Recipient Information

1. Recipient Name
SCRIPPS RESEARCH INSTITUTE, THE
130 SCRIPPS WAY

JUPITER, FL 33458

2. Congressional District of Recipient
18

3. Payment System Identifier (ID)
1330435954A5

4. Employer Identification Number (EIN)
330435954

5. Data Universal Numbering System (DUNS)
148230662

6. Recipient's Unique Entity Identifier
MGUCX773RNJ8

7. Project Director or Principal Investigator
Mauricio de Aguiar Martins, PHD
Assistant Professor
mmartins@scripps.edu
Redacted by agreement

8. Authorized Official
Redacted by agreement
Redacte@scripps.edu
(858) 784-8653

Federal Agency Information

9. Awarding Agency Contact Information
PAULA Acevedo
Grants Management Specialist
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
paula.acevedo@nih.gov
301-435-2860

10. Program Official Contact Information
James E. Cummins
Program Official
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
cumminsje@niaid.nih.gov
240-292-4800

Federal Award Information

11. Award Number
5R21AI157929-02

12. Unique Federal Award Identification Number (FAIN)
R21AI157929

13. Statutory Authority
42 USC 241 42 CFR 52

14. Federal Award Project Title
A nonhuman primate model to study the immunological effects of feminizing hormone therapy in transgender women

15. Assistance Listing Number
93.855

16. Assistance Listing Program Title
Allergy and Infectious Diseases Research

17. Award Action Type
Non-Competing Continuation

18. Is the Award R&D?
Yes

Summary Federal Award Financial Information

19. Budget Period Start Date 12/01/2021 **– End Date** 11/30/2022	
20. Total Amount of Federal Funds Obligated by this Action	$205,562
20 a. Direct Cost Amount	$173,329
20 b. Indirect Cost Amount	$32,233
21. Authorized Carryover	$0
22. Offset	$0
23. Total Amount of Federal Funds Obligated this budget period	$205,562
24. Total Approved Cost Sharing or Matching, where applicable	$0
25. Total Federal and Non-Federal Approved this Budget Period	$205,562
26. Project Period Start Date 12/01/2020 **– End Date** 11/30/2022	
27. Total Amount of the Federal Award including Approved Cost Sharing or Matching this Project Period	$478,188

28. Authorized Treatment of Program Income
Additional Costs

29. Grants Management Officer - Signature
Nicole A Guidetti

30. Remarks

Acceptance of this award, including the "Terms and Conditions," is acknowledged by the recipient when funds are drawn down or otherwise requested from the grant payment system.

EXPLORATORY/DEVELOPMENT GRANT
Department of Health and Human Services
National Institutes of Health

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

SECTION I – AWARD DATA – 5R21AI157929-02

Principal Investigator(s):
Mauricio de Aguiar Martins, PHD

Award e-mailed to: grants@scripps.edu

Dear Authorized Official:

The National Institutes of Health hereby awards a grant in the amount of $205,562 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to SCRIPPS FLORIDA in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award, including the "Terms and Conditions," is acknowledged by the recipient when funds are drawn down or otherwise requested from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R21AI157929. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please direct questions to the Federal Agency contacts.

Sincerely yours,

Nicole A Guidetti
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

Cumulative Award Calculations for this Budget Period (U.S. Dollars)

Federal Direct Costs	$173,329
Federal F&A Costs	$32,233
Approved Budget	$205,562
Total Amount of Federal Funds Authorized (Federal Share)	$205,562
TOTAL FEDERAL AWARD AMOUNT	$205,562
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$205,562

SUMMARY TOTALS FOR ALL YEARS (for this Document Number)		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
2	$205,562	$205,562

Fiscal Information:

Payment System Identifier:	1330435954A5
Document Number:	RAI157929A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2022

IC	CAN	2022
AI	8472297	$205,562

NIH Administrative Data:
PCC: A22F / **OC**: 41025 / **Released**: Guidetti, Nicole 11/12/2021
Award Processed: 11/15/2021 12:03:38 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 5R21AI157929-02

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – STANDARD TERMS AND CONDITIONS – 5R21AI157929-02

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but

non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R21AI157929. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

This award represents the final year of the competitive segment for this grant. See the NIH Grants Policy Statement Section 8.6 Closeout for complete closeout requirements at: http://grants.nih.gov/grants/policy/policy.htm#gps.

A final expenditure Federal Financial Report (FFR) (SF 425) must be submitted through the eRA Commons (Commons) within 120 days of the period of performance end date; see the NIH Grants Policy Statement Section 8.6.1 Financial Reports, http://grants.nih.gov/grants/policy/policy.htm#gps, for additional information on this submission requirement. The final FFR must indicate the exact balance of unobligated funds and may not reflect any unliquidated obligations. There must be no discrepancies between the final FFR expenditure data and the Payment Management System's (PMS) quarterly cash transaction data. A final quarterly federal cash transaction report is not required for awards in PMS B subaccounts (i.e., awards to foreign entities and to Federal agencies). NIH will close the awards using the last recorded cash drawdown level in PMS for awards that do not require a final FFR on expenditures or quarterly federal cash transaction reporting. It is important to note that for financial closeout, if a grantee fails to submit a required final expenditure FFR, NIH will close the grant using the last recorded cash drawdown level. If the grantee submits a final expenditure FFR but does not reconcile any discrepancies between expenditures reported on the final expenditure FFR and the last cash report to PMS, NIH will close the award at the lower amount. This could be considered a debt or result in disallowed costs.

A Final Invention Statement and Certification form (HHS 568), (not applicable to training, construction, conference or cancer education grants) must be submitted within 120 days of the expiration date. The HHS 568 form may be downloaded at: http://grants.nih.gov/grants/forms.htm. This paragraph does not apply to Training grants, Fellowships, and certain other programs—i.e., activity codes C06, D42, D43, D71, DP7, G07, G08, G11, K12, K16, K30, P09, P40, P41, P51, R13, R25, R28, R30, R90, RL5, RL9, S10, S14, S15, U13, U14, U41, U42, U45, UC6, UC7, UR2, X01, X02.

Unless an application for competitive renewal is submitted, a Final Research Performance Progress Report (Final RPPR) must also be submitted within 120 days of the period of performance end date. If a competitive renewal application is submitted prior to that date, then an Interim RPPR must be submitted by that date as well. Instructions for preparing an Interim or Final RPPR are at: https://grants.nih.gov/grants/rppr/rppr_instruction_guide.pdf. Any other specific requirements set forth in the terms and conditions of the award must also be addressed in the Interim or Final RPPR. *Note that*

data reported within Section I of the Interim and Final RPPR forms will be made public and should be written for a lay person audience.

NIH strongly encourages electronic submission of the final invention statement through the Closeout feature in the Commons, but will accept an email or hard copy submission as indicated below.

Email: The final invention statement may be e-mailed as PDF attachments to:
NIHCloseoutCenter@mail.nih.gov.

Hard copy: Paper submissions of the final invention statement may be faxed to the NIH Division of Central Grants Processing, Grants Closeout Center, at 301-480-2304, or mailed to:

National Institutes of Health
Office of Extramural Research
Division of Central Grants Processing
Grants Closeout Center
6705 Rockledge Drive
Suite 5016, MSC 7986
Bethesda, MD 20892-7986 (for regular or U.S. Postal Service Express mail)
Bethesda, MD 20817 (for other courier/express deliveries only)

NOTE: If this is the final year of a competitive segment due to the transfer of the grant to another institution, then a Final RPPR is not required. However, a final expenditure FFR is required and should be submitted electronically as noted above. If not already submitted, the Final Invention Statement is required and should be sent directly to the assigned Grants Management Specialist.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.
Treatment of Program Income:
Additional Costs

SECTION IV – AI SPECIFIC AWARD CONDITIONS – 5R21AI157929-02

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

This Notice of Award (NoA) includes funds for activity with **University of Wisconsin-Madison**.

SPREADSHEET SUMMARY
AWARD NUMBER: 5R21AI157929-02

INSTITUTION: SCRIPPS FLORIDA

Facilities and Administrative Costs	Year 2
F&A Cost Rate 1	85%
F&A Cost Base 1	$37,921
F&A Costs 1	$32,233

A. COVER PAGE

Project Title: A nonhuman primate model to study the immunological effects of feminizing hormone therapy in transgender women	
Grant Number: 5R21AI157929-02	**Project/Grant Period:** 12/01/2020 - 11/30/2022
Reporting Period: 12/01/2020 - 11/30/2021	**Requested Budget Period:** 12/01/2021 - 11/30/2022
Report Term Frequency: Annual	**Date Submitted:** 09/30/2021
Program Director/Principal Investigator Information: MAURICIO DEAGUIAR MARTINS , PHD **Phone Number:** Redacted by agreement **Email:** mmartins@scripps.edu	**Recipient Organization:** SCRIPPS FLORIDA 130 Scripps Way JUPITER, FL 334585284 **DUNS:** 148230662 **EIN:** 1330435954A5 **RECIPIENT ID:**
Change of Contact PD/PI: NA	
Administrative Official: Redacted by agreement 10550 N. Torrey Pines Rd TPC-7 La Jolla, CA 92037 **Phone number:** 858-784-8653 **Email:** Redacted@scripps.edu	**Signing Official:** Redacted by agreement 10550 N. Torrey Pines Rd TPC-7 La Jolla, CA 92037 **Phone number:** 858-784-8653 **Email:** Redacted@scripps.edu
Human Subjects: No	**Vertebrate Animals:** Yes
hESC: No	**Inventions/Patents:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Specific aim 1: Determine if feminizing hormone therapy (FHT) increases the availability of HIV-susceptible CD4+ T cells in vivo.

Specific aim 2: Determine if FHT interferes with AAV-mediated delivery of eCD4-Ig.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File Uploaded : B.2 20210921 Transgender R21 Progress Report_PH.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File Uploaded : IDP.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

With the pilot study to determine appropriate dosages of spironolactone (SP) and 17-□ estradiol (E2) completed, we can move on to a determination of immune consequences in circulating blood and gut biopsies of rhesus macaques treated with both E2 and SP. Additionally, we will determine if AAV9-driven expression of eCD4-Ig is affected by SP and E2 administration in AAV9-seronegatice rhesus macaques.

To determine if feminizing hormone therapy (FHT) affects the availability of HIV-susceptible CD4+ T cells in the rhesus macaque, a pilot study was performed to determine the appropriate dosages of spironolactone (SP) and 17-β estradiol (E2). E2 is prescribed in the regimen of FHT to increase estrogen levels to the female physiological range. Spironolactone is typically prescribed in tandem as an androgen competitor to lower testosterone levels. In order to determine the appropriate dosages of both E2 and SP, a pilot study was conducted using two animals per condition (**Fig. 1**). Proprietary Info

Proprietary Info

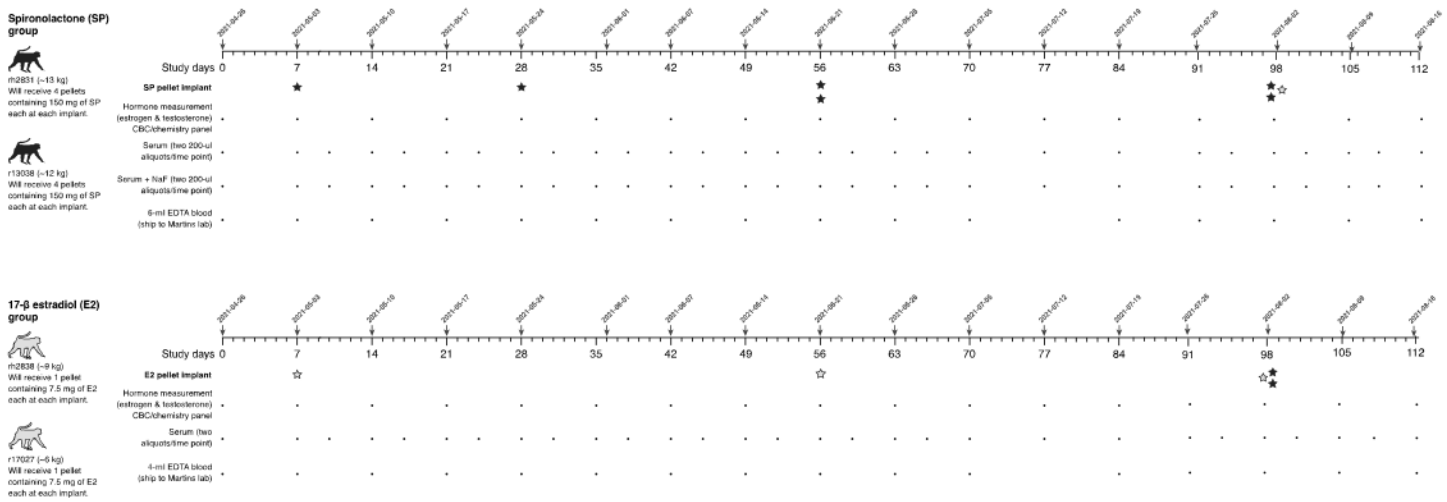

Figure 1. Experimental layout. The pilot experiment consisted of two groups of two biological male rhesus macaques. The first group was dosed using subcutaneously implanted slow-release drug pellets of 50 mg/kg spironolactone (SP), then later dosed with an increased 100 mg/kg of SP. The second group was dosed using similarly implanted slow-release pellets of 1 mg/kg 17-β estradiol (E2), then later dosed with a decreased 0.3 mg/kg of E2. At the final time point of implantation, all four rhesus macaques were dosed with 100 mg/kg of SP in combination with 0.3 mg/kg of E2.

Proprietary Info

Proprietary Info

Proprietary Info

Proprietary Info

INDIVIDUAL DEVELOPMENT PLANS DESCRIPTION

Annual performance reviews and Individual Development Plans (IDPs) are widely recognized as effective tools for setting and achieving Ph.D.-level training goals. They also encourage productive communication between trainees and their mentors.

The Scripps Research Institute strongly encourages graduate students and postdocs to create and revisit IDPs, and to seek regular feedback on their performance from their mentor. IDP templates are available from the Career and Postdoctoral Services Office website and are provided to trainees as part of the onboarding process. The Career and Postdoctoral Services Office also arranges biannual IDP workshops to help trainees interpret self-assessment information, explore career options, and set goals using *myIDP* from AAAS/ScienceCareers.org.

Mentors are encouraged to work with their trainees to create personalized IDPs. To provide necessary feedback, TSRI recommends that mentors conduct annual reviews with their assigned postdocs to discuss lab obligations, research goals, skills development, and career planning.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

No

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

C.5 OTHER PRODUCTS AND RESOURCE SHARING

NOTHING TO REPORT

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
eRA Commons User Name	Y	Martins, Mauricio de Aguiar	PHD	PD/PI	EFFORT					NA
	Y	Redacted by agreement		Co-Investigator						NA
	N			Graduate Student (research assistant)						NA
	N			Technician						NA

Glossary of acronyms:
S/K - Senior/Key
DOB - Date of Birth
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RE - Reentry Supplement
DI - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Will there be, in the next budget period, either (1) a reduction of 25% or more in the level of effort from what was approved by the agency for the PD/PI(s) or other senior/key personnel designated in the Notice of Award, or (2) a reduction in the level of effort below the minimum amount of effort required by the Notice of Award?

No

D.2.b New Senior/Key Personnel

Are there, or will there be, new senior/key personnel?

No

D.2.c Changes in Other Support

Has there been a change in the active other support of senior/key personnel since the last reporting period?

Yes

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D.2.d New Other Significant Contributors

Are there, or will there be, new other significant contributors?

No

D.2.e Multi-PI (MPI) Leadership Plan

Will there be a change in the MPI Leadership Plan for the next budget period?

NA

OTHER SUPPORT 5 R21 AI157929-02

Name of Individual: Martins, Mauricio
Commons ID: eRA Commons User Name

ACTIVE

Title: rAAV-mediated delivery of HIV-specific bnMAbs in newborn rhesus macaques

Major Goals: The overarching goal of this project is to develop a practical immune intervention for preventing mother-to-childtransmission (MTCT) of HIV via breastfeeding by evaluating the safety of AAV-mediated gene transfer to deliverthe broadly-reactive neutralizing monoclonal antibody (bnMAb) 3BNC117 to newborns.

Status: Active

Project Number: Private Source

Name of PD/PI: Martins, Mauricio

Source of Support: Private Source

Primary Place of Performance: Scripps Florida

Project / Proposal Start and End Date: (MM/YYYY) (if available): 07/12/18 - 03/31/22 NCE

Total Award Amount (including Indirect Costs): $1,044,704

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.#)
4. 2021	EFFORT calendar

Title: **An AAV-mediated functional cure and its impact on the reservoir**
Major Goals: We show that AAV-expressed entry inhibitors can establish functional cures in rhesus macaques. We will make these functional cures safer, more consistent, and more robust, use these cures to study "kick-and kill" eradication strategies, and ask whether a "kick" is necessary if the "kill" is potent and sustained.

Status of Support: Active

Project Number: 5 U19 AI149646-02

Name of PD/PI: Redacted by agreement

Source of Support: NIAID

Primary Place of Performance: Scripps Florida

Project/Proposal Start and End Date: (if available): 04/15/2020 - 03/31/2025

Total Award Amount (including Indirect Costs): $14,215,400 ($2,312,500 Martins' Lab Portion)

Person Months (Calendar/Academic/Summer) per budget period.

OTHER SUPPORT 5 R21 AI157929-02

Name of Individual: Martins, MauricioCommons ID: [eRA Commons User Name]

Year (YYYY)	Person Months (##.##)
2. 2021	[EFFORT] calendar
3. 2022	calendar
4. 2023	calendar
5. 2024	calendar

Title: A nonhuman primate model to study the immunological effects of feminizing hormone therapy intransgender women

Major Goals: This project will investigate whether feminizing hormone therapy (FHT) can contribute to the high incidence of HIV infection among transgender women. To that end, FHT will be modeled in male rhesus macaques to determine whether it increase the availability of HIV "target" cells in the gut mucosa and whether FHT interferes with a prophylactic immune intervention. Ultimately, this project will advance our understanding of the pharmacodynamics of FHT and how it affects immune responses in biological males.
(THIS AWARD)

Status of Support: Active

Project Number: 5R21AI157929-02

Name of PD/PI: Martins, Mauricio

Source of Support: NIH / NIAID

Primary Place of Performance: Scripps Florida

Project / Proposal Start and End Date: (MMNYYY) 2/01/2020 — 11/30/2022

Award Amount (including Indirect Costs): $478,188 ($207,808 Martins' lab portion)

Person Months (Calendar / Academic / Summer) per budget period.

Year (YYYY)	Person Months (##.##)
2.2021	[EFFORT] calendar

Title: AAV-mediated delivery of eCD4-Ig for prevention and treatment of perinatal HIV infection

Major Goals: Develop immune-based interventions to combat perinatal HIV infection. Here we will use the pediatric rhesus macaque model of HIV/AIDS to assess whether gene therapy with the antibody-like entry inhibitor eCD4-Ig canprotect infants from immunodeficiency virus acquisition and treat an established infection. If successful, the proposed research will establish the pre-clinical foundation for using gene therapy as prophylaxis and therapy for perinatal HIV infection.
(NEW FUNDING)

Status of Support: Active

Project Number: 5 R01 HD102252-02

Name of PD/PI: Martins, Mauricio

OTHER SUPPORT 5 R21 AI157929-02

Name of Individual: Martins, MauricioCommons ID: eRA Commons User Name

Source of Support: NICHD (Primary) / NIAID

Primary Place of Performance: Scripps Florida

Project *I* Proposal Start and End Date: (MM/YYYY) (if available): 08/07/20 - 05/31/25

Total Award Amount (including Indirect Costs): $4,115,922 ($1,536,163 Martins' lab portion)

Person Months (Calendar / Academic / Summer) per budget period.

Year (YYYY)	Person Months (##.##)
2.2021	EFFORT calendar
3. 2022	calendar
4.2023	calendar
5.2024	calendar

Title: eCD4-Ig for preventing and treating obstetric HIV infection

Major Goals: Young women in sub-Saharan Africa, who are already twice as likely to acquire HIV than their male counterparts, become even more susceptible to HIV infection during pregnancy and the postpartum periods.Because current prophylactic and treatment measures are unlikely to end the morbidity and mortality of HIV/AIDS in women of reproductive age, this project will explore the safety and antiviral properties of the HIVentry inhibitor eCD4-Ig in pregnant and postpartum rhesus macaques. If successful, this project will establishthe pre-clinical foundation for testing the ability of eCD4-Ig to combat obstetric HIV infection.
(NEW FUNDING)

Status of Support: Active

Project Number: 5 R01 HD103494-02

Name of PD/PI: Martins, Mauricio

Source of Support: NICHD (Primary) / NIAID

Primary Place of Performance: Scripps Florida

Project / Proposal Start and End Date: (MM/YYYY) (if available): 08/13/20- 05/31/25

Total Award Amount (including Indirect Costs): $4,042,316 ($1,116,902 Martins' lab portion)

Name of Individual: Martins, Mauricio Commons ID: eRA Commons User Name

Person Months (Calendar / Academic / Summer) per budget period

Year (YYYY)	Person Months(##.##)
2.2021	EFFORT calendar
3.2022	calendar
4.2023	calendar
5.2024	calendar

OTHER SUPPORT 5 R21 AI157929-02

Name of Individual: Martins, MauricioCommons ID: eRA Commons User Name

PENDING

Pending Support

OTHER SUPPORT 5 R21 AI157929-02

Pending Support

IN KIND

None

OVERLAP

None

I, Mauricio Martins, certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

Signature: Personal Info

Mauricio Martins

Date: 09/29/2021

Name of Individual: Redacted by
Commons ID: eRA Commons User

Other Support – 5 R21 AI57929-02

ACTIVE

Title: Wisconsin National Primate Research Center Support

Major Goals: The goals of the Scientific Protocol Implementation Unit (SPI) is to serve as the gateway for utilization of the WNPRC resources and service units that are available to core scientists, University of Wisconsin researchers, and external researchers from other institutions. SPI coordinates the research activities of the animal portion of WNPRC approved research programs by integrating research needs with resources and services available and by providing a competent team of technical staff supported by the scientific and veterinary unit heads. The scientific head is responsible for developing research plans with outside investigators and connecting them with other scientific expertise as needed.

Status of Support: Active

Project Number: 5 P51 OD 011106 60

Name of PD/PI: Redacted by agreement

Source of Support: OD

Primary Place of Performance: University of Wisconsin-Madison Project/Proposal

Start and End Date: (MM/YYYY) (if available): 07/21/2017– 04/30/2022

Total Award Amount (including Indirect Costs): $50,810,630

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
60. 2021	EFFORT calendar

Title: Zika virus pathophysiology during pregnancy

Major Goals: The key hypothesis of this Program Project grant is that prolonged maternal viremia during pregnancy predicts fetal risk of congenital Zika syndrome.

Status of Support: Active

Project Number: 5 P01 AI 132132 04

Name of Individual: Redacted by
Commons ID: eRA Commons User Name

Name of PD/PI: Redacted by agreement

Source of Support: NIAID

Primary Place of Performance: University of Wisconsin-Madison

Project/Proposal Start and End Date: (MM/YYYY) (if available): 08/01/2018 – 07/31/2023

Total Award Amount (including Indirect Costs): $10,192,689

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
4. 2021	EFFORT calendar
5. 2022	calendar

Title: A Multi-Modality, Multi-Scale Approach to Understanding Parturition

Major Goals: The overall goal of this project is to address the large knowledge gap in the basic understanding of parturition by constructing patient-specific biomechanical models that delineate the structure-function relationship of the cervix and other tissues that support the fetus (membranes, uterus), based on specific measurements of cervical microstructure, potential minor extracellular matrix (ECM) and non-ECM informants of cervical remodeling and maternal anatomy in the Rhesus macaque model for pregnancy.

Status of Support: Active

Project Number: 5 R01 HD 072077 09

Name of PD/PI: Redacted by

Source of Support: NICHD

Primary Place of Performance: University of Wisconsin-Madison

Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/01/2018 – 06/30/2023

Total Award Amount (including Indirect Costs): $3,321,260

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
4. 2021	EFFORT calendar
5. 2022	calendar

Title: An AAV-Mediated Functional Cure and Its Impact on the Reservoir

Major Goals: The overall goal is to establish and use an integrated non-human primates (NHP) pipeline for testing concepts relevant to an AAV-based functional cure in NHPs, used

Name of Individual: Redacted by
Commons ID: eRA Commons

by every project. Key personnel of this core, together with the project and other core leaders, form a governing board that collectively determine allocation of NHP resources, and help direct individual projects.

Status of Support: Active

Project Number: 5 U19 AI 149646 02

Name of PD/PI: Redacted by agreement

*Source of Support: NIA

Primary Place of Performance: The Scripps Research Institute, FL Project/Proposal

Start and End Date: (MM/YYYY) (if available): 04/15/2020 – 03/31/2025

Total Award Amount (including Indirect Costs): $3,807,715

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2021	EFFORT calendar
3. 2022	calendar
4. 2023	calendar
5. 2024	calendar

Title: Functional Role of O-glycosylation of HIV-1

Major Goals: The proposed studies will better define the characteristics of V1 region sequences that are predictive of whether an individual sequence is likely to be O-glycosylated. We will determine whether there is specificity to the blocking effects, i.e. whether one O-glycosylated V1 region potently blocks recognition by one V3-glycan mAb but not another while a different O-glycosylated V1 region may have different specificities to its blocking effects. We will use experimental SHIV infection of rhesus monkeys to examine whether there is selective disadvantage to such long O-glycosylated V1 regions in the absence of such antibodies and to examine the types of selective pressure that may drive elongation and O-glycosylation of V1 regions.

Status of Support: Active

Project Number: 5 R01 AI 104523 08

Name of PD/PI: Redacted by agreement

Source of Support: NIAID

Primary Place of Performance: University of Miami

Project/Proposal Start and End Date: (MM/YYYY) (if available): 02/15/2013 – 02/28/2025

Total Award Amount (including Indirect Costs): $776,508

Person Months (Calendar/Academic/Summer) per budget period.

Name of Individual: Redacted by
Commons ID: eRA Commons

Year (YYYY)	Person Months (##.##)
9. 2021	EFFORT calendar
10. 2022	calendar
11. 2023	calendar
12. 2024	calendar

(THIS AWARD)

Title: A nonhuman primate model to study the immunological effects of feminizing hormone therapy in transgender women

Major Goals: The overall goal is to model the effects of transfeminine (male-to-female) hormone therapy on active and passive immunization regimens against HIV. The study aims to determine if hormone therapy used for gender reassignment impact HIV vaccine immunogenicity in similar ways, and if hormone therapy can affect the immunogenicity and pharmacokinetics of monoclonal antibodies that are currently being used to prevent and treat HIV infection.

Status of Support: Active

Project Number: 5 R21 AI157929 02

Name of PD/PI: Martins, Mauricio

Source of Support: NIAID

Primary Place of Performance: Scripps Research Institute, FL

Project/Proposal Start and End Date: (MM/YYYY) (if available): 12/01/2020 – 11/30/2022

Total Award Amount (including Indirect Costs): $270,380

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2021	EFFORT calendar

(NEW)

Title: Preclinical Safety of Human Induced Pluripotent Stem Cell-Derived Cardiac Lineage Cells in a Nonhuman Primate Model of Right Ventricular Pressure Overload

Major Goals: The specific objective of this proposed work is to evaluate safety of the engraftment of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) after injection into an immunosuppressed primate diseased right ventricle (banded pulmonary artery). The banding of the pulmonary artery results in a condition that closely models the diseased, overloaded right heart that occurs in hypoplastic left heart syndrome

Name of Individual: Redacted by
Commons ID: eRA Commons

(HLHS). The aim of our proposed study is to investigate whether the hiPSC-CM cell product will engraft into the myocardium of the immunosuppressed macaque using a model of right ventricular disease induced by pulmonary artery banding.

Status of Support: Active

Project Number: N/A

Name of PD/PI: Redacted by agreement

Source of Support: Private Source

Primary Place of Performance: Private Source

Project/Proposal Start and End Date: (MM/YYYY) (if available): 10/01/2020 – 12/31/2021

Total Award Amount (including Indirect Costs): $999,125

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2020	EFFOR calendar

(NEW)

Title: WHICH ISOTYPE OF ECD4-IG MOST EFFECTIVELY SUPPRESSES VIRUS REPLICATION

Major Goals: eCD4-Ig is an exceptional HIV-1 entry inhibitor that may help prevent new HIV-1 infections and maintain a drug-free state of HIV-1 remission in infected persons. Here we will extend its half-life, increase its neutralization potency, and reduce its immunogenicity, thereby improving its safety, efficacy, and cost.

Status of Support: Active

Project Number: 5 R44 AI 145491 03

Name of PD/PI: Redacted by agreement

Source of Support: NIAID

Primary Place of Performance: Emmune, Inc.

Project/Proposal Start and End Date: (MM/YYYY) (if available): 05/19/2019 – 04/30/2023

Total Award Amount (including Indirect Costs): $671,921

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
3. 2021	EFFOR calendar
4. 2022	T calendar

Name of Individual: Redacted by eRA Commons
Commons ID:

Title: Therapeutic Use of an Enhanced Form of CD4-Ig

Major Goals: eCD4-Ig is a very broad and potent HIV-1 entry inhibitor that works well with a safe and established gene-therapy vector. We will determine whether eCD4-Ig, as a passively administered protein or with this gene-therapy vector, can suppress infection in rhesus macaques. We will also improve eCD4-Ig and its delivery system, and lay the foundations for human studies of eCD4-Ig.

Status of Support: Active

Project Number: 4 R37 AI 091476 11

Name of PD/PI: Redacted by agreement

Source of Support: NIAID

Primary Place of Performance: The Scripps Research Institute, FL

Project/Proposal Start and End Date: (MM/YYYY) (if available): 12/01/2019 – 11/30/2021 *UW sub*

Total Award Amount (including Indirect Costs): $689,073

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2020	EFFORT calendar

(NEW)

Title: RSV infection and immune transmission to offspring

Major Goals: Despite the significant global burden of respiratory syncytial virus (RSV) infection, there are limited therapeutic options for RSV infection and no vaccine to prevent the disease. The overall goals of the project are to test the efficacy of maternal vaccination and passive transfer of RSV immunity using novel vaccine candidates in a highly relevant, pre-clinical model. We expect to contribute critical information regarding the effect of pregnancy on vaccine-induced immunity and susceptibility to RSV infection of a pre-clinical model RSV infection.

Status of Support: Active

Project Number: 1 R01 AI 141648 – 1A1

Name of PD/PI: Redacted by agreement

Source of Support: NIAID

Primary Place of Performance: University of Georgia

Project/Proposal Start and End Date: (MM/YYYY) (if available): 02/01/2021 – 01/31/2022 *UW sub*

Total Award Amount (including Indirect Costs): $108,884

Person Months (Calendar/Academic/Summer) per budget period.

Name of Individual: Redacted by
Commons ID: eRA Commons

Year (YYYY)	Person Months (##.##)
1. 2021	EFFORT calendar

(NEW)

Title: PK/PD Q-122

Major Goals: Sponsor is contracting with University under this Agreement to conduct a pharmacokinetic/pharmacodynamic study with cynomolgus monkeys, using Sponsor's proprietary drug referred to as Q-122, and a control drug. The purpose of this research is to allow Sponsor to more fully understand the mechanism of action of Q-122 by observing the relationship of pharmacokinetics (PK) and pharmacodynamics (PD), using data generated through the research.

Status of Support: Active

Project Number: N/A

Name of PD/PI: Redacted by

Source of Support: Private Source

Primary Place of Performance: Private Source

Project/Proposal Start and End Date: (MM/YYYY) (if available): 05/01/2021 – 10/31/2021

Total Award Amount (including Indirect Costs): $162,958

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2021	EFFORT calendar

(NEW)

Title: KSHV Subunit Vaccine Candidates to Elicit Potent Humoral Immune Responses against KSHV Infection

Major Goals: we will use wild-type and humanized mice and common marmoset (Callithrix jacchus) models to test the hypothesis that purified KSHV LPs delivered directly or through immunization with a modified vaccinia Ankara vector (MVA-KSHV-LPs) will elicit robust protective nAb responses to KSHV infection and its associated malignancies.

Status of Support: Active

Project Number: 1 R01 AI 151013 01A1

Name of PD/PI: Redacted by agreement

Source of Support: NIAID

Primary Place of Performance: Beckman Research Institute/City of Hope

Project/Proposal Start and End Date: (MM/YYYY): 3/22/2021 – 2/28/2026

Name of Individual: Redacted by
Commons ID: eRA Commons

Total Award Amount (including Indirect Costs): $863,275 *(subaward forthcoming)*

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2021	EFFORT calendar
2. 2022	calendar
3. 2023	calendar
4. 2024	calendar
5. 2025	calendar

(NEW)

Title: Unmasking the roles of viral glycoproteins in oral transmission of KSHV

Major Goals: Kaposi sarcoma-associated herpesvirus (KSHV) is the causal agent of Kaposi sarcoma, a common cancer in immunocompromised people (such as HIV/AIDS and transplant patients) and two lymphoid malignancies. We have designed a study to uncover the role of key KSHV glycoproteins in oral and systemic transmission of KSHV ex vivo and in vivo. We expect to generate critical data that will inform future design of an effective prophylactic KSHV vaccine candidate to prevent KSHV infection and its associated cancers.

Status of Support: Active

Project Number: 1 R01 CA 264911 01

Name of PD/PI: Redacted by agreement

Source of Support: NCI

Primary Place of Performance: Beckman Research Institute/City of Hope

Project/Proposal Start and End Date: (MM/YYYY): 09/01/2021 – 8/31/2026

Total Award Amount (including Indirect Costs): $863,275 *(subaward forthcoming)*

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2021	EFFORT calendar
2. 2022	calendar
3. 2023	calendar
4. 2024	calendar
5. 2025	calendar

Name of Individual: [Redacted by agreement]
Commons ID: [eRA Commons User Name]

PENDING

Pending Support

Pending Support

Pending Support

D.2.c (OS Doc MM_Redacted...pdf)

Pending Support

D.2.c (OS Doc MM_Redacted..pdf)

Pending Support

Name of Individual: Redacted by
Commons ID: eRA Commons User Name

IN-KIND

None

OVERLAP

There is no potential scientific or commitment overlap for Redacted by agreement, effort on theP51 center grant will be reduced accordingly should any of his Pending Support be awarded.

I, PD/PI or other senior/key personnel, certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

Signature: Personal Info

Redacted by agreement

Date: September 23, 2021

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?
Not Applicable
E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?
NOTHING TO REPORT
E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?
Not Applicable
E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
NOTHING TO REPORT

F. CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

The COVID19 pandemic resulted in major disruptions to everyday life. During lockdowns, it was difficult to get lab work done and National Primate Research Centers had to prioritize NHPs for COVID19-related research. The start of our experiment was also delayed due to a longer-than-usual review of our IACUC protocol. Despite these challenges, we were able to optimize our FHT regimen in four animals, which have provided critical parameters for the larger monkey study planned for 2022.

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subject

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. SPECIAL REPORTING REQUIREMENTS SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name	DUNS	Congressional District	Address
Primary: SCRIPPS FLORIDA	148230662	FL-018	130 Scripps Way JUPITER, FL 334585284
The Board of Regents of the University of Wisconsin System	161202122	WI-002	University of Wisconsin-Madison, Research & Sponsored P

			21 N. Park Street, Suite 6401 Madison, WI 537151218

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period? No

G.12 F&A COSTS

Is there a change in performance sites that will affect F&A costs?

No

From: Acevedo, Paula (NIH/NIAID) [E]
Sent: Tue, 9 Nov 2021 13:26:22 +0000
To: Cummins, James (NIH/NIAID) [E]
Subject: RE: DELINQUENT FCOI Reports for Grant # 5 R21 AI 157929-02 PI: Martins , Mauricio de Aguiar

Yes, that's in fact what happened. The problem has now be resolved.

Paula

From: Cummins, James (NIH/NIAID) [E] <cumminsje@niaid.nih.gov>
Sent: Tuesday, November 9, 2021 8:24 AM
To: Acevedo, Paula (NIH/NIAID) [E] <paula.acevedo@nih.gov>
Subject: RE: DELINQUENT FCOI Reports for Grant # 5 R21 AI 157929-02 PI: Martins , Mauricio de Aguiar

Hi Paula –

I checked FCOI# 21644 in eRA and it looks like it's complete – just wanted to make sure based on the email thread below.

I assume that the redundant FCOI entry must have triggered the system that there was an outstanding FCOI on the grant, but that it's now okay with the deletion of the redundant entry.

Thanks,
James

James E. Cummins, Jr., Ph.D
Chief, Preclinical Microbicide and Prevention Research Branch
PSP/DAIDS/NIAID/NIH
5601 Fishers Lane
Room 8B53 MSC 9831
Bethesda, MD 20892-9831
Office Tel: 240-292-4800
FAX: (240) 627-3465

From: Acevedo, Paula (NIH/NIAID) [E] <paula.acevedo@nih.gov>
Sent: Monday, November 8, 2021 4:45 PM
To: Redacted by agreement @scripps.edu>
Cc: Mauricio Martins <mmartins@scripps.edu>; Redacted by agreement @scripps.edu>; Cummins, James (NIH/NIAID) [E] <cumminsje@niaid.nih.gov>; Redacted by agreement @scripps.edu>
Subject: RE: DELINQUENT FCOI Reports for Grant # 5 R21 AI 157929-02 PI: Martins , Mauricio de Aguiar

Hi Redacted by agreement

Thanks the alert has in fact disappeared.

Paula

From: Redacted by agreement @scripps.edu>
Sent: Monday, November 8, 2021 3:11 PM
To: Acevedo, Paula (NIH/NIAID) [E] <paula.acevedo@nih.gov>
Cc: Mauricio Martins <mmartins@scripps.edu>; Redacted by agreement @scripps.edu>; Cummins, James (NIH/NIAID) [E] <cumminsje@niaid.nih.gov>; Redacted by agreement @scripps.edu>
Subject: RE: DELINQUENT FCOI Reports for Grant # 5 R21 AI 157929-02 PI: Martins , Mauricio de Aguiar

Dear Paula,

I would like to confirm that FCOI report # 21644 for grant # 1R21AI157929-01 was previously submitted in ERA Commons on August 25, 2021. There are no additional FCOI reports for Dr. Martins on this grant. After receiving your email below we realized that a redundant FCOI entry in ERA Commons inadvertently remained open. The issue should now be corrected, as the redundant entry has been deleted. Please do not hesitate to let me know if you need further information to ensure this issue is resolved. You can reach me directly at Redacted by agreement

Best,

Redacted by agreement
Scripps Research

From: Acevedo, Paula (NIH/NIAID) [E] <paula.acevedo@nih.gov>
Sent: Monday, November 8, 2021 10:52 AM
To: Redacted by agreement @scripps.edu>
Cc: Mauricio Martins <mmartins@scripps.edu>; Cummins, James (NIH/NIAID) [E] <cumminsje@niaid.nih.gov>
Subject: DELINQUENT FCOI Reports for Grant # 5 R21 AI 157929-02 PI: Martins , Mauricio de Aguiar
Importance: High

Good afternoon,

I am writing to you as the FCOI Officer with an urgent request that the delinquent Financial Conflict of Interest reports for the above–referenced grant be filed immediately.

We will be unable to issue the next award which has a **12/01/2021** start date if we have not received and approved those missing reports.

The individual with reported conflicts on this grant and the FCOI report number is listed below:

Martins , Mauricio de Aguiar - FCOI Report # 21644

Given the short time remaining before the next award is due and the need for review, please have these FCOI reports filed electronically by no later than cob, **11/15/2021.**

Feel free to contact me if you have any questions on this request.

Thank you for your assistance.

Regards,
Paula

Paula Acevedo, MPA
Grants Management Specialist
DHHS/NIH/NIAID/DEA/GMP
5601 Fishers Lane Room 4G28, MSC 9833, Rockville, Maryland 20852
For US Mail use: Bethesda, MD 20892 | For FedEx/UPS use: Rockville, MD 20852
Phone (301) 435-2860 | Fax (301) 493-0597 | Paula.Acevedo@nih.gov

From: Machuca, Jorge (NIH/NIAID) [E]
Sent: Wed, 10 Nov 2021 18:01:17 +0000
To: Redacted by agreement
Cc: Acevedo, Paula (NIH/NIAID) [E]; Guidetti, Nicole (NIH/NIAID) [E]
Subject: RE: DELINQUENT FCOI Reports for Grant # 5 R21 AI157929-02 PI: Mauricio Martins

Redacted by agreement

Thank you.

Jorge Machuca

From: Redacted by agreement @scripps.edu>
Sent: Wednesday, November 10, 2021 12:41 PM
To: Machuca, Jorge (NIH/NIAID) [E] <jorge.machuca@nih.gov>
Cc: Acevedo, Paula (NIH/NIAID) [E] <paula.acevedo@nih.gov>; Guidetti, Nicole (NIH/NIAID) [E] <nicole.guidetti@nih.gov>
Subject: RE: DELINQUENT FCOI Reports for Grant # 5 R21 AI157929-02 PI: Mauricio Martins

Dear Jorge Machuca,

Thank you for clarifying this issue, and I apologize for the misunderstanding. The Annual Report referenced below has now been submitted. Please let me know if you need any further information.

Best,

Redacted by agreement

Scripps Research

From: Machuca, Jorge (NIH/NIAID) [E] <jorge.machuca@nih.gov>
Sent: Wednesday, November 10, 2021 5:59 AM
To: Redacted by agreement @scripps.edu>
Cc: Acevedo, Paula (NIH/NIAID) [E] <paula.acevedo@nih.gov>; Guidetti, Nicole (NIH/NIAID) [E] <nicole.guidetti@nih.gov>
Subject: DELINQUENT FCOI Reports for Grant # 5 R21 AI157929-02 PI: Mauricio Martins

Dear Redacted by agreement ;

I am writing to you as the FCOI Officer with an urgent request that the delinquent Financial Conflict of Interest reports for the above–referenced grant be filed immediately.

There is one FCOI reports due for conflicts related to this funding. Our records indicate that this report has still not been received.

We will be unable to issue the next award which has a **12/01/2021** start date if we have not received and approved those missing reports.

The individual with reported conflicts on this grant and the FCOI report number are listed below:

Mauricio Martins - FCOI Report # 21644

Given the short time remaining before the next award is due and the need for review, please have these FCOI reports filed electronically by no later than cob, **11/15/2021**

Feel free to contact me if you have any questions on this request.

For future reference:
Here are is the FAQs that address this question:

> H.39. If an Original FCOI report is submitted during the last 75 days of a budget period for an ongoing grant but before the annual progress report due date, is an Annual FCOI Report required for submission before the start of the next type 5 award? (Institution)
>
> Yes. The Annual FCOI Report link will appear the day after the Original FCOI Report is submitted. The Annual FCOI Report is due at the same time as when the current *annual progress* report is due or at the time of grant extension, whichever is applicable.

Thank you for your assistance.

Regards,

Jorge Machuca
Grants Management Specialist
DHHS|NIH|NIAID|GMP
Tel: 240-669-2933
jorge.machuca@nih.gov

From: Cummins, James (NIH/NIAID) [E]
Sent: Thu, 20 Jan 2022 00:01:53 +0000
To: Mauricio Martins
Subject: RE: Follow-up on Grant # R21AI157929

Hi Mauricio –

Unfortunately, these are the times in which we live. I am amazed at the misinformation that is so quickly spread on social media. Your work is incredibly important since we need to know whether biological factors play a role in transgender health and susceptibility to HIV.

I look forward to seeing the data from your studies.

All the best,
James

James E. Cummins, Jr., Ph.D
Chief, Preclinical Microbicide and Prevention Research Branch
PSP/DAIDS/NIAID/NIH
5601 Fishers Lane
Room 8B53 MSC 9831
Bethesda, MD 20892-9831
Office Tel: 240-292-4800
FAX: (240) 627-3465

From: Mauricio Martins <mmartins@scripps.edu>
Sent: Wednesday, January 19, 2022 12:36 PM
To: Cummins, James (NIH/NIAID) [E] <cumminsje@niaid.nih.gov>
Subject: [EXTERNAL] Re: Follow-up on Grant # R21AI157929

Hi James,

Thank you for your email. Yes, I am aware that my R21 grant was recently featured in right-wing media segments. I was disappointed to see my research being twisted and berated by people who are not qualified to judge its merit. To my surprise, the segments in question prompted a backlash on Twitter by the transgender community, who stands to gain the most by the research outlined in my R21. Sadly, my graduate student who is leading the R21 project in

question has expressed concerns about publishing our results, since their identity will be made public as the first author in the paper.

Despite the baseless comments that have circulated on Twitter, I stand by my R21 proposal as it addresses a critical knowledge gap in gender-affirming hormone therapy. We will continue with our research, hoping that it will be more fairly reviewed once it is published.

Best,

Mauricio

Mauricio Martins, Ph.D.
Assistant Professor
Department of Immunology and Microbiology
Scripps Research - Florida
130 Scripps Way
Jupiter, FL 33458
Phone: Redacted by agreement
mmartins@scripps.edu

From: Cummins, James (NIH/NIAID) [E] <cumminsje@niaid.nih.gov>
Sent: Wednesday, January 19, 2022 11:44 AM
To: Mauricio Martins <mmartins@scripps.edu>
Subject: Follow-up on Grant # R21AI157929

Dear Dr. Martins –

In case you are unaware, NIAID was recently contacted from an outside group about the use of animals in your funded research grant (Grant # R21 AI 157929: "A nonhuman primate model to study the immunological effects of feminizing hormone therapy in transgender women").
I am reaching out to let you know that NIAID is taking the inquiry seriously and issuing a response to this outside group. My intent is to keep you aware in case you or anyone in your group has also been contacted.

If you have any concerns, please feel free to reach out to me.

Best regards,
James

James E. Cummins, Jr., Ph.D
Chief, Preclinical Microbicide and Prevention Research Branch
PSP/DAIDS/NIAID/NIH
5601 Fishers Lane
Room 8B53 MSC 9831
Bethesda, MD 20892-9831

Office Tel: 240-292-4800
FAX: (240) 627-3465

From: Mauricio Martins
Sent: Wed, 3 Mar 2021 22:35:54 +0000
To: Cummins, James (NIH/NIAID) [E]
Subject: Re: New Program Officer for Grant #1R21AI157929-01, "A nonhuman primate model to study the immunological effects of feminizing hormone therapy in transgender women"

Hi James,
Thanks for reaching out.
I look forward to working with you!
Best,
M.

Mauricio Martins, Ph.D.
Assistant Professor
Department of Immunology and Microbiology
Scripps Research - Florida
130 Scripps Way
Jupiter, FL 33458
Phone: Redacted by agreement
mmartins@scripps.edu

From: Cummins, James (NIH/NIAID) [E] <cumminsje@niaid.nih.gov>
Sent: Wednesday, March 3, 2021 5:30 PM
To: Mauricio Martins <mmartins@scripps.edu>
Subject: New Program Officer for Grant #1R21AI157929-01, "A nonhuman primate model to study the immunological effects of feminizing hormone therapy in transgender women"

Dear Dr. Martins (Mauricio) –
As you well know by now, Jim Turpin retired last week from the Division of AIDS (DAIDS) in NIAID at NIH. Like many of us, I am sure that you will miss working with him in those key scientific areas that impact the field of HIV prevention. In 2009 I joined Jim to develop a preclinical program within the newly formed Prevention Sciences Program in DAIDS, and it has been an amazing journey. While much of my time has been spent on developing extramural contract resources to support the preclinical development of biomedical prevention products, over the years I have assisted Jim in the development of multiple grant programs within our Preclinical Microbicide and Prevention Research Branch. Thus, I have some familiarity with the work of most of our grantees – including you.

With this in mind, I am reaching out to introduce myself as the new Program Officer for your grant (1R21AI157929-01). If you have any current issues or items related to the grant that need discussion, please let me know. While I may not have an immediate answer for certain grant-specific items, I will certainly work with Grants Management and others at NIH to address any problems and find potential solutions. In addition, I welcome the opportunity to discuss the science and hear about the exciting new data that our innovative grantees (like you) are generating every day.
I look forward to working with you.
All the best,
James

Acting Branch Chief
Preclinical Microbicide and Prevention Research Branch
PSP/DAIDS/NIAID/NIH

5601 Fishers Lane
Room 8B53 MSC 9831
Bethesda, MD 20892-9831
Office Tel: 240-292-4800
FAX: (240) 627-3465

From: Allen, Racine (NIH/NIAID) [C]
To: Redacted by
Subject: RE: JIT Docs - Grant Number: 1R21AI157929 - 01 PI Name: Martins, Mauricio de Aguiar
Date: Thursday, November 12, 2020 11:31:00 AM
Attachments: image001.png

Yes, I received it.

Thanks,
Racine Allen, MPH
Grants Management Specialist
National Institute of Allergy and Infectious Diseases (NIAID), Contractor
5601 Fishers Lane
Rockville, MD 20892-9824

From: Redacted by agreement @scripps.edu>
Sent: Wednesday, November 11, 2020 6:17 PM
To: Allen, Racine (NIH/NIAID) [C] <racine.allen@nih.gov>
Subject: FW: JIT Docs - Grant Number: 1R21AI157929 - 01 PI Name: Martins, Mauricio de Aguiar

Good afternoon Ms. Allen,

I sent the e-mail below to you yesterday but had a bounce back e-mail in my inbox this morning.

I wanted to make sure you are aware that the JIT information has been submitted.

Regards,

Redacted by agreement

Redacted by agreement
Manager, Pre-Award
Office of Sponsored Programs
(858) 784-8024

10550 North Torrey Pines Road
La Jolla, CA 92037

Scripps Research
Science Changing Life

From: Redacted by agreement
Sent: Tuesday, November 10, 2020 4:09 PM

To: racine.allen@nih.gov
Cc: jturpin@niaid.nih.gov; Mauricio Martins <mmartins@scripps.edu>; Redacted by agreement
Redacted by agreement @scripps.edu>; grants <grants@scripps.edu>
Subject: RE: JIT Docs - Grant Number: 1R21AI157929 - 01 PI Name: Martins, Mauricio de Aguiar

Dear Ms. Allen,

The requested JIT material has been submitted via the JIT feature in eRA Commons. This included submission of updated Other Support pages for Drs. Martins and Kurian.

If there area any questions, or if additional information is needed, please let us know.

Regards,

Redacted by agreement

Redacted by agreement

Office of Sponsored Programs
(858) 784-8024

10550 North Torrey Pines Road
La Jolla, CA 92037

Scripps Research
Science Changing Life

From: Allen, Racine (NIH/NIAID) [C] <racine.allen@nih.gov>
Sent: Wednesday, November 4, 2020 11:19 AM
To: Mauricio Martins <mmartins@scripps.edu>; Turpin, Jim (NIH/NIAID) [E] <jturpin@niaid.nih.gov>; grants <grants@scripps.edu>
Subject: Grant Number: 1R21AI157929 - 01 PI Name: Martins, Mauricio de Aguiar

Hello,

The above referenced application is being considered for funding by the National Institute of Allergy and Infectious Diseases. Please note that this request is not a guarantee of funding. Official notification of funding is only made by issuance of a Notice of Award (NoA).
The following Just-In-Time information (JIT) identified is requested:

___X____ Verification that the other support submitted on 08/25/2020 is still current. If not, current other support should be submitted.

___X____ IACUC approval date *(NIH does not require a copy of the IACUC certification/approval).* Pending or out-of-date approvals are not acceptable**.**
If IACUC has not met, provide anticipated meeting date.
Information regarding IACUCs can be found at http://grants.nih.gov/grants/olaw/faqs.htm

___X____ Other

1. Confirm institution's Entity Identification Number (EIN) is 1330435954A5.
2. Confirm the latest F&A agreement is dated 6/16/2020 and the F&A rate is 85%. **If this is not correct, send a copy of your latest F&A rate agreement with your JIT response.**

The requested Just In Time (JIT) information must be submitted via eRA Commons (NIH Guide Notice NOT-OD-12-101) by **11/11/2020**. If unable to submit the requested information through eRA Commons, please contact your Grants Management Specialist. Timely submission of the above information will enable us to expedite the issuance of an award should the application be identified for funding.

Thanks,
Racine Allen, MPH
Grants Management Specialist
National Institute of Allergy and Infectious Diseases (NIAID), Contractor
5601 Fishers Lane
Rockville, MD 20892-9824

Scripps Research

Science Changing Life

Recipient Information

1. Recipient Name
SCRIPPS RESEARCH INSTITUTE, THE
130 SCRIPPS WAY

JUPITER, FL 33458

2. Congressional District of Recipient
18

3. Payment System Identifier (ID)
1330435954A5

4. Employer Identification Number (EIN)
330435954

5. Data Universal Numbering System (DUNS)
148230662

6. Recipient's Unique Entity Identifier

7. Project Director or Principal Investigator
Mauricio de Aguiar Martins, PHD
Assistant Professor
mmartins@scripps.edu
Redacted by agreement

8. Authorized Official
Redacted by agreement
grants@scripps.edu
(858) 784-8653

Federal Agency Information

9. Awarding Agency Contact Information
RACINE ANN-MARIE Allen

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
racine.allen@nih.gov
301-761-7004

10. Program Official Contact Information
Jim A Turpin
Program Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
jturpin@niaid.nih.gov
301-451-2732

Federal Award Information

11. Award Number
1R21AI157929-01

12. Unique Federal Award Identification Number (FAIN)
R21AI157929

13. Statutory Authority
42 USC 241 42 CFR 52

14. Federal Award Project Title
A nonhuman primate model to study the immunological effects of feminizing hormone therapy in transgender women

15. Assistance Listing Number
93.855

16. Assistance Listing Program Title
Allergy and Infectious Diseases Research

17. Award Action Type
New Competing

18. Is the Award R&D?
Yes

Summary Federal Award Financial Information

19. Budget Period Start Date 12/01/2020 **– End Date** 11/30/2021	
20. Total Amount of Federal Funds Obligated by this Action	$272,626
20a. Direct Cost Amount	$197,893
20b. Indirect Cost Amount	$74,733
21. Authorized Carryover	$0
22. Offset	$0
23. Total Amount of Federal Funds Obligated this budget period	$272,626
24. Total Approved Cost Sharing or Matching, where applicable	$0
25. Total Federal and Non-Federal Approved this Budget Period	$272,626
26. Project Period Start Date 12/01/2020 **– End Date** 11/30/2022	
27. Total Amount of the Federal Award including Approved Cost Sharing or Matching this Project Period	$272,626

28. Authorized Treatment of Program Income
Additional Costs

29. Grants Management Officer - Signature
Regina E. Kitsoulis

30. Remarks

Acceptance of this award, including the "Terms and Conditions," is acknowledged by the recipient when funds are drawn down or otherwise requested from the grant payment system.

Version:200 - 11/17/2020 12:25 PM | Generated on: 11/24/2020 12:31 AM

EXPLORATORY/DEVELOPMENT GRANT
Department of Health and Human Services
National Institutes of Health

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

SECTION I – AWARD DATA – 1R21AI157929-01

Principal Investigator(s):
Mauricio de Aguiar Martins, PHD

Award e-mailed to: grants@scripps.edu

Dear Authorized Official:

The National Institutes of Health hereby awards a grant in the amount of $272,626 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to SCRIPPS RESEARCH INSTITUTE, THE in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award, including the "Terms and Conditions," is acknowledged by the recipient when funds are drawn down or otherwise requested from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R21AI157929. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please direct questions to the Federal Agency contacts.

Sincerely yours,

Regina E. Kitsoulis
Grants Management Officer
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES

Additional information follows

Cumulative Award Calculations for this Budget Period (U.S. Dollars)

Federal Direct Costs	$197,893
Federal F&A Costs	$74,733
Approved Budget	$272,626
Total Amount of Federal Funds Authorized (Federal Share)	$272,626
TOTAL FEDERAL AWARD AMOUNT	$272,626
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$272,626

SUMMARY TOTALS FOR ALL YEARS (for this Document Number)		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$272,626	$272,626
2	$205,562	$205,562

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

Payment System Identifier: 1330435954A5
Document Number: RAI157929A
PMS Account Type: P (Subaccount)
Fiscal Year: 2021

IC	CAN	2021	2022
AI	8472297	$272,626	$205,562

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: A22B / **OC**: 41021 / **Released**: Kitsoulis, Regina 11/13/2020
Award Processed: 11/24/2020 12:31:34 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1R21AI157929-01

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – STANDARD TERMS AND CONDITIONS – 1R21AI157929-01

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget period.
e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2**.** As such, auditees

should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) R21AI157929. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SECTION IV – AI SPECIFIC AWARD CONDITIONS – 1R21AI157929-01

Clinical Trial Indicator: No
This award does not support any NIH-defined Clinical Trials. See the NIH Grants Policy Statement Section 1.2 for NIH definition of Clinical Trial.

This is a Modular Award without direct cost categorical breakdowns in accordance with the guidelines published in the NIH Grants Policy Statement, see https://grants.nih.gov/grants/policy/nihgps/HTML5/section_13/13.5_post-award_administration.htm. Recipients are required to allocate and account for costs related to this award by category within their institutional accounting system in accordance with applicable cost principles.

****

This Notice of Award (NoA) includes funds for activity with **University of Wisconsin-Madison**

SPREADSHEET SUMMARY
AWARD NUMBER: 1R21AI157929-01

INSTITUTION: SCRIPPS RESEARCH INSTITUTE, THE

Budget	Year 1	Year 2
TOTAL FEDERAL DC	$197,893	$173,329
TOTAL FEDERAL F&A	$74,733	$32,233
TOTAL COST	$272,626	$205,562

Facilities and Administrative Costs	Year 1	Year 2
F&A Cost Rate 1	85%	85%
F&A Cost Base 1	$87,921	$37,921
F&A Costs 1	$74,733	$32,233

PI: **Martins, Mauricio de Aguiar**	Title: A nonhuman primate model to study the immunological effects of feminizing hormone therapy in transgender women	
Received: 05/11/2020	FOA: PAR20-054 Clinical Trial:Not Allowed	Council: 10/2020
Competition ID: FORMS-E	FOA Title: Transgender People: Immunity, Prevention, and Treatment (R21 Clinical Trial Not Allowed)	
1 R21 AI157929-01	Dual:	Accession Number: 4437314
IPF: 10005569	Organization: SCRIPPS FLORIDA	
Former Number:	Department: Immunology & Microbiology	
IRG/SRG: ZRG1 AARR-M (55)R	AIDS: Y	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 150,000 Year 2: 125,000	Animals: Y Humans: N Clinical Trial: N Current HS Code: Evaluative HESC: N HFT: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
Redacted by agreement	University of Wisconsin-Madison	Co-Investigator
	Scripps Florida	Other (Specify)-Other Significant Contributor
	Scripps Florida	Other (Specify)-Other Significant Contributor
Mauricio Martins	Scripps Florida	PD/PI
Redacted by agreement	University of Massachusetts Medical School	Other (Specify)-Other Significant Contributor

OMB Number: 4040-0010
Expiration Date: 12/31/2022

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

❍ Pre-application ❍ Application ● Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number
GRANT13100861

5. APPLICANT INFORMATION

Organizational DUNS*: 1482306620000

Legal Name*: Scripps Florida
Department:
Division:
Street1*: 130 Scripps Way
Street2:
City*: Jupiter
County:
State*: FL: Florida
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 33458-5284

Person to be contacted on matters involving this application
Prefix: First Name*: Redacted by agreement Middle Name: Redacted by Last Name*: Redacted by Suffix:
Position/Title: Senior Director, Office of Sponsored Programs
Street1*: 10550 North Torrey Pines Road
Street2: TPC-7
City*: La Jolla
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 92037-1000
Phone Number*: 858-784-8653 Fax Number: 858-784-8037 Email: grants@scripps.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1330435954A5

7. TYPE OF APPLICANT* M: Nonprofit with 501C3 IRS Status (Other than Institution of Higher Education)

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
A nonhuman primate model to study the immunological effects of feminizing hormone therapy in transgender women

12. PROPOSED PROJECT
Start Date* Ending Date*
12/01/2020 11/30/2022

13. CONGRESSIONAL DISTRICTS OF APPLICANT
FL-018

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Mauricio Middle Name: de Aguiar Last Name*: Martins Suffix:
Position/Title: Assistant Professor
Organization Name*: Scripps Florida
Department: Immunology & Microbiology
Division:
Street1*: 130 Scripps Way
Street2:
City*: Jupiter
County:
State*: FL: Florida
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 33458-5284
Phone Number*: Redacted by agreement Fax Number: Email*: mmartins@scripps.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$478,188.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$478,188.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Redacted by Middle Name: Redacted Last Name*: Redacted Suffix:
Position/Title*: Senior Director
Organization Name*: The Scripps Research Institute
Department: Office of Sponsored Programs
Division:
Street1*: 10550 North Torrey Pines Road
Street2: TPC-7
City*: La Jolla
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 92037-1000
Phone Number*: 858-784-8653 Fax Number: 858-784-8037 Email*: grants@scripps.edu

Signature of Authorized Representative*
Redacted by

Date Signed*
05/11/2020

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	SCRIPPS FLORIDA
Duns Number:	1482306620000
Street1*:	130 Scripps Way
Street2:	
City*:	JUPITER
County:	
State*:	FL: Florida
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	33458-5284
Project/Performance Site Congressional District*:	FL-018

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	The Board of Regents of the University of Wisconsin System
DUNS Number:	1612021220000
Street1*:	University of Wisconsin-Madison, Research & Sponsored P
Street2:	21 N. Park Street, Suite 6401
City*:	Madison
County:	
State*:	WI: Wisconsin
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	53715-1218
Project/Performance Site Congressional District*:	WI-002

Additional Location(s) File Name:

Funding Opportunity Number: PAR-20-054. Received Date: 2020-05-11T14:50:59.000-04:00

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number A4460-01

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	20200511_ABSTRACT.pdf
8. Project Narrative*	20200511_PROJECT_NARRATIVE.pdf
9. Bibliography & References Cited	20200511_R21_references_figures.pdf
10.Facilities & Other Resources	FACILITIES.pdf
11.Equipment	EQUIPMENT.pdf

Funding Opportunity Number: PAR-20-054. Received Date: 2020-05-11T14:50:59.000-04:00

PROJECT SUMMARY/ABSTRACT

HIV/AIDS thrives in the margins of society, where low education, unstable housing, and poverty heighten people's vulnerability to HIV. No population is more affected by these social injustices than transgender persons. A case in point is transgender women (TGW)–individuals who were assigned a male sex at birth but express their gender along a feminine spectrum. Sadly, TGW have some of the highest concentrated HIV epidemics in the world, with a pooled global prevalence of 19% and a 49-fold higher odds ratio of acquiring HIV than non-transgender adults. A key part of gender affirmation in TGW is feminizing hormone therapy (FHT), of which the main drug is the hormone estradiol. Although the physical female traits triggered by FHT are well established, less is known about the immunological effects of FHT in TGW. It is noteworthy that estradiol can modulate immune responses in many ways, including by upregulating CCR5 expression on CD4+ T-cells. Since activated CCR5+ CD4+ T-cells are highly permissive to HIV infection, a better understanding of how FHT impacts the male immune system may provide new insights into how to prevent HIV infection in TGW. In this regard, here we will model FHT in nonhuman primates to prospectively address two knowledge gaps about the impact of FHT on the male immune system. 1) Does FHT increase the availability of HIV-susceptible CD4+ T-cells in vivo? To answer this question, we will use flow cytometry to assess the frequency and phenotype of memory CD4+ T-cells in blood and gut biopsies from male rhesus macaques receiving FHT (Group 1) or placebo (Group 2). By comparing the levels of activated CD4+ T-cells between animals in Groups 1 and 2, this analysis will reveal whether FHT modulates a crucial marker of HIV susceptibility in biological males. 2) Does FHT interfere with adeno-associated virus (AAV)-vectored immunoprophylaxis? Considering TGW's high risk of acquiring HIV, they stand to benefit greatly from AAV-mediated delivery of immunoglobulins as this approach can provide durable anti-HIV immunity after a one-time administration. Indeed, a single dose of an AAV vector encoding the potent and extremely broad HIV entry inhibitor eCD4-Ig resulted in persistent expression of eCD4-Ig and protection against stringent immunodeficiency virus challenges in rhesus macaques. However, given the immunoenhancing properties of estradiol, FHT may undermine AAV-driven eCD4-Ig expression in TGW by amplifying host anti-drug antibodies (ADAs)–a major limiting factor for AAV delivery of biologics. To address this possibility, all animals in Groups 1 and 2 will be inoculated with AAV/eCD4-Ig after 6 months of FHT or placebo therapy. We will then compare their serum levels of eCD4-Ig and ADAs to determine the impact of FHT on AAV-mediated delivery of eCD4-Ig in males. Ultimately, the experiments proposed here will begin to uncover how FHT can affect HIV susceptibility and the outcome of immune interventions in TGW.

PROJECT NARRATIVE

This project will investigate whether feminizing hormone therapy (FHT) can contribute to the high incidence of HIV infection among transgender women. To that end, FHT will be modeled in male rhesus macaques to determine whether it increases the availability of HIV “target” cells in the gut mucosa and whether FHT interferes with a prophylactic immune intervention. Ultimately, this project will advance our understanding of the pharmacodynamics of FHT and how it affects immune responses in biological males.

FACILITIES AND RESOURCES (Scripps Research FL)

Scripps Florida is a state-of-the-art biomedical research facility located in Palm Beach County in the town of Jupiter, Florida. The campus consists of three buildings (Buildings A, B, and C) comprising Redacted by gross square feet, with approximately Redacted by square feet of laboratory and lab support and Redacted by square feet of office space. Basic biomedical research is conducted on a wide variety of fields, including immunology, infectious diseases, medicinal chemistry, cancer, molecular therapeutics, bioinformatics, metabolism and aging, and neuroscience. The campus is also supported by a range of state-of-the-art core facilities, including state-of-the art bioinformatics and statistical services.

The small size of Scripps Florida makes for a highly collaborative and interactive environment. The setting is informal and administration is kept at a minimum. The facilities and cores at the Florida campus are state-of-the-art. The Florida members of the Department of Immunology and Microbiology also benefit from bicoastal intra-departmental collaborations with their La Jolla colleagues. Departmental seminar series and faculty chalk talks are broadcast from both coasts, and there are multiple opportunities to interact and collaborate as a single department. Outstanding genomic, proteomic, NMR, crystallography, histology and flow cytometry cores complement the work of the laboratories.

The Martins's lab: Dr. Martins's lab and office are located Redacted by agreement

Redacted by agreement

Statistical Services: Redacted by agreement

Redacted by agreement

Features of the Martins lab:

- Redacted by sq. ft. of dedicated lab space
- Two BSL-2 lab benches
- Separate tissue culture room for infectious BSL-2 work
- Adjacent office space

Redacted by agreement

Resources and Environment - WNPRC Animal Services Division

Facilities:
Currently, the Wisconsin Primate Research Center (WPRC) houses animals at four facilities, supporting ongoing research and a breeding colony. All animal rooms meet or exceed USDA and AAALAC regulations. In Building Redacted by agreement we occupy approximately Redacted by agreement sq. ft. of animal housing space, including cage wash areas, storage rooms, procedure rooms and a surgery suite. Building 1 houses approximately 450 animals. Redacted by agreement we occupy approximately Redacted by agreement sq. ft. of animal housing space, including cage wash areas, storage rooms and procedure rooms. Building 2 currently houses approximately 1050 animals. The Wisconsin Institutes for Medical Research (WIMR) NHP vivarium, which was be completed in April of 2009, is a Redacted by agreement of the UW-M campus. The facility consists of staff offices, animal housing rooms, animal husbandry support space, and ample clinical, surgical, and experimental procedure space and has the capacity to hold 275 adult macaques. The unique design of this facility allows scientists to perform their experiments in close proximity to the housing areas of their experimental subjects thus increasing efficiency and promoting collaboration with the veterinarians and animal caretakers that care for the animals. Directly adjacent to the WIMR vivarium is a two floor imaging suite that is equipped with an array of state-of-the-art imaging equipment (e.g., MRI, CT, PET, Fluoroscopy, etc.). The WNPRC utilizes the Blue Mounds Quarantine and Holding Facility, a 10-year old, Redacted by agreement sq. ft. facility, as a NHP quarantine and holding facility. This facility consists of four, 40-animal quarantine rooms equipped with anterooms, individual showers, mobile caging, and a procedure room between quarantine rooms 1 and 2; 2 NHP holding rooms equipped with mobile caging and large enough to hold approximately 100 animals each; 2 additional clinical procedure rooms; 2 laboratories; a cage wash suite; a freezer/refrigerator room for sample storage; an animal food prep and storage area, 2 offices, and a large equipment and mechanical area. The building is also equipped with ample mobile NHP housing and contemporary diagnostic, clinical, and surgical veterinary equipment.

Laboratory:
The clinical pathology laboratory provides timely diagnostic testing for both nonhuman primates species (rhesus macaques and common marmosets) at WNPRC in support of veterinary, pathology, scientific and affiliate staff. Testing is provided on site for hematology, microbiology and parasitology. The laboratory also coordinates sample submission to a growing number of reference and specialty labs, including routine chemistry. One registered medical technologist with considerable experience in nonhuman primate clinical pathology staffs the laboratory.

Clinical:
The WNPRC Veterinary Services Unit is led by Redacted by agreement and consists of eight clinical veterinarians, two veterinary pathologists, eight veterinary technicians, and two necropsy technicians. Members of the veterinary staff are present at the center from 8:00AM - 5:00 PM Monday through Friday and are reachable by cell phone at all times during the workday. A clinical veterinarian and a veterinary pathologist are on-call and reachable by cell phone after before 8:00AM and after 5:00PM on weekdays and 24 hours per day on weekends. This unit responds to all concerns raised by animal caretakers and researchers, evaluates and diagnoses cases, and prescribes care and treatment to accomplish better understanding of morbidity within the colony and related to research use. The information gained is then used in treatment of future cases and considered in the ongoing preventive medicine program. The preventive medicine program continues to use semi-annual TB testing, physical exams and dental prophylaxis as its core procedures. It has expanded to include periodic serological surveys of the conventional colony. This is done to track the presence of select infectious agents including Macacine Herpes Virus-1, SRV, STLV and SIV.

All surgery is performed by experienced veterinarians or other scientists who are trained to perform aseptic surgeries. Surgical patients are given post procedural analgesic agents, determined by the veterinarians, to assure patient comfort.

A staff of experienced caretakers managed by a colony manager and four animal care supervisors provides for the daily care of the animals and maintains the animal areas. Complete clinical and experimental histories are readily available for all animals from a computerized recordkeeping system. The WNPRC has full AAALAC accreditation through the Research and Graduate Education Division of the University of Wisconsin-Madison.

Animal:
The current nonhuman primate colony at the Primate Center is composed of 1030 rhesus macaques, 291 cynomolgus macaques, and 260 common marmosets.

Computer:
Our Electronic Health Records System EHR uses LabKey Server, a free, open source platform designed to manage and store scientific data. LabKey Server was developed by LabKey Software, a consultancy comprised of professional software developers, many of whom have previously worked for world-class companies including Microsoft and Amazon. By building EHR on the LabKey platform, we were able to take advantage of many pre-existing capabilities, including robust security and data sharing functions, without dedicating any EHR resources. We have been able to focus our development specifically on those elements unique to the management of primate data. EHR is focused primarily on clinical data; however, it also contains diagnostic and other laboratory data, administrative information, and manages requests for services such as blood draws and other clinical procedures. All data are available through a uniform web interface, with securable, tiered access to both WNPRC staff, researchers associated with WNPRC, and collaborators at other institutions.
EHR currently contains over 5.5 million historical records on > 7000 animals housed at the WNPRC since 1982. The database contains the following information:

- Demographic data, e.g., species, birth date, country or facility of origin, dam, and sire, etc.)
- Housing and transfer history
- Experimental assignment history
- Physical examination results, e.g., body condition, weight, alopecia, etc.
- Diagnostic test results, e.g., tuberculin skin tests, serum chemistry, hematology, bacteriology, parasitology, virology, urinalyses, and immunology
- Experimental and clinical procedure history, e.g., blood draws, drug administration, virus inoculation
- Surgical procedure history
- Clinical comments and treatments
- Genetic data including MHC typing

Each veterinarian has a desktop personal computer, currently an Apple iMac 21", with an intel core i3 processor, and 500 GByte disk drive. The computers are connected to the Center's high speed local area network and have access to a central storage server. Each veterinarian also has a laptop computer, currently an Apple MacBook 13" for use when away from their desks. All veterinarians have access to Apple iPads that allow them to do cage-side observations and make clinical entries directly into the EHR database.

The Colony Manager and each ART supervisor has a desktop personal computer, currently an Apple iMac 21", with an intel core i3 processor, and 500 GByte disk drive. The computers are connected to the Center's high speed local area network and have access to a central storage server. Additionally, similar computers are locate throughout the animal areas to facilitate real-time data entry by the ARTs. Each animal holding floor is also equipped with at least one Apple iPAD to facilitate the performance of health observations and direct syncing into the Electronic Health Records System.

Each SPI staff member has a 20” iMac computer. In addition, SPI staff share one intel MacBook for research use behind the animal barrier and Redacted by agreement also have an intel MacBook each. Student workers within SPI have access to a 20” iMac and may also access computers of permanent staff when needed.

Each member of the Compliance and Training Unit is provided with an iMac desktop computer. In addition, the Instructional Laboratory is equipped with two iMac desktop computers. The compliance coordinator and the trainers are also provided with laptop computers. The staff is provided with HP LaserJet laser printers.

All activity and behavioral recording equipment is operated by personal computers (PC or MAC platforms). Three laptop PCs equipped with Noldus Observer 10.5™ software for ethogram development have been purchased recently for behavioral data collection and analysis. PC based handheld devices (Palm2) are also used in the

collection for some types of behavioral data. All laboratory personnel have a computer for word processing, data manipulation or accounting purposes. All personnel have access to the internet and e-mail. All laboratory data and research materials are backed-up nightly on the WNPRC server. In additional, all electronic materials are periodically backed-up on a portable hard-drive.

MAJOR EQUIPMENT (Scripps Florida)

Equipment in the Martins lab includes two biosafety cabinets (where all the blood and colorectal biopsies) will be processed), four incubators, one microscope, and miscellaneous items for molecular biology (e.g., microcentrifuge, water baths, etc.). Additionally, the Martins lab contains two –20 ºC and one refrigerator. As stated in Redacted by agreement s letter of support, Dr. Martins will have full access to the equipment in the Redacted by agreement lab, which is listed below.

5 laminar flow tissue culture hoods
1 chemical hood
2 Beckman Coulter Allegra X-15R centrifuges
6 CO_2 incubators (VRW and Thermo)
3 thermocyclers (one gradient thermocycler)
1 Accuri flow cytometer
2 –80 ºC freezers
8 microcentrifuges
Electrophoresis equipment and power supplies
1 LightCycler 2.0 instrument (real-time PCR)
1 Nanodrop 2000C
1 Olympus bright-field and fluorescent microscope
1 Berthold Tristar2 LB942 microplate Reader
1 Sorvall T-21 centrifuge
1 Sorvall RC5C Plus centrifuge
1 Sorvall RC5B superspeed centrifuge
1 ultracentrifuge
2 sample K Series liquid nitrogen cryostorage systems
1 Kodak GelLogic 200 Imaging System
1 Victor microplate reader
1 Olympus IX50 inverted fluorescence microscope with DP70 camera
1 Millipore Elix5-MilliQ A10 water purification system
2 floor shaker-incubators

Shared and core-associated equipment. The Florida campus of the Scripps Research Institute makes available a number of resources through its core facilities (see list below).

2 confocal microscopes
1 Kalypsys High Throughput Screening System
1 Hamilton Microlab STARlet Robotic Liquid Handler
1 Analyst and Tecan M200 multimode reader
1 Envision fluorescence and luminescence reader
1 Matrix Platemate screen system
Several HPLC systems
1 Thermo Finnigan LTQ and 1 Thermo Finnigan Orbitrap, both equipped with micro electrospray ionization sources
3 Bruker NMR instruments
2 Avance 400 MHz ULTRAShield instruments
1 Avance III 700 MHz ULTRAShield instrument.

The Genomics Core maintains and operates the following equipment. The core also provides bioinformatics services using a range of commercial, public domain, and in-house programs

1 Affymetrix GeneChip System
1 Illumina NextSeq 500 and miSeq platforms
1 Life Technologies SOID4 and SOLID5500
1 Ion Torrent Ion Proton, Ion Personal Genome Machine
1 Applied Biosystems Sequence Detection System 7900HT

2 sonicators (Bioruptor and S2 Covaris)
2 real time PCR machines (Step One plus and Light Cycler 480)
2 Agilent 2100 Bioanalyzer.

The Histology Core maintains and operates the following equipment.

1 VIP Tissue Processor
1 Rotary Microtome
1 Leica Cryostat
1 Leica stereomicroscope
1 Gryphon crystallization tobot
Roboincubators.

The Flow Cytometry Core maintains and operates the following equipment.
1 four-laser FACS ARIA3 system for cell sorting
3 flow-cytometry systems for analysis (BD LSR II, BD Canto and Beckman Coulter Gallios, systems)
1 laser capture microdissection system (Leica LMD 7000),
1 Hemavet 950FS animal blood cell counter and analyzer.

Finally, the Department of Immunology and Microbiology equipment rooms contain freezers, high-speed and ultra-centrifuges, shakers, gel readers and scintillation counters, and several large cold rooms, a freezer farm, and server storage rooms.

Equipment-WNPRC Animal Services Division

Major Equipment:
Veterinary and Pathology Services:
Buildings 1 and 2 and the WIMR vivarium are all equipped with surgical suites that consist of each of the following components:

- animal prep room
- surgeon's scrub area
- instrument cleaning and preparation room
- operating room(s)
- recovery room

Each surgical suite is equipped with state-of-the art equipment to ensure the safety of the animals and the success of the procedure being performed Other specialized equipment used in the surgical suites includes the following:

- 5 VSSI model 64836 heated surgery tables
- 2 MDS Matrix VMS anesthesia machines with an isoflurane vaporizers
- 2 Fraser Sweatman VMS anesthesia machines with an isoflurane vaporizers
- 3 Engler A.D.S. 100 anesthesia machines with isoflurane vaporizers
- 3 Engler ADS1000 electronic ventilators
- 2 FLO-GARD 6200 volumetric infusion pump
- 2 Bair Hugger 200 patient warming systems
- 2 Heska Vet/Ox Plus 4800 pulse oximeters
- 2 Surgivet V3395 TPR temperature, SP02, pulse oximeters
- 2 Nonin 7500FO pulse oximeters
- 1 Surgivet V9204 Advisor monitor
- 1 Zeiss OP-Mi6 surgical microscope

The smaller surgery suite in Building 2 is equipped with a laparoscope tower that includes a digital camera, C02 insufflator, digital image printer, and a monitor.

The dental suite in Building 2 is equipped with a Progeny intra-oral dental radiography unit, stainless steel dental tub table, an ultrasonic cleaner, and numerous automated and manual dental instruments. The equipment in the dental suite allow the veterinary staff to perfom procedures ranging from simple dental prophylaxis to major dental extractions. The dental radiography unit is utilized to facilitate the diagnosis of clinical and occult dental disease (e.g., tooth root abscesses, dental caries, osteomyelitis, and maxilla/mandibular fractures).

The WNPRC owns two modern portable ultrasound units (GE logiq 7, GE logiq e) and three aging but functional units (Hitachi EUB-410 system, Aloka SSD-1400, Hewlett Packard AG-6300). These ultrasound units are used for NHP cardiac evaluations, abdominal organ evaluations, abdominal organ biopsies, reproductive organ evaluations, aspiration of endometriomas, fetal viability evaluations, fetal measurements, amniocentesis, chorionic villus sampling, and percutaneous umbilical blood sampling.

The WNPRC's radiographic suite is equipped with a Universal Uni-Matic 325 X-Ray Unit, and an AgFa CP1000 automatic processor. The Uni-Matic unit is used to perform quarantine screen-out thoracic radiographs on NHPs to determine the presence or absence of pulmonary lesions consistent with *Mycobacterium tuberculosis/bovis* and to perform routine diagnostic radiographs.

The Veterinary Services Unit relies on specialized equipment in the Clinical Pathology laboratory of the Pathology Services Unit (i.e., a Sysmex xs10001 CBC analyzer, Bayer Clintek 50 Urine Chemistry analyzer) for quick turn around times on diagnostic samples. The unit also sends diagnostic samples to outside laboratories (e.g., chemistry panels, bacterial cultures, viral samples for ELISA & PCR) that are beyond the capabilities of the pathology Service Unit.

Colony Management:
Building 2 Cage Washer - Getinge Castle Model 2130
WIMR Cage Washer – Lynx Series 400
Blue Mounds Quarantine Facility Cage Washer – Basil Model RW4602

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Mauricio Middle Name de Aguiar Last Name*: Martins Suffix:

Position/Title*: Assistant Professor
Organization Name*: Scripps Florida
Department: Immunology & Microbiology
Division:
Street1*: 130 Scripps Way
Street2:
City*: Jupiter
County:
State*: FL: Florida
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 33458-5284

Phone Number*: Redacted by agreement Fax Number:

E-Mail*: mmartins@scripps.edu

Credential, e.g., agency login: eRA Commons User Name

Project Role*: PD/PI Other Project Role Category:

Degree Type: PHD,BS Degree Year: 2011,2006

Attach Biographical Sketch*: File Name: 20200504_BIOSKETCH_MARTINS.pdf

Attach Current & Pending Support: File Name:

Funding Opportunity Number: PAR-20-054 . Received Date: 2020-05-11T14:50:59.000-04:00

PROFILE - Senior/Key Person

Redacted by agreement

PROFILE - Senior/Key Person

Redacted by agreement

PROFILE - Senior/Key Person

Redacted by agreement

PROFILE - Senior/Key Person

Redacted by agreement

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors. Follow this format for each person. ***DO NOT EXCEED FIVE PAGES****.*

NAME: Martins, Mauricio, A.

eRA COMMONS USER NAME (credential, e.g., agency login): eRA Commons User Name

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Universidade Federal de Minas Gerais, Brazil	B.Sc.	2006	Biochemistry/Immunology
University of Wisconsin-Madison, Madison, WI	Ph.D.	2011	Immunology
University of Miami, Miami, FL	Fellow	2017	Immunology

A. Personal Statement

Here we will utilize male rhesus macaques to study whether feminizing hormone therapy (FHT) increases the availability of HIV "target" cells in the gut mucosa and whether FHT interferes with AAV-vectored immunoprophylaxis. I am well qualified to lead the proposed experiments given my extensive background in nonhuman primate biology, immunology, and the 13 years I have spent testing HIV vaccine concepts in the SIV/rhesus macaque model. As a graduate student at UW-Madison, I evaluated the extent to which vaccine-elicited T-cells targeting non-Env proteins could control SIV replication in rhesus macaques. As a post-doctoral associate at the University of Miami, my research provided new insights into the immunological basis of elite control of SIVmac239 infection. Later, as an Assistant Professor at the University of Miami, my work led to the development of a vaccine modality that afforded significant protection against SIVmac239 acquisition in rhesus macaques. The latter work is noteworthy because it was funded by my K01 HIV/AIDS Vaccine Scholars Award and resulted in two publications, one in PNAS and the other, my first senior author paper, in Plos Pathogens.

I have recently moved to the Scripps Research Institute in Jupiter FL, where I have established a productive collaboration with Redacted by agreement. His group developed the eCD4-Ig molecule that will be used in specific aim 2. Despite this close collaboration with Redacted by agreement I must emphasize that my laboratory is independent from his. I have my own laboratory space and my own funding. Although Redacted by agreement s mentorship and expertise on the biology of eCD4-Ig will be welcome in this project, I will be the one leading its research operations.

B. Positions and Honors

Positions and Employment

03/2012-04/2014 Postdoctoral Associate, University of Miami Miller School of Medicine, Miami, FL

05/2014-02/2017 Assistant Scientist, University of Miami Miller School of Medicine, Miami, FL

02/2017-09/2019 Assistant Professor, University of Miami Miller School of Medicine, Miami, FL

09/2019-present Assistant Professor, Scripps Research, Jupiter, FL

Honors and Awards

2010 Invited speaker for the 2010 Keystone Symposia on HIV Vaccines and Viral Immunity, workshop #2 on HIV Vaccines. Banff, Alberta, Canada.

2011 Invited speaker for the VI Advanced course on HIV pathogenesis at the University of São Paulo. São Paulo, Brazil.

2011 Early Career Investigator, OCTAVE Workshop on *Statistical Methods in HIV Vaccine Trial Design and Evaluation*. São Paulo, Brazil.

2016 *Vaccine*'s Young Investigator.

2018 Invited speaker for the 2018 36th Annual Symposium on Nonhuman Primate Models for AIDS. Seattle, WA.

2019 Invited speaker for the 2019 Keystone Symposia on HIV Vaccines and Functional Cures and the Eradication of HIV, workshop #1 on Lessons from Nonhuman Primate Studies. Whistler, British Columbia, Canada.

2019 Invited speaker for the 2019 15th PEGSBoston Meeting, Section on "Emerging Indications for Therapeutic Antibodies". Boston, Massachusetts, USA.

C. Contributions to Science

1) **Vaccine protection against SIVmac239 acquisition in rhesus macaques.** As part of studies funded by K01 HIV/AIDS Vaccine Scholars Program award, we generated a near-full-length SIVmac239 genome that retains >95% of the coding capacity of SIV, expresses all nine SIV gene products, and assembles noninfectious SIV particles. To evaluate the immunogenicity and efficacy of SIVnfl vaccination, we inserted SIVnfl into DNA plasmids and live recombinant rhesus monkey rhadinovirus (RRV) vectors. We then showed that I) monkeys inoculated with RRV-SIVnfl become persistently infected, mount durable anti-Env antibodies, and develop effector-differentiated CD8+ T-cell responses against all nine SIV proteins. Crucially, these features are also observed following inoculation with live-attenuated SIV strains, the most effective vaccine modality against pathogenic SIV challenge in nonhuman primates. We have also shown that the use of RRV-SIVnfl as a prime or as a boost for DNA-SIVnfl vaccinations induces SIV-specific immune responses capable of significantly protecting rhesus macaques against repeated, marginal-dose intravenous (II) and intrarectal (III) challenges with SIVmac239. Together, these studies comprise the first demonstration of vaccine-mediated protection against acquisition of SIVmac239 by any active immunization strategy other than live-attenuated strains of SIV.

   I. Shin YC, Bischof GF, Lauer WA, Gonzalez-Nieto L, Rakasz EG, Hendricks GM, Watkins DI, **Martins MA***, Desrosiers RC*. A recombinant herpesviral vector containing a near-full-length SIVmac239 genome produces SIV particles and elicits immune responses to all nine SIV gene products. PLoS Pathog. 2018. *** Co-corresponding authors.**

   II. **Martins MA**, Bischof GF, Shin YC, Lauer WA, Gonzalez-Nieto L, Watkins DI, Rakasz E, Lifson JD, Desrosiers RC. Vaccine protection against SIVmac239 acquisition. Proc Natl Acad Sci U S A. 2019 Jan 29;116(5):1739-1744. doi: 10.1073/pnas.1814584116.

   III. Gonzalez-Nieto L, Castro IM, Bischof GF, Shin YC, Ricciardi MJ, Bailey VK, Dang CM, Pedreño-Lopez N, Magnani DM, Ejima K, Allison DB, Gil HM, Evans DT, Rakasz EG, Lifson JD, Desrosiers RC, **Martins MA***. Vaccine protection against rectal acquisition of SIVmac239 in rhesus macaques. PLoS Pathog. 2019. * **Corresponding author.**

2) **Which viral proteins should be targeted by vaccine-induced HIV-specific T-cells?** Multiple studies have reported discordant associations between HIV/SIV viremia and virus-specific T-cell responses, illustrating the uncertainty that still exists as to which viral proteins comprise the best targets for vaccine-induced HIV-specific T-cell responses. I attempted to address this issue in several studies and reached the following conclusions: (I) increasing the number of vaccine-encoded immunogens enhances control of SIV replication in rhesus macaques, especially when Gag and Env are present; (I) Total vaccine-elicited antigen-specific CD8+ T-cells are poised to proliferate until a "ceiling" level is reached, regardless of the number or size of vaccine-encoded inserts; II) The pre-challenge breadth of vaccine-induced T-cell responses against Gag and Vif can predict control of viremia in SIV-infected rhesus macaques.

   I. **Martins MA***, Shin YC, Gonzalez-Nieto L, Domingues A, Gutman MJ, Maxwell HS, Castro I, Magnani DM, Ricciardi M, Pedreño-Lopez N, Bailey V, Betancourt D, Altman JD, Pauthner M, Burton DR, von Bredow B, Evans DT, Yuan M, Parks CL, Ejima D, Allison DB, Rakasz E, Barber

GN, Capuano S 3rd, Lifson JD, Desrosiers RC, Watkins DI. Vaccine-induced immune responses against both Gag and Env improve control of simian immunodeficiency virus replication in rectally challenged rhesus macaques. PLoS Pathog. 2017 ***Corresponding author.**

II. **Martins MA**, Wilson NA, Reed JS, Ahn CD, Klimentidis YC, Allison DB, Watkins DI. T-cell correlates of vaccine efficacy after a heterologous simian immunodeficiency virus challenge. J Virol. 2010.

3) **Spontaneous control of SIV replication in rhesus macaques expressing the MHC class I alleles *Mamu-B*08* and *Mamu-B*17* rely on different immunological mechanisms.** Elite control of chronic phase viremia is a classic example of an effective immune response against HIV. It is widely accepted that CD8+ T-cells are key mediators of this phenomenon. Since the attributes of effective T-cell responses against HIV remain incompletely defined, elucidating why CD8+ T-cells in elite controllers can control viral replication while T-cells in most other individuals cannot may inform HIV vaccine development. Interestingly, expression of the MHC class I alleles *Mamu-B*08* and *Mamu-B*17* predisposes SIVmac239-infected rhesus macaques to control viral replication. Both Mamu-B*08 and Mamu-B*17 molecules bind immunodominant SIV epitopes in Vif and Nef. Notably, the peptide binding motifs of Mamu-B*08 and Mamu-B*17 resemble those of HLA-B*27 and HLA-B*57, respectively, which are associated with elite control of HIV infection. Given these similarities, SIV-infected *Mamu-B*08*+ and *Mamu-B*17*+ macaques might inform the study of elite control of HIV-1 infection. My contributions in this area include the discovery that CD8+ T-cells focused on Vif and Nef epitopes (I & II), but not on Nef epitopes alone (III), are sufficient to increase the incidence of elite control in *Mamu-B*08*+ macaques. Curiously, however, the incidence of elite controllers in *Mamu-B*17*+ animals vaccinated with Vif and Nef epitopes was indistinguishable from that in unvaccinated MHC class I-matched animals (IV), suggesting that the underlying mechanisms of elite control differ between *Mamu-B*08*+ and *Mamu-B*17*+ macaques.

I. Mudd PA, **Martins MA**, Ericsen AJ, Tully DC, Power KA, Bean AT, Piaskowski SM, Duan L, Seese A, Gladden AD, Weisgrau KL, Furlott JR, Kim YI, Veloso de Santana MG, Rakasz E, Capuano S 3rd, Wilson NA, Bonaldo MC, Galler R, Allison DB, Piatak M Jr, Haase AT, Lifson JD, Allen TM, Watkins DI. Vaccine-induced CD8+ T cells control AIDS virus replication. Nature. 2012.

II. **Martins MA*,** Gonzalez-Nieto L, Shin YC, Domingues A, Gutman MJ, Maxwell H, Magnani DM, Ricciardi MJ, Pedreño-Lopez N, Bailey VK, Altman JD, Parks CL, Allison DB, Ejima K, Rakasz EG, Capuano S, Desrosiers RC, Lifson JD, Watkins DI. 2019. The frequency of vaccine-induced T-cell responses does not predict the rate of acquisition after repeated intrarectal SIVmac239 challenges in *Mamu-B*08*+ rhesus macaques. J Virol 2019. Feb 19;93(5). pii: e01626-18. doi: 10.1128/JVI.01626-18. Print 2019 Mar 1. PMID: 30541854. ***Corresponding author.**

III. **Martins MA***, Tully DC, Cruz MA, Power KA, Veloso de Santana, MG, Bean DJ, Ogilvie CB, Gadgil R, Lima NS, Magnani DM, Ejima K, Allison DB, Piatak M Jr, Altman JD, Parks CL, Rakasz EG, Capuano S 3rd, Galler R, Bonaldo MC, Lifson JD, Allen TM, Watkins DI. Vaccine-induced SIV-specific CD8+ T-cell responses focused on a single Nef epitope select for escape variants shortly after infection. J Virol. 2015. ***Corresponding author.**

IV. **Martins MA***, Tully DC, Pedreño-Lopez N, von Bredow B, Pauthner MG, Shin YC, Yuan M, Lima NS, Bean DJ, Gonzalez-Nieto L, Domingues A, Gutman MJ, Maxwell HS, Magnani DM, Ricciardi MJ, Bailey VK, Altman JD, Burton DR, Ejima K, Allison DB, Evans DT, Rakasz EG, Parks CL, Bonaldo MC, Capuano S 3rd, Lifson JD, Desrosiers RC, Allen TM, Watkins DI. *Mamu-B*17*+ rhesus macaques vaccinated with *env*, *vif*, and *nef* manifest early control of SIVmac239 replication. J Virol. 2018 ***Corresponding author.**

4) **Gene fragmentation is not sufficient to overcome CD8+ T-cell immunodominance in nonhuman primates.** It is widely accepted that HIV vaccines must elicit broadly-targeted cellular immune responses to cope with the diversity of circulating isolates. However, eliciting such responses by vaccination is

complicated by immunodominance, the preferential targeting of only a few of the many possible epitopes of a given antigen. Previous studies in murine models have suggested that delivering vaccine immunogens as fragments instead of full-length genes might circumvent immunodominance and thereby broaden the repertoire of antigen-specific T-cell responses. Moreover, those studies showed that the co-expression of dominant and subdominant targets in the same antigen presenting cell (APC) was central to immunodominance. However, when these targets were delivered separately and therefore presented to the immune system by different APCs, immunized mice mounted equal frequencies of T-cell responses to the dominant and subdominant sequences. I have addressed this hypothesis in rhesus macaques utilizing vaccine vectors encoding SIV minigenes. My major finding was that gene fragmentation did not substantially broaden the repertoire of vaccine-induced T-cells compared to the standard practice of delivering vaccine sequences as full-length genes. These results underscore the difficulty of inducing subdominant CD8+ T-cells by vaccination and indicate that strategies other than gene fragmentation may be required to alter immunodominance in primates.

I. **Martins MA**, Wilson NA, Piaskowski SM, Weisgrau KL, Furlott JR, Bonaldo MC, Veloso de Santana MG, Rudersdorf RA, Rakasz EG, Keating KD, Chiuchiolo MJ, Piatak M Jr, Allison DB, Parks CL, Galler R, Lifson JD, Watkins DI. Vaccination with Gag, Vif, and Nef gene fragments affords partial control of viral replication after mucosal challenge with SIVmac239. Journal of Virology. 2014 Jul;88(13):7493-516.

II. **Martins MA**, Bonaldo MC, Rudersdorf RA, Piaskowski SM, Rakasz EG, Weisgrau KL, Furlott JR, Eernisse CM, Veloso de Santana MG, Hidalgo B, Friedrich TC, Chiuchiolo MJ, Parks CL, Wilson NA, Allison DB, Galler R, Watkins DI. Immunogenicity of seven new recombinant yellow fever viruses 17D expressing fragments of SIVmac239 Gag, Nef, and Vif in Indian rhesus macaques. PLoS One. 2013;8(1):e54434.

A full list of my publications can be accessed at the following URL:

https://www.ncbi.nlm.nih.gov/myncbi/mauricio.martins.1/bibliography/public/

D. Research Support
Ongoing Research Support

U19 AI149646 Redacted by agreement 04/15/20 – 03/31/25
NIH-NIAID Role: Project 1 leader
An AAV-mediated functional cure and its impact on the reservoir
This program-project combines the expertise of four group leaders Redacted by agreement Martins, Redacted by agreement in four projects and two cores to optimize the delivery of bnAbs or bnAb-like molecules via AAV-mediated gene therapy. The overarching goal is to stably suppress an ongoing SHIV infection in rhesus macaques.

Private Source Martins (PI) 07/01/18 – 06/30/22
Private Source Role: PI
rAAV-mediated delivery of HIV-specific bnMAbs in newborn rhesus macaques
The goal of this project is to evaluate whether neonatal delivery of AAV vectors expressing anti-HIV bnAbs or eCD4-Ig protects infant rhesus macaques against oral SHIV challenge.

Completed Research Support

1K01OD023032-01 Martins (PI) 08/05/16 – 04/30/20
NIH-Office of the Director Role: PI
Ipilimumab as an adjuvant for HIV vaccines
The goal of this project was to evaluate whether CTLA-4 blockade during antigen priming enhances the immunogenicity and efficacy of a novel SIV vaccine regimen in rhesus macaques.

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Withheld pursuant to exemption

Redacted by agreement

of the Freedom of Information and Privacy Act

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ○ Yes ● No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

○ Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period *Anticipated Amount ($) *Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

5. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Modular Budget

OMB Number: 0925-0001
Expiration Date: 02/28/2023

Budget Period: 1

Start Date: 12/01/2020 End Date: 11/30/2021

A. Direct Costs

	Funds Requested ($)
Direct Cost less Consortium Indirect (F&A)*	150,000.00
Consortium Indirect (F&A)	47,893.00
Total Direct Costs*	197,893.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	MTDC	85.00	87,921.00	74,733.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number): HHS Region IX DHHS, Division of Cost Allocation 90 7th Street San Francisco, CA 94103-6701 (415) 437-7820

Indirect (F&A) Rate Agreement Date: 11/07/2019

Total Indirect (F&A) Costs: 74,733.00

C. Total Direct and Indirect (F&A) Costs (A + B)

Funds Requested ($): 272,626.00

Funding Opportunity Number: PAR-20-054. Received Date: 2020-05-11T14:50:59.000-04:00

PHS 398 Modular Budget

Budget Period: 2			
Start Date: 12/01/2021 End Date: 11/30/2022			
A. Direct Costs			Funds Requested ($)
		Direct Cost less Consortium Indirect (F&A)*	125,000.00
		Consortium Indirect (F&A)	48,329.00
		Total Direct Costs*	173,329.00

B. Indirect (F&A) Costs

	Indirect (F&A) Type	Indirect (F&A) Rate (%)	Indirect (F&A) Base ($)	Funds Requested ($)
1.	MTDC	85.00	37,921.00	32,233.00
2.				
3.				
4.				

Cognizant Agency (Agency Name, POC Name and Phone Number)	HHS Region IX DHHS, Division of Cost Allocation 90 7th Street San Francisco, CA 94103-6701 (415) 437-7820		
Indirect (F&A) Rate Agreement Date	11/07/2019	Total Indirect (F&A) Costs	32,233.00

C. Total Direct and Indirect (F&A) Costs (A + B)	Funds Requested ($)	205,562.00

Funding Opportunity Number: PAR-20-054. Received Date: 2020-05-11T14:50:59.000-04:00

PHS 398 Modular Budget

Cumulative Budget Information

1. Total Costs, Entire Project Period

Section A, Total Direct Cost less Consortium Indirect (F&A) for Entire Project Period ($)	275,000.00
Section A, Total Consortium Indirect (F&A) for Entire Project Period ($)	96,222.00
Section A, Total Direct Costs for Entire Project Period ($)	371,222.00
Section B, Total Indirect (F&A) Costs for Entire Project Period ($)	106,966.00
Section C, Total Direct and Indirect (F&A) Costs (A+B) for Entire Project Period ($)	478,188.00

2. Budget Justifications

Personnel Justification	20200504_PERSONNEL_JUSTIFICATION.pdf
Consortium Justification	050520_Consortium_Justification.pdf
Additional Narrative Justification	

Funding Opportunity Number: PAR-20-054. Received Date: 2020-05-11T14:50:59.000-04:00

PERSONNEL JUSTIFICATION

Senior/Key Personnel

Mauricio A. Martins, Ph.D., Principal Investigator EFFORT **months).** Dr. Martins will be responsible for performing some of the experiments outlined in this application. Dr. Martins will be aided by Redacted by agreement a research technician in the Martins lab. Dr. Martins has 13 years of experience on flow cytometry and immunological analyses in rhesus macaques.

Other Personnel

Redacted by agreement **Research Technician** EFFORT **months).** Redacted by agreement is a technician in the Martins lab who will be in charge of quantifying eCD4-Ig and ADA concentrations in monkey serum, as part of the experiments described in aim 2. Redacted by agreement will also aid Dr. Martins in the sample processing and flow cytometric analyses planned for aim 1.

Other Significant Contributors

Redacted by agreement **Other Significant Contributor (**EFFORT **months effort).** Redacted by agreement developed the eCD4-Ig molecule that will be used in aim 2. Redacted by agreement is also at Scripps Research in Florida and his office is adjacent to that of Dr. Martins. In addition to providing mentorship and scientific insights, he will also provide the DNA plasmids needed for production of the eCD4-Ig and TPST2-expressing AAV vectors needed for aim 2.

Redacted by agreement **Statistical Collaboratory Services (**EFFORT **months effort).** Redacted by agreement will consult and assist with all experimental design and study analysis of data from the non-human primate studies of this program. Redacted by agreement has extensive published background in the appropriate use of statistics in human and animal studies, and also applies this expertise to large-scale genomics and proteomics platforms. Redacted by agreement s position at the Institute is ensuring statistical rigor for investigators of the Florida campus of the Scripps Research Institute.

Redacted by agreement **Other Significant Contributor (**EFFORT **months effort).** Redacted by agreement is the director of the Gene Therapy Center and Viral Vector Core at the University of Massachusetts Medical School. His group has extensive experience in the production, purification, formulation, and quality control testing of large-scale preparations of recombinant AAV vectors expressing various transgene products. The Redacted by aboratory will be in charge of producing the AAV/eCD4-Ig and AAV/TPST2 constructs for aim 2.

Consortium Budget Justification

Consortium Approximately $135,000 Total Costs per year (55% Yr 1 F&A, 55.5% Yr 2 F&A; $87,000 direct costs)

Consortium with The Board of Regents of the University of Wisconsin System, Wisconsin National Primate Research Center (WNPRC) {X} Domestic { } Foreign

Personnel

Redacted by agreement **Co-Investigator** EFFORT **months).** Redacted by agreement will manage the nonhuman primate (NHP) portion of the project the at the Wisconsin National Primate Research Center (WNPRC). He will arrange for identification of suitable animals for the study design. In addition, he will oversee all technical experimental plans and oversee the WNPRC Scientific Protocol Implementation Unit technicians and veterinary staff assisting with blood collections, biopsies, hormone capsule placements, vaccinations, and treatments. Working with the WNPRC Assay Services and Pathology Services Units, Redacted by agreement will coordinate the measurement of plasma hormone levels, CBC/Chem panels and Anti-nuclear antibody evaluations. Redacted by agreement will be responsible for the IACUC and Biosafety protocols at the University of Wisconsin, discuss experimental plans with Dr. Martins, and also assist in data analysis and manuscript preparation as needed.

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 02/28/2023

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	20200511_MARTINS_R21_SPECIFIC_AIMS.pdf
3. Research Strategy*	20200511_MARTINS_R21_RESEARCH_STRATEGY.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	20200508_MARTINS_R21_VERTEBRATE_ANIMALS.pdf
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	Consortium_Agreement_Letters.pdf
9. Letters of Support	20200508_LOS.pdf
10. Resource Sharing Plan(s)	20200508_MARTINS_R21_RESOURCE_SHARING_PLAN.pdf
11. Authentication of Key Biological and/or Chemical Resources	20200508_R21_AUTHENTICATION_KEY_RESOURCES.pdf
Appendix	
12. Appendix	

Tracking Number: GRANT13100885
Funding Opportunity Number: PAR-20-054. Received Date: 2020-05-11T14:50:59.000-04:00

SPECIFIC AIMS

Transgender women (TGW) bear a disproportionately heavy burden of HIV/AIDS, with a pooled global prevalence of 19% and a 49-fold higher odds ratio of acquiring HIV than non-transgender adults[1]. Tackling the HIV/AIDS epidemic in the TGW community is hampered by stigma and high-risk behavior. Indeed, TGW frequently engage in condomless receptive anal sex, which appears to be the primary method of HIV transmission in this population[2]. Additionally, because of employment discrimination, sex work is often one of the few income opportunities available to TGW, thereby increasing their exposure to HIV[3]. Many TGW also avoid pre-exposure prophylaxis because of fear that antiretroviral drugs can interfere with feminizing hormone therapy (FHT)–a key component of gender affirmation in TGW[4]. Thus, given TGW's unique challenges to optimal HIV prevention, new TGW-tailored HIV prophylactic tools are urgently needed.

Efforts to prevent HIV infection in TGW are further complicated by our poor understanding of how FHT impacts immune responses in biological males. The hormone estradiol (E2), for instance, the main drug in FHT, can modulate immune responses in many ways[5,6], including by shaping CD4+ T-cell development and function[7]. Importantly, E2 has also been shown to increase CCR5 expression in CD4+ T-cells[8]. Consistent with this notion, Giltay *et al.* have previously reported a significant rise in circulating CCR5+ CD4+ T-cells in TGW following FHT initiation[9]. Together, these observations raise the possibility that FHT can trigger the expansion of optimal HIV "target" cells *in vivo*, thereby increasing TGW's susceptibility to HIV infection. However, crucial questions remain unanswered, as it is unknown whether FHT-driven changes on CD4+ T-cells in blood are reflected in the rectal mucosa–the presumed portal of HIV entry in TGW. It is also unclear whether FHT affects other subsets of HIV-susceptible cells, like Th17 cells[10]. Given the difficulties of prospectively addressing these knowledge gaps in clinical studies, here we will model FHT in rhesus macaques (RMs) to facilitate in-depth analyses of FHT-driven immune changes. To that end, 12 adult male RMs will be enrolled in this study. The animals in Group 1 (n = 6) will be continuously dosed for nearly one year with a popular FHT regimen. The monkeys in Group 2 (n = 6) will be treated with placebo and serve as controls. Animals in both groups will undergo frequent blood draws and gut biopsies. This study design will allow us to answer two key questions about the immune effects of FHT.

1) Does FHT increase the availability of HIV-susceptible CD4+ T-cells *in vivo*? Activated CD4+ T-cells in the gut, particularly those expressing CCR5 or biased toward the Th17 phenotype, are the major early targets for HIV/SIV replication and amplification[10–13]. In fact, the susceptibility of RMs to rectal SIV infection is directly proportional to the numbers of CCR5+ CD4+ T-cells in the rectal mucosa[14–17]. Since E2 treatment has been linked to CCR5 upregulation on CD4+ T-cells[8,9], we will use our monkey model of FHT to characterize the kinetics and tissue distribution of CD4+ T-cells following FHT initiation. To that end, we will use flow cytometry to assess the frequency and phenotype of memory CD4+ T-cells in blood and gut biopsies from the Group 1 and Group 2 animals throughout the FHT and placebo regimens. By comparing the levels of activated CD4+ T-cells in tissues from FHT-treated (Group 1) and untreated (Group 2) animals, this analysis will reveal whether FHT modulates a crucial marker of HIV susceptibility in biological males.

2) Does FHT interfere with adeno-associated virus (AAV)-vectored immunoprophylaxis? Considering TGW's high risk of acquiring HIV, they stand to benefit greatly from AAV-mediated delivery of immunoglobulins as this approach can provide durable antiviral immunity after a one-time administration[18–26]. In this regard, the potent and extremely broad HIV entry inhibitor eCD4-Ig is well suited for AAV-vectored immunoprophylaxis. eCD4-Ig is a chimeric molecule consisting of the ectodomain of CD4, an IgG Fc portion, and a coreceptor-mimetic peptide[19]. Because eCD4-Ig emulates the receptor (CD4) and coreceptors (CCR5 & CXCR4) of primate lentiviruses, it binds avidly to and neutralizes virtually any HIV or SIV Env proteins. Remarkably, RMs treated once with an AAV/eCD4-Ig vector developed persistent levels of eCD4-Ig for >1 year and resisted challenges with viruses as divergent in their Env proteins as SHIV-AD8 and SIVmac239[(ref. 19,20)]. Given the unique capacity of a single dose of AAV/eCD4-Ig to confer persistent and universal immunity against HIV, AAV-mediated gene therapy with eCD4-Ig constitutes a powerful approach for fighting HIV/AIDS in TGW. However, considering the immunoenhancing properties of E2[(Ref. 5,6)], FHT may undermine AAV-driven eCD4-Ig expression by amplifying host anti-drug antibodies (ADAs)–a major limiting factor for AAV-vectored immunoprophylaxis[27]. To address this possibility, all animals in Groups 1 and 2 will receive a single dose of AAV/eCD4-Ig six months into their FHT and placebo regimens. Serum will then be collected in the ensuing weeks for quantification of eCD4-Ig and ADA. By comparing the levels of eCD4-Ig and ADAs in samples from FHT-treated (Group 1) and untreated (Group 2) animals, this analysis will reveal the impact of FHT on AAV-mediated delivery of eCD4-Ig in biological males.

In sum, this project will advance our understanding of the pharmacodynamics of FHT and how it affects immune responses in biological males. The data generated here may also pave the way for future studies aimed at addressing the impact of gender reassignment hormone therapy on immunodeficiency virus containment.

RESEARCH STRATEGY

Significance

The HIV/AIDS crisis in transgender people. HIV/AIDS thrives in the margins of society, where low educational achievement, unstable housing, and poverty render community members vulnerable to acquiring HIV. No population is more affected by these social injustices than transgender persons[28,29]. A case in point is transgender women (TGW)–individuals who were assigned a male sex at birth but express their gender along a feminine spectrum. Compared to non-transgender adults, TGW bear a disproportionate burden of HIV/AIDS that is fueled by stigma and high-risk sexual behaviors[1,4,28]. However, as outlined below, we posit that feminizing hormone therapy (FHT)–a key component of gender affirmation–can also predispose TGW to contracting HIV.

TGW face unique barriers to optimal HIV prevention. Many TGW are concerned that antiretroviral therapy (ART) can interfere with FHT, a possibility that is only now beginning to be explored[30–32]. This is relevant because TGW may prioritize gender-affirming care over HIV treatment[33]. Indeed, while pre-exposure prophylaxis (PrEP) can prevent HIV infection in non-transgender men and women[34–36], a sub-group analysis of the iPrEx trial showed that PrEP was ineffective in TGW[37]. Moreover, consistent with TGW's hesitation toward to ART, TGW were more likely to have undetectable drug levels than non-TGW persons in the iPrEx trial[37]. Thus, given the limited efficacy of current prophylactic tools in TGW, new methods for combating HIV infection in TGW are urgently needed.

Biological consequences of FHT in TGW. FHT generally includes an estrogen (i.e., 17-β estradiol or E2) plus a testosterone-lowering agent, like spironolactone[38]. FHT leads to reduction in facial hair, breast development, and redistribution of fat and muscle to a more a feminine pattern[38]. Despite these profound physical alterations, the immune effects of FHT in biological males remain largely unexplored. In specific aim (SA) 1, we will explore whether FHT increases the availability of activated CD4+ T-cells *in vivo*, a notion that is based on several observations linking female sex hormones and HIV susceptibility (see below).

- The increased risk of HIV transmission in pregnant and postpartum women has been associated with hormonal alterations on the immune system[39–41].
- Hormonal contraceptives have been repeatedly associated with high frequencies of activated cervical CD4+ T-cells and increased susceptibility to HIV infection in women[42–47].
- Exogenous E2 treatment has been shown to upregulate CCR5 expression in CD4+ T-cells from ovariectomized female mice[8].
- Giltay *et al.* have previously reported a significant rise in circulating CCR5+ CD4+ T-cells in TGW following FHT initiation[9].

Strengths and weaknesses of prior research. While the study by Giltay *et al.* provides actual clinical evidence for the ability of E2 to upregulate CCR5 expression, that study was conducted 20 years ago and had several caveats: the cohort was relatively small, the CCR5 analysis included only two timepoints, and mucosal samples were not examined. In view of these limitations, more in-depth studies on FHT-driven immune consequences are needed to guide the development of anti-HIV interventions tailored for TGW.

Adeno-associated virus (AAV)-vectored immunoprophylaxis is emerging as a promising and viable HIV vaccine alternative[18–26]. AAV-vectored immunoprophylaxis deviates from conventional HIV vaccines in that host cells, after receiving the relevant immunoglobulin (Ig) genes through AAV transduction, can immediately begin to produce HIV-specific broadly-reactive (b) neutralizing (n) antibodies (Abs) or bnAb-like molecules. AAV vectors are well suited for this approach because of their non-pathogenicity and ability to efficiently transduce a wide range of cells[48]. The AAV vector genome persists in the nucleus of the transduced cell in episomal format, with little or no integration occurring. Additionally, the only protein expressed from AAV vectors is the transgene product; as long as it is non-toxic and does not trigger host immunity, transgene expression can persist for long periods of time. Skeletal muscle is a preferred tissue for AAV-mediated Ig gene transfer because of the long lifespan of muscle cells (~15 years)[49]. Indeed, Martinez-Navio *et al*. have recently reported that a RM maintained serum concentrations of an SIV-specific monoclonal (m)Ab in the 240-350 ug/ml range for >6 years after receiving a single intramuscular (IM) AAV vector administration[50].

One limitation of AAV-vectored immunoprophylaxis is that the transgene product often becomes an immune target, leading to the development of anti-drug Abs (ADAs) that clear the delivered Ig from circulation[27]. This issue is especially detrimental for anti-HIV bnAbs, as their hypermutated nature and unusual structural features make them highly immunogenic in the context of AAV gene transfer[51]. Notably, ADAs can emerge even when there is complete species match between the AAV-delivered anti-HIV bnAb and the host[23,52,53]. Indeed, ~50% of participants of recent clinical trials of AAV/bnAb vectors mounted ADAs[52,53], underscoring the potential of ADA responses to limit sustained AAV-driven bnAb expression.

The remarkable properties of eCD4-Ig. Our SA 2 is to assess the impact of FHT on AAV-mediated gene therapy with eCD4-Ig in male RMs. eCD4-Ig was developed by Redacted by agreement, a collaborator in this project

[see Redacted by agreement letter of support (LOS)]. eCD4-Ig is a fusion protein consisting of the outer domains of CD4, an IgG Fc portion, and a short tyrosine-sulfated coreceptor-mimetic peptide that resembles the tyrosine-sulfated regions of all HIV and SIV coreceptors (Fig. 1)[19,54]. eCD4-Ig has several key advantages over HIV-specific bnAbs.

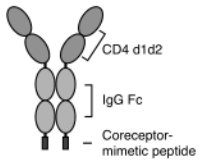

Figure 1. Scheme of eCD4-Ig molecule.

- *Breadth*: eCD4-Ig is broader than any single anti-HIV bnAb described to date[19]. Indeed, eCD4-Ig neutralizes all 273 HIV-1, HIV-2, and SIV isolates it has been tested against. Remarkably, a single IM dose of AAV/eCD4-Ig to RMs resulted in continuous production of eCD4-Ig for >1 year and conferred significant protection against challenge with viruses as divergent in their Env proteins as SHIV-AD8 and SIVmac239(ref. 19,20).
- *Potency*: eCD4-Ig rivals the best anti-HIV bnAbs for neutralization potency, with IC_{80} values below 9.0 μg/ml for all HIV isolates it has been tested against.
- *Escape*: It is harder for HIV to escape neutralization by eCD4-Ig than by bnAbs. In fact, despite rigorous efforts, full escape from eCD4-Ig has not yet been observed[20,55].
- *Synergy*: Because eCD4-Ig binding leads to conformational changes in Env, it results in the exposure of vulnerability sites that are normally occluded in the native Env trimer. Consequently, eCD4-Ig, but not bnAbs, enhances the NK cell-mediated Ab-dependent cell-mediated cytotoxicity (ADCC) activities of non-neutralizing anti-HIV Abs in sera from HIV-infected patients[56].
- *Immunogenicity*: Because eCD4-Ig is more "self-like" than most anti-HIV bnAbs, eCD4-Ig triggers ADAs less frequently than bnAbs following AAV gene transfer[19]. Furthermore, even when ADAs are induced, they usually wane over time and do not completely abrogate eCD4-Ig expression (Fig. 2A&B).

Given these remarkable properties, achieving persistent AAV-driven expression of eCD4-Ig in TGW would be a game-changer for combating HIV/AIDS in the transgender community.

FHT may interfere with AAV-driven eCD4-Ig expression in biological males. Sex hormones have been known to impact the immune system for nearly a century. A study from the 1930s reported that exogenous estrogen treatment increased the baseline levels of antibacterial Abs in male and female rabbits in a dose-dependent way[57]. Likewise, a 1942 study showed that estrogen dosing increased Ab responses to a killed-bacterial vaccine in rabbits of both sexes[58], suggesting that estrogen could work as a vaccine adjuvant. Furthermore, a 1967 analysis of human serum Ig levels revealed higher Ab levels in women than in men[59]. These observations are in line with the current paradigm that women are more resistant to infections than men, partly because of the immunoenhancing effects of estrogen[5,6].

Given the ability of estrogen to amplify adaptive immune responses, FHT may inadvertently interfere with AAV-driven expression of eCD4-Ig in TGW by increasing the immunogenicity of eCD4-Ig and triggering ADAs. In support of this idea, a recent study reported that ADAs occur more frequently in women than in men following passive transfer of therapeutic mAbs[60]. The NHP model of FHT described here will allow us to study the extent to which FHT can affect AAV-vectored immunoprophylaxis in males. RMs are well suited for this analysis because of their close phylogenetic relation to humans and the fact that critical variables, such as adherence to FHT and sampling schedule, can easily be controlled in NHPs. In short, this project is significant because it will advance our knowledge of the pharmacodynamics of FHT and how it affects immune interventions.

Innovation

This project will be the first to model FHT in NHPs. Our experimental outline will allow a detailed analysis of the immune consequences of FHT in blood and gut biopsies of biological males. This project will also be the first to assess whether FHT can interfere with AAV-vectored immunoprophylaxis. The use of eCD4-Ig is also novel, given its high potency and unmatched breadth against HIV.

Approach

The 12 RMs needed for this project will be purchased from

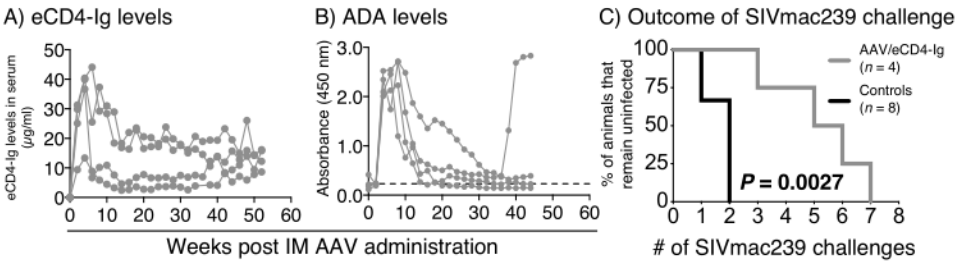

Figure 2. Inoculation of RMs with AAV/eCD4-Ig results in persistent expression of eCD4-Ig and protection against SIVmac239 challenge. Four RMs received one IM dose of AAV-1 vectors encoding eCD4-Ig or TPST2. Serum levels of eCD4-Ig (A) and ADAs (B) were measured by ELISA. The AAV/eCD4-Ig-treated RMs were eventually challenged intravenously with escalating doses of SIVmac239 until all animals became infected (C). Note that all control RMs (n = 8) became infected before any of the experimental monkeys did (C). As expected, the AAV/eCD4-Ig-treated animal that experienced the surge in ADAs at week 38 also had the lowest levels of eCD4-Ig in serum and was the first experimental animal to acquire SIV infection. Adapted from [20].

and housed at the Wisconsin National Primate Research Center (WNPRC). Since the experiments described here do not require euthanasia, the animals will be leased for the duration of this project and then returned to the WNPRC colony once the study is completed. All animal procedures will be done at the WNPRC under the supervision of Redacted by agreement whose expertise on NHP endocrinology will provide an additional layer of rigor to this project. Biological specimens intended for immunoassays will be harvested at the WNPRC and sent overnight to the Martins lab in temperature-controlled packages. This arrangement has been used in many prior NHP studies led by Dr. Martins[61–69], with no overt effects on cell viability or assay performance.

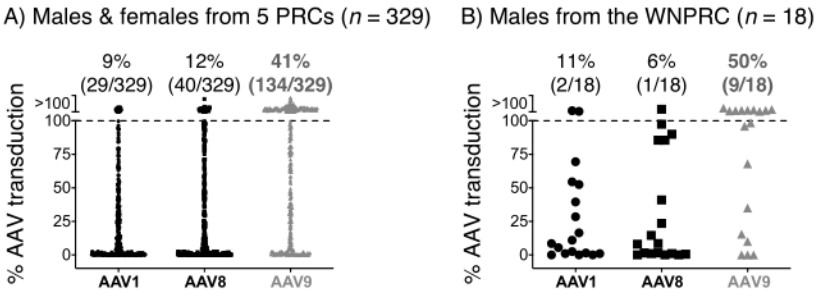

Figure 3. AAV seroprevalence among RMs in 5 PRCs. Serum samples from 329 RMs from the Wisconsin, Oregon, California, Tulane, and Caribbean PRCs were screened for nAbs against AAV-1, AAV-8, and AAV-9 using a luciferase-based neutralization assay. In short, monkey sera were diluted 5-fold and incubated with luciferase-expressing AAV vectors of the indicated serotypes for 30 min at 37 °C. The samples were then added to 293T cells alongside the appropriate controls. The luciferase signal was read 48 hrs later. The "percent AAV transduction" values shown in the *y*-axes were calculated based on the luciferase signal in the presence or absence of serum. The data from all animals (males and females) are shown in panel A. The subgroup analysis of males from the WNPRC is shown in B. The percentages and absolute numbers of animals that are seronegative for each AAV vector are shown on top of each panel. Courtesy of Redacted by agreement

Relevant biological variables. To model FHT in TGW, only male monkeys will be used in this project. Note that both humans and RMs can become naturally infected with wild-type AAV[70]. As a result, pre-existing Abs to WT AAV may cross-react with some of the capsids commonly used for AAV gene therapy and reduce their transduction efficiency[27]. With this in mind, we examined the prevalence of nAbs against AAV-1, AAV-8, and AAV-9 in 329 RMs from 5 Primate Research Centers (PRCs). This survey revealed that only 9%-12% of the animals were seronegative for AAV-1 and AAV-8 (Fig. 3A). In contrast, 41% of the cohort was seronegative for AAV-9, a serotype with low seroprevalence in humans that can also transduce skeletal muscle[70,71]. Importantly, we found a similar pattern of AAV seronegativity in male RMs from the WNPRC, where half of the animals lacked detectable anti-AAV-9 nAbs (Fig. 3B). Based on these data, we will use AAV-9 for our SA 2. Thus, only AAV-9 seronegative male RMs will be used in this project.

FHT regimen. FHT helps TGW achieve hormone levels that match their gender identity. This includes reducing testosterone levels from the male range (300-1,000 ng/dl) to the female range (<50 ng/dl) and increasing E2 levels to 100-200 pg/ml[38]. The key component of FHT is E2, an estrogen that by itself can suppress testosterone levels through a central feedback mechanism. However, to avoid supraphysiological levels of E2 (>200 pg/ml), FHT generally includes a testosterone-lowering agent as well, like the potassium sparing diuretic spironolactone, so that lower doses of E2 can be used[38].

To ensure consistent delivery of E2 and spironolactone across animals, these drugs will be formulated in custom-made biodegradable pellets (Innovative Research of America; see LOS) that allow for sustained drug release over a 3-week period. Separate pellets for E2 and spironolactone will be produced for Group 1, while a placebo pellet will be ordered for Group 2. These pellets will be implanted subcutaneously over the shoulder blade bone every 3 weeks (Fig. 4), as described previously[72]. It is worth noting that female sex hormones in RMs fluctuate in the same range as those in humans[73]. Hence, given that the present experiment will be the first to model FHT in RMs, we will tailor the FHT dose to achieve the aforementioned human concentrations of E2 (100-200 pg/ml) and testosterone (<50 ng/dl). To that end, the Group 1 animals will first be treated with pellets that release E2 at a rate of 4.0 μg/kg/day. In a previous study, a one-time administration of these pellets to ovariectomized female RMs resulted in E2 concentrations in the 50-150 pg/ml range over a 3-week period[72]. Spironolactone will be delivered at a lower dose (1.0 μg/kg/day). Depending on the hormone levels measured in the ensuing weeks, we may increase or decrease the doses of E2 and spironolactone delivered in subsequent pellets. Ultimately, our goal will be to dose the Group 1 animals with the minimal concentration of FHT required to maintain E2 and testosterone levels within female intervals.

Safety considerations. Exogenous E2 therapy can increase triglyceride levels and the risk of thromboembolism in TGW[74]. Spironolactone can also result in supraphysiological levels in blood[38]. In light of these potential adverse events, all animals in Groups 1 and 2 will undergo regular measurements of hormones (testosterone and E2) in serum, as well as blood chemistry tests and complete blood cell count.

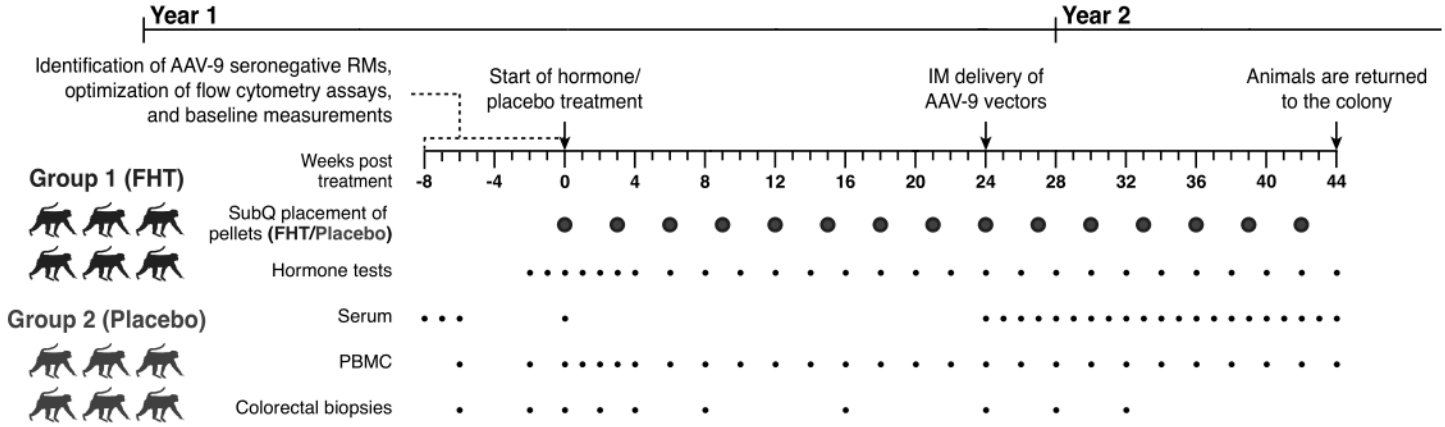

Figure 4. Experimental layout and sampling schedule.

SA 1) Does FHT increase the availability of HIV-susceptible CD4+ T-cells *in vivo*? Value added: The discovery that FHT triggers phenotypical and migratory properties on CD4+ T-cells that render TGW more vulnerable to HIV would provide a biological basis for the unusually high burden of HIV/AIDS among TGW. Such a discovery would also open up new opportunities for interventions to prevent HIV infection in TGW.

Approach: We will use polychromatic flow cytometry to characterize the kinetics of memory (CD95+) CD4+ T-cells in blood and colorectal biopsies from the Group 1 and Group 2 monkeys throughout the FHT/placebo regimens. We will also determine the proportion of memory CD4+ T-cells that display the phenotypes below since they have been linked to mucosal HIV/SIV transmission and early virus amplification[10].

- Transitional memory (T_{RM}; CD28+/CCR7–/CCR5+) CD4+ T-cells[11,12].
- Effector memory (T_{EM}; CD28–/CCR7–/CCR5dim) CD4+ T-cells[11,12].
- Th17 (CCR6+) CD4+ T-cells[13].

Sample processing: Blood draws and colorectal biopsies will be harvested from the Group 1 and Group 2 animals at baseline and regular time points thereafter (Fig. 4). The colorectal specimens will consist of 10-12 punch biopsies from the rectum and colon (20-24 biopsies total) from each animal. These samples will be weighed as soon as they arrive in the lab. In an attempt to increase the cell yields from these biopsies, rectal and colon biopsies from each animal will be combined and processed together using collagenase digestion and mechanic disruption. Note that previous studies have reported no significant differences in tissue density or phenotype between rectal and colonic CD4+ T-cells[75,76]. PBMCs will be isolated from EDTA-treated vacutainer tubes by standard Ficoll density centrifugation. Ultimately, one million PBMCs or 85% of the gut cells isolated from each animal will be suspended in 100 ul of PBS and added to their respective flow cytometry tubes. The remaining 15% of gut cells from each animal will be pooled and used in isotype mAb control tubes. PBMC isotype mAb control tubes will contain pooled PBMCs from multiple animals.

Flow cytometric staining: The first step will be to stain the cells with a viability dye (LIVE/DEAD Aqua) for 20 min. Then, to enable parallel quantification of cell counts in the PBMC and mucosal samples, we will spike the tubes with defined amounts of SPHERO AccuCount Rainbow Fluorescent beads. The PBMC tubes will be spiked with 50,000 beads, while the gut cell tubes will receive 1,000 beads/mg of tissue weight (measured before processing), as described previously[77]. We will then add the fluorescent mAb cocktail (Table 1) to the cells and fix them with 500 ul of BD FACS Lyse solution after a 30-min incubation. We will then place the tubes on ice until acquisition. Because each tube must retain its original number of beads for the absolute cell quantification, the tubes will not be washed. The samples will be acquired on a BD LSRII flow cytometer equipped with 5 lasers (See LOS by Redacted by agreement Redacted by agreement

Table 1. Surface staining panel for delineating memory CD4+ T-cell subsets in RMs.

mAb/ARD	Clone	Fluorochrome	Excitation laser	Emission Max	
LIVE/DEAD ARD		AQUA	Violet (405 nm)	526 nm	Dump
CD14	M5E2	BV510	Violet (405 nm)	510 nm	Dump
CD16	3G8	BV510	Violet (405 nm)	510 nm	Dump
CD20	2H7	BV510	Violet (405 nm)	510 nm	Dump
CD3	SP34-2	BB700	Blue (488 nm)	693 nm	
CD4	L200	FITC	Blue (488 nm)	520 nm	
CD45	D058-1283	Texas Red	Yellow (561 nm)	615 nm	
CD28	CD28.2	APC	Red (633 nm)	660 nm	
CD95	DX2	PE Cy7	Yellow (561 nm)	785 nm	
CCR5 (CD195)	3A9	PE	Yellow (561 nm)	578 nm	
CCR6 (CD196)	11A9	BUV805	UV (355 nm)	805 nm	
CCR7 (CD197)	150503	BV421	Violet (405 nm)	421 nm	

Data analysis: We will use FlowJo v.10 to analyze the data. First, we will record the number of Rainbow Fluorescent beads in each tube based on their distinctly high side scatter properties. We will then proceed to excluding doublets and creating a time gate including only the events recorded within the 5th and 90th percentiles. Next, we will gate on dump channel (ARD AQUA, CD14, CD16, and

CD20) negative cells, followed by CD3+ CD45+ cells. At this stage, we will delineate the lymphocyte population based on its forward and side scatter properties. Next, we will gate on CD3+ CD4+ cells, followed memory (CD95+ CD28–/+) ones. We will delineate the relative frequencies of the three CD4+ T-cell subsets mentioned above within this memory gate. Absolute cell counts will be calculated based on cell-to-bead ratios, as described previously[77,78].

Statistical power calculation: The primary endpoint of SA 1 is the difference in CD4+ T-cell frequency between Groups 1 and 2. Based on the distribution of blood and gut CCR5+ CD4+ T-cells in RMs (Fig. 5), six RMs per group will provide ≥92-93% power to detect differences of ≥2 standard deviations (SDs) between Groups 1 and 2 using a 2-sided α of 0.05.

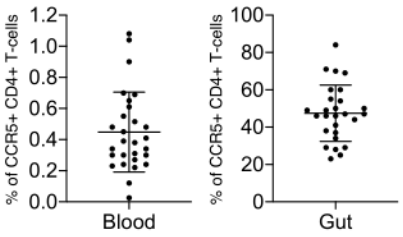

Figure 5. Flow cytometric analysis of blood and gut CCR5+ CD4+ T-cells in RMs. Flow cytometry was used to determine the relative frequencies of CCR5+ CD4+ T-cells in blood (A) or gut cells (B). Horizontal bars correspond to means and error bars denote the SD. Adapted from reference [15].

Key question to be addressed: *Does FHT increase the frequency of HIV-susceptible CD4+ T-cells* in vivo*?* We will address this question in two ways. The first one will involve intragroup comparisons of the relative and absolute frequencies of CD4+ T_{RM}, T_{EM}, and Th17 subsets between baseline and subsequent time points. The second one will consist of intergroup comparisons of the same subsets between Groups 1 and 2. For gut cells, these comparisons will be done every time biopsies are collected (Fig. 4). For PBMC, these comparisons will be done on a per time point basis until week 8. For later time points, the cumulative changes in peripheral CD4+ T-cell levels will be compared based on the area under the curve (AUC) values for each group. In all cases, *P* values will be determined by two-tailed *t*-tests. Redacted by agreement Redacted by agreement will assist in these analyses (see Redacted by LOS).

Possible outcomes: If FHT increases the levels of activated CD4+ T-cell levels *in vivo*, it will be important to establish the kinetics of these changes following FHT so that TGW receive anti-HIV interventions when they are most vulnerable to HIV. Consistent with this notion, the timing of administering an immune intervention may vary depending on whether activated CD4+ T-cell numbers rise slowly over time or undergo a sharp but transient increase following FHT initiation. To account for these two different outcomes, the sampling schedule will be more intense during the first 2 months of treatment compared to the remainder of the experiment (Fig. 4). It is also possible that no significant difference in CD4+ T-cell levels is observed between Groups 1 and 2.

Potential caveats: We have considerable experience with the workflow proposed here. In fact, nearly all papers published by Dr. Martins include flow cytometry analysis from monkey tissues[61–69,79–82]. While low cell yields can be a potential pitfall to flow cytometric analysis of gut lymphocytes, we are taking several precautions to minimize this caveat. Namely, we will obtain punch biopsies from two adjacent intestinal compartments (rectum and colon), all gut lymphocytes from each animal will be stained in one experimental tube, and the cells will not be washed prior to acquisition. Importantly, we will also obtain blood and colorectal biopsies from animals during the AAV9 screening process to validate the fluorescent mAb staining panel proposed here (Fig. 4). These "practice runs" will allow us to polish our assays prior to the final selection of animals for Groups 1 and 2.

While our approach to calculate absolute numbers of CD4+ T-cell subsets will strengthen our intragroup and intergroup comparisons, its accuracy depends on not washing the tubes prior to flow cytometric acquisition. Without washing, we will not be able to permeabilize the cells for staining intracellular molecules, such as ki-67 (a common marker for T-cell activation/proliferation) and IL-17, the signature cytokine for Th17 cells. Note, however, that the absence of ki-67 staining will not preclude us from identifying memory CCR5+ CD4+ T-cells–the main targets for HIV/SIV. Moreover, surface expression of CCR6 is a well-established marker of Th17 cells[83,84]. Although not all CCR6+ CD4+ T-cells produce IL-17 upon stimulation, the vast majority of them are prone to acquire Th17 effector functions[85]. Thus, the proposed analysis of surface markers will enable the detection of significant FHT-driven changes in HIV/SIV target cell availability regardless of intracellular markers.

SA 2) Does FHT interfere with AAV-mediated delivery of eCD4-Ig? Value added: Understanding whether and how FHT impacts AAV-mediated Ig delivery in monkeys may enable adjustments to the FHT regimen to maximize AAV-driven Ig expression in TGW.

Approach: If AAV/eCD4-Ig vectors are ever to be tested in TGW, we envision that most trial participants will be established users of FHT. To simulate this scenario within the budget of this grant, the RMs in Groups 1 and 2 will receive AAV/eCD4-Ig at week 24 post treatment and will be monitored for an additional 20 weeks (Fig. 4). As a reference, it takes at least 6 months of FHT for physical changes to begin to appear in humans[38].

AAV vectors. Two AAV-9 vectors will be used in this project. One of them will encode an eCD4-Ig molecule consisting of the ectodomain of rhesus CD4 with an I39N substitution intended to improve the potency of eCD4-Ig[86]. The Fc portion will be that of rhesus IgG2 and will contain the "LS" mutations for extending the serum half-life of eCD4-Ig[87]. We selected the Fc domain of IgG2 because it is less immunogenic than its IgG1 counterpart

for AAV-mediated Ig delivery while also being able to direct Fc-dependent effector functions[88,89]. Furthermore, the tyrosine-sulfated coreceptor-mimetic peptide will be Emm58, which is derived from "self" proteins that are conserved between humans and RMs. Transgene expression will be under the control of the chicken β-actin (CBA) promoter, with a CMV enhancer placed upstream of it (Fig. 6). To ensure that the tyrosines in Emm58 are properly sulfated in muscle cells, the AAV-9/eCD4-IgG2 vector will be co-delivered with a separate AAV-9 vector expressing rhesus tyrosine-protein sulfotransferase 2 (TPST2) at a 4:1 ratio[19]. This two-vector approach has been used in all previous AAV/eCD4-Ig-related experiments conducted in RMs. Since increasing AAV vector dispersal among different muscle sites seems to enhance transgene expression[23], a solution containing 2.0×10^{12} genome copies (GC)/kg of AAV-9/eCD4-IgG2 and 5.0×10^{11} GC/kg of AAV-9/TPST2 will be split among 8 IM sites. Importantly, AAV-9 can efficiently transduce skeletal muscle tissue[70].

Figure 6. Genomic scheme of AAV-9 vectors.

Quantification of eCD4-IgG2 and ADA levels by ELISA. Serum will be collected according to the schedule in Figure 4. These samples will be used to quantify eCD4-IgG2 by ELISA using plates coated with recombinant gp120 from the HIV-1 BG505 isolate (Immune Tech). Defined amounts of recombinant eCD4-IgG2 produced in house will serve as standards. The lower limit of detection for these ELISAs is ~20 ng/ml. The concentration of eCD4-Ig measured at each time point will be expressed as μg/ml of serum. We will compare Groups 1 and 2 based on the cumulative amounts of eCD4-Ig produced by each animal during the 20 weeks of follow-up (weeks 24-44; Fig. 4). To that end, we will calculate the AUC of eCD4-Ig concentrations and use these AUC values for the group comparisons.

We will monitor ADAs throughout the experiment by semi-quantitative ELISA using a fixed serum dilution of 1:20. The plates will be coated with recombinant eCD4-IgG2 (the exact same protein delivered by the AAV vectors) and rhesus Abs against these molecules will be detected by goat secondary Abs specific for human λ and κ light chains. Note that eCD4-Ig has no Ig light chain. Because there is no rhesus eCD4-Ig-specific mAb available that could be used as the standard for ADA ELISAs, we will not be able to quantify ADAs in μg/ml. To overcome this caveat and obtain a quantitative measure of ADAs in Groups 1 and 2, we will assess the reactivity of serially diluted serum to plate-bound eCD4-Ig. This will allow us to calculate the midpoint titer of ADAs in each animal, that is, the reciprocal serum dilution that reduces the signal in the ELISA by 50%. We will determine ADAs titers in Groups 1 and 2 at 4-week intervals following the AAV-9/eCD4-Ig inoculation, which should encompass the initial rise and fall of ADAs (Fig. 2B). We will compare the levels of ADAs measured in Groups 1 and 2 using the same approach described above for eCD4-Ig. That is, we will calculate the AUC of ADA titers for each animal and then use these AUC values for the intergroup comparisons.

Assessment of the biological activity of eCD4-Ig. We will measure the ability of serum to neutralize SHIV-AD8 and direct ADCC against SHIV-AD8-infected cells at 4-week intervals using established assays[90,91].

Statistical power calculation: The primary endpoint of SA 2 is the difference in eCD4-Ig and ADA levels between Groups 1 and 2. Based on the mean and SD of the cumulative levels of AAV-expressed eCD4-Ig (i.e., AUC) in a recent study (Fig. 2), 6 RMs per group will provide ≥90% power to detect differences of ≥2 SDs between Groups 1 and 2 using a 2-sided α of 0.05. Although we do not have reference ADA titers for a statistical power calculation, we expect that 6 animals per group will be sufficient to detect meaningful differences.

Key question to be addressed: *Does FHT affect the levels of eCD4-Ig and ADAs following AAV-9/eCD4-Ig inoculation?* We will address this question by comparing eCD4-Ig concentrations and ADA titers between Groups 1 and 2. These variables will be expressed as means and *P*-values will be calculated using Welch's t-test. To complement this analysis, we will also compare Groups 1 and 2 on the basis of serum concentrations of eCD4-Ig measured at peak and the last follow up. Again, Redacted by agreement will assist in these comparisons.

Potential outcomes: If FHT results in higher ADAs following AAV/eCD4-Ig inoculation, we expect that the cumulative levels of eCD4-Ig in serum will be significantly lower in Group 1 than in Group 2. Depending on the extent of this reduction, this finding would have important implications for designing clinical trials of AAV-vectored immunoprophylaxis in TGW, as FHT would need to be interrupted–at least transiently–in order for AAV transgene expression to occur unabatedly. Alternatively, FHT may not impact AAV-driven expression in Group 1, a scenario that would clear the path for AAV/eCD4-Ig to be tested in TGW.

Potential caveats: While pre-existing immunity to AAV is typically a limiting factor for NHP experiments involving AAV gene therapy, this should not be an issue in the present project based on the relatively low AAV-9 seroprevalence in the WNPRC.

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	❍ Yes ● No
Is the Project Exempt from Federal regulations?	❍ Yes ❍ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Does the proposed research involve human specimens and/or data	❍ Yes ● No
Other Requested information	

VERTEBRATE ANIMALS

1. Description of procedures.

Because this project seeks to develop a nonhuman primate model to study the immunological effects of feminizing hormone therapy (FHT) in transgender women (TGW), only sexually mature (6-8 years of age) male rhesus macaques (RMs; *Macaca mulatta*) will be used in this project. The 12 RMs needed for this project will be purchased from and housed at the Wisconsin National Primate Research Center (WNPRC). Since the experiments described here do not require euthanasia, the animals will be leased for the duration of this project and then returned to the WNPRC colony once the study is completed. The main drug in FHT is the female sex hormone estradiol (E2), which TGW must take continuously to maintain their female sexual traits. FHT generally includes a testosterone-lowering agent as well, like the potassium sparing diuretic spironolactone, to allow for lower doses of estradiol to be used. To ensure consistent delivery of E2 and spironolactone across animals, these drugs will be formulated in custom-made biodegradable pellets (Innovative Research of America; see letter of support) that allow for sustained drug release over a 3-week period. Separate pellets for E2 and spironolactone will be produced for Group 1, while a placebo pellet will be ordered for Group 2. These pellets will be implanted subcutaneously over the scapula (i.e., the shoulder bone), with animals under anesthesia, via a superscapular incision that will be subsequently closed with permanent suture. Additional animal procedures will include blood collections by venipuncture; colorectal biopsies; and intramuscular injection of AAV-9 vectors.

2. Justifications.

There are no appropriate *in vitro* model systems to simulate the immunological consequences of exogenous hormone therapy. Although rodents have been used to study hormonal regulation of immune responses, these models tend to be artificial and rarely predict the outcome of clinical interventions. Additionally, female sex hormones in RMs, but in mice, fluctuate in the same range as those in humans. One example that is particularly relevant to this project is the fact that mice tend to mount low levels of anti-drug antibodies (ADAs) following AAV-mediated gene therapy with anti-HIV broadly reactive neutralizing antibodies (bnAbs). In contrast, AAV-expressed anti-HIV bnAbs and other monoclonal antibodies often trigger ADAs in monkeys and humans, even when there is complete species match between the antibody and the host. As a result, the impact of FHT on AAV-driven transgene expression and ADAs cannot be adequately modeled in rodents.

3. Minimization of pain and distress.

Both physical and chemical restraint methods will be used to minimize pain and distress. Whenever physical restraint is insufficient, general anesthesia (ketamine, 10-30 mg/kg IM; and Dexmedetomidine, 0.0075-0.015 mg/kg IM) is used and administered under the direction of a WNPRC veterinarian, to minimize discomfort during experimental procedures. The state of the anesthetized animals is constantly monitored (movement, respiratory rate, etc.), and additional ketamine or another anesthetic is given as needed. To minimize any discomfort associated with subcutaneous placement of hormone pellets, the side of the superscapular incision (left versus right) will be alternated after each 3-week interval. Importantly, no surgeries or procedures that that might lead to severe discomfort, distress, pain, or injury are planned for this study.

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Scripps Research

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May 7, 2020

National Institute of Health
9000 Rockville Pike
Bethesda, MD 20892

Dear Sir or Madam:

The appropriate programmatic and administrative personnel of The Scripps Research Institute and The Board of Regents of the University of Wisconsin System involved in the application of the grant titled, "A nonhuman primate model to study the immunological effects of feminizing hormone therapy in transgender women" are aware of the NIH consortium grant policy and are prepared to establish the necessary inter-institutional agreement consistent with the policy.

Sincerely,

Personal Info

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April 22, 2020

National Institutes of Health
9000 Rockville Pike
Bethesda, MD 20892

Re: Application Titled - A nonhuman primate model to study the immunological effects of feminizing hormone therapy in transgender women

Prime Institute: Scripps Florida

Consortium Institute: The Board of Regents of the University of Wisconsin System

Dear Sir or Madam:

The appropriate programmatic and administrative personnel of the Prime and Consortium Institutes indicated above, involved in the referenced application, are aware of the NIH consortium grant policy and are prepared to establish the necessary inter-institutional agreement consistent with the policy.

Sincerely,

Personal Info

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University of Wisconsin Madison
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RESOURCE SHARING PLAN

Model organisms: No model organisms will be generated as part of this project.

Genomic data: No genomic data will be generated as part of this project.

Final research data: Our research findings will be made available through publication and presentations at scientific meetings in the form of oral talks or posters. Upon reasonable request, we will make the raw data available to the scientific community, as well as any tables or graphs generated from the data. We will answer questions regarding the data from the scientific research community by e-mail or telephone.

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

Biological resources:

Cell lines: The TZM-bl cells needed for SIV neutralization assays and CEM.NKR-CCR5-sLTR-Luc target cells and KHYG-1 cells needed for ADCC assays will be authenticated by short tandem repeat fingerprinting services offered by various companies. All cells will also be tested regularly for *Mycoplasma* contamination. The cell line source will be indicated in any publication that results from the proposed research, as well as the exact culture conditions.

Hormone/placebo pellets: Feminizing hormone therapy will be modeled in male rhesus macaques by periodic implants of pellets containing estradiol, spironolactone, or placebo. These drugs will be purchased from Innovative Research of America, a reputable vendor that has provided similar pellets for many prior studies.

AAV vectors: The AAV vectors that will be used in this project will be produced at the Gene Therapy Center and Viral Vector Core at the University of Massachusetts Medical School, under the supervision of Redacted Redacted by agreement has extensive quality controls in place on the production and quantitation of rAAV preparations. Redacted by agreement uses several procedures, including electron micrograph, to verify that the purity of the prep is satisfactory and that the concentration of empty capsids is not too high.

Plasmids: The AAV expression cassettes (eCD4-Ig and TPST2) will be synthesized and expanded at Genscript. These constructs will be authenticated by restriction enzyme digestion and nucleic acid sequencing.