Juramentado - CPF 240 259 689-91

Avenida Ivaí, 498 — Fone: (044) 238-1210 — Doutor Camargo — Paraná

Nascimento N.º 2.528

CERTIFICO que, às fls. 214 do Livro nº A - 9 - de Registro de Nascimentos, foi lavrado hoje o assento de "PEDRO HENRIQUE CAETANO DA SILVA" nascido aos vinte e oito (28) - de Fevereiro (02) de mil novecentos e noventa e seis (1996) - às 08: horas e 45 minutos, em Hospital e Maternidade da Cidade de Ivatuba-Pr. do sexo masculino Filho de Valdevino Justino da Silva, Comerciante. natural de Maringá-Pr. e de Dona Zenaide de Fatima Caetano, do lar, c/ 34 anos de idade. natural de Ivatuba-Pr. Brasileiros, ele casado e ela solteira, residentes e domiciliados neste Municipio de Dr.Camargo-Pr.

Sendo avós paternos Franquelino Justino da Silva e Dona Maria José da Silva e avós maternos Celestino Caetano e Dona Saturnina Condes Caetano

Foi declarante Os Pais do Registrando. e serviram de testemunhas Srs. José Bozza e Otavio Raduvir Romanezi, Brasileiros, maiores, capazes, aqui residentes e domiciliados.

Observações:

O referido é verdade e dou fé,

Dr.Camargo-Pr. 06 de Março de 1996

OFICIAL
José Deuslene Jardim Nocchi
TITULAR
CPF. 206 208 619-91

GF510121

Assinatura do titular / Signature du titulaire
Bearer's signature / Firma del titular

Este passaporte deve ser assinado pelo titular, salvo em caso de incapacidade.

Ce passeport doit être signé par le titulaire, sauf en cas d'incapacité.

This passport must be signed, except where the bearer is unable to do so.

Este pasaporte debe ser firmado por el titular, salvo en caso de incapacidad.

REPÚBLICA FEDERATIVA DO BRASIL

PASSAPORTE
PASSPORT

TIPO / TYPE: P
PAÍS EMISSOR / ISSUING COUNTRY: BRA
PASSAPORTE Nº / PASSPORT No: GF510121

SOBRENOME / SURNAME: CAETANO DA SILVA

NOME / GIVEN NAMES: PEDRO HENRIQUE

NACIONALIDADE / NATIONALITY: BRASILEIRO(A)

DATA DO NASCIMENTO / DATE OF BIRTH: 28 FEV/FEB 1996

IDENTIDADE Nº / PERSONAL No:

SEXO / SEX: M

NATURALIDADE / PLACE OF BIRTH: IVATUBA/PR

FILIAÇÃO / FILIATION: VALDEVINO JUSTINO DA SILVA

ZENAIDE DE FATIMA CAETANO

DATA DE EXPEDIÇÃO / DATE OF ISSUE: 07 NOV/NOV 2022

AUTORIDADE / AUTHORITY: DPF/MGA/PR

VALIDO ATÉ / DATE OF EXPIRY: 06 NOV/NOV 2032

P<BRACAETANO<DA<SILVA<<PEDRO<HENRIQUE<<<<<<<
GF510121<7BRA9602289M3211061<<<<<<<<<<<<<<00

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For: **PEDRO HENRIQUE CAETANO DA SILVA**

U.S. Customs and Border Protection
Securing America's Borders

Most Recent I-94

Note to employers, local, state or federal agency granting benefits:
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Admission I-94 Record Number: 517916026A4

Arrival/Issued Date: 2025 May 03

Class of Admission: O3

Admit Until Date: 2028 January 10

Details provided on the I-94 Information form:

Last/Surname: CAETANO DA SILVA

First (Given) Name: PEDRO HENRIQUE

Birth Date: 1996 February 28

Document Number: GF510121

Country of Citizenship: Brazil

- Effective April 26, 2013, DHS began automating the admission process. An alien lawfully admitted or paroled into the U.S. is no longer required to be in possession of a preprinted Form I-94/I-95. A record of admission printed from the CBP website constitutes a lawful record of admission. See 8 CFR § 1.4(d).
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- For security, close your browser after retrieving your I-94/I-95 number.

OMB No. 1651-0111
Expiration Date: 08/31/2025

View Travel History

Travel history includes up to 100 arrivals and departures spanning the last ten years

Travel History Results

Document Number: **GF510121**

Document Country of Issuance: **Brazil**

Row	DATE	TYPE	LOCATION
1	2025-05-03	Arrival	CHI

OMB No. 1651-0111 Expiration Date: 08/31/2025