NIDA AVANT-GARDE AWARD
Department of Health and Human Services
National Institutes of Health

NATIONAL INSTITUTE ON DRUG ABUSE

Federal Award Date: 02/11/2016

Grant Number: 1DP1DA037979-01 REVISED
FAIN: DP1DA037979

Principal Investigator(s):
Nichole Rose Klatt, PHD

Project Title: Impact of Cannabis on Inflammation and Viral Persistence in Treated HIV/SIV

Arias, Lynette
Director, Office of Sponsored Programs
4333 Brooklyn Ave NE
Box 359472
SEATTLE WASHINGTON, WA 981959472

Award e-mailed to: osp@uw.edu

Period Of Performance:
Budget Period: 03/01/2015 – 01/31/2016
Project Period: 03/01/2015 – 01/31/2020

Dear Business Official:

The National Institutes of Health hereby revises this award (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to UNIVERSITY OF WASHINGTON 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 grantee when funds are drawn down or otherwise obtained 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 On Drug Abuse of the National Institutes of Health under Award Number DP1DA037979. 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 contact the individual(s) referenced in Section IV.

Sincerely yours,

Carol Alderson
Grants Management Officer
NATIONAL INSTITUTE ON DRUG ABUSE

Additional information follows

SECTION I – AWARD DATA – 1DP1DA037979-01 REVISED

Award Calculation (U.S. Dollars)

Federal Direct Costs	$500,000
Federal F&A Costs	$390,000
Approved Budget	$890,000
Total Amount of Federal Funds Obligated (Federal Share)	$890,000
TOTAL FEDERAL AWARD AMOUNT	$890,000

AMOUNT OF THIS ACTION (FEDERAL SHARE) $0

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$890,000	$890,000
2	$890,000	$890,000
3	$890,000	$890,000
4	$890,000	$890,000
5	$890,000	$890,000

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

Fiscal Information:

CFDA Name:	Drug Abuse and Addiction Research Programs
CFDA Number:	93.279
EIN:	1916001537A1
Document Number:	DDA037979A
PMS Account Type:	P (Subaccount)
Fiscal Year:	2015

IC	CAN	2015	2016	2017	2018	2019
DA	8472630	$890,000	$890,000	$890,000	$890,000	$890,000

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

NIH Administrative Data:
PCC: MC/JHK / **OC**: 414A / **Released**: eRA Commons User Name 02/10/2016
Award Processed: 02/11/2016 12:02:26 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1DP1DA037979-01 REVISED

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 – TERMS AND CONDITIONS – 1DP1DA037979-01 REVISED

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).

This institution is a signatory to the Federal Demonstration Partnership (FDP) Phase VI Agreement which requires active institutional participation in new or ongoing FDP demonstrations and pilots.

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) DP1DA037979. 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/.

Treatment of Program Income:
Additional Costs

SECTION IV – DA Special Terms and Conditions – 1DP1DA037979-01 REVISED

REVISION This award is revised for two reasons:

1. This award is revised to acknowledge receipt and acceptance of the IACUC approval date for this project, and remove the restriction which prohibited the drawdown and obligation of funds for research involving live vertebrate animals. This revision supersedes Notice of Award (NoA) issued 02/20/2015.
2. This revised award reflects NIDA's acceptance of the certification of Institutional Review Board (IRB) approval for the grantee organization and releases the restriction on the Notice Grant Award issued on 2/20/2015. Accordingly, the special condition prohibiting

research involving human subjects is removed, effective as of the date of IRB approval. See the NIH Grants Policy Statement, Part II.4.1.15 Human Subjects Protections http://grants.nih.gov/grants/policy/nihgps/nihgps.pdf for specific requirements related to the protection of human subjects, which are applicable to this term and condition of award.

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REVISION: This Notice of Award (NoA) is revised to correct the F&A rate. This NoA supercedes the NoA issued on 02/03/2015.

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Recycling Start Date This award is funded at the recommended level adjusted to an 11-month budget period. Future year anniversary dates for this grant will be February 1 and the Research Performance Progress Report (RPPR) is due on December 15.

NIDA Avant-Garde Award Program for HIV/AIDS and Drug Use Research The principal investigator is required to commit the major portion (at least 35% or 4.2 person months) of their research effort to activities supported by the Avant-Garde Award program.

Under governing regulations, grant funds shall not be expended for research projects or activity involving human subjects or animals without the approval of the Institutional Review Board (IRB) or Institutional Animal Care and Use Committee (IACUC) in accordance with the approved Assurance on file with the Office of Human Research Protection (OHRP) or the Office of Laboratory Animal Welfare (OLAW), respectively. IT IS THE INSTITUTION'S RESPONSIBILITY TO ENSURE THAT ALL RESEARCH INVOLVING HUMAN SUBJECTS OR ANIMAL SUBJECTS HAVE IRB OR IACUC APPROVAL, OR NOTICE OF EXEMPTION, IN ACCORDANCE WITH THE APPROVED ASSURANCE ON FILE WITH OHRP OR OLAW.

In addition, all research that involve human subjects and meet the definition of an NIH Clinical Trial, must obtain PO approval of a co-signed Data and Safety Monitoring (DSM) plan for any new clinical trial that is to be conducted under this grant (e.g. treatment or intervention research in humans including feasibility trials or patient focus groups.).

No funds may be provided for projects involving foreign sites unless specifically indicated on a revised Notice of Award. This includes pilot studies that have foreign involvement. If pilot project core studies involve international studies, prior approval must be received by NIDA Program and Grants Management Staff.

NIDA AWARD TERMS All grantees must acknowledge funding received from the National Institute on Drug Abuse at the National Institutes of Health when issuing statements, press releases, requests for proposals, bid solicitations, and other documents describing projects or programs funded in whole or in part with NIDA money. (NIH Grants Policy Statement, October 2013, http://grants.nih.gov/grants/policy/nihgps_2013/nihgps_ch8.htm#_Toc271264948).

In conjunction with this requirement, in order to most effectively disseminate research results, advance notice should be given to NIDA that research finds are about to be published so that we may coordinate accurate and timely release to the media. This information will be embargoed until the publication date. Any press notification should be coordinated with the NIDA Press Officer who can be reached at (301) 443-6245.

NIDA has an interest in supporting HIV/AIDS and infectious disease research. The purpose of this support is to develop effective prevention, treatment, and service strategies for drug abusing youth and adults. To that end, awardees are encouraged to make every effort to incorporate scientific questions related to HIV/AIDS and other infectious diseases into research protocols. Principal Investigators will be required to provide information related to the development of research in this area in annual progress reports to allow NIDA to assess progress regarding HIV/AIDS research.

The National Institute on Drug Abuse (NIDA) encourages data harmonization to increase comparability, collaboration, and scientific yield of research on drug abuse. Towards that end, NIDA strongly encourages human-subject studies to incorporate a series of measures from the

Substance Abuse and Addiction Core and Specialty collections, which are available in the PhenX Toolkit (www.phenxtoolkit.org). For more information about NIDA's data harmonization efforts, please see NOT-DA-12-008 at http://grants.nih.gov/grants/guide/notice-files/NOT-DA-12-008.html.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Carol Alderson
Email: aldersoc@nida.nih.gov **Phone**: 301-933-6196 **Fax**: (301) 594-6849

Program Official: Jagjitsingh H. Khalsa
Email: jk98p@nih.gov **Phone**: (301) 443-2159

SPREADSHEET SUMMARY
GRANT NUMBER: 1DP1DA037979-01 REVISED

INSTITUTION: UNIVERSITY OF WASHINGTON

Budget	Year 1	Year 2	Year 3	Year 4	Year 5
TOTAL FEDERAL DC	$500,000	$500,000	$500,000	$500,000	$500,000
TOTAL FEDERAL F&A	$390,000	$390,000	$390,000	$390,000	$390,000
TOTAL COST	$890,000	$890,000	$890,000	$890,000	$890,000

Facilities and Administrative Costs	Year 1	Year 2	Year 3	Year 4	Year 5
F&A Cost Rate 1	78%	78%	78%	78%	78%
F&A Cost Base 1	$500,000	$500,000	$500,000	$500,000	$500,000
F&A Costs 1	$390,000	$390,000	$390,000	$390,000	$390,000

PI: **Klatt, Nichole Rose**	Title: Impact of Cannabis on Inflammation and Viral Persistence in Treated HIV/SIV	
Received: 11/04/2013	FOA: DA14-008	Council: 05/2014
Competition ID: FORMS-C	FOA Title: FY14 NIDA AVANT-GARDE AWARD PROGRAM FOR HIV/AIDS RESEARCH (DP1)	
1 DP1 DA037979-01	Dual:	Accession Number: 3635697
IPF: 9087701	Organization: UNIVERSITY OF WASHINGTON	
Former Number:	Department: Pharmaceutics	
IRG/SRG: ZDA1 SXC-E (04)	AIDS: Y	Expedited: Y
Subtotal Direct Costs (excludes consortium F&A) Year 1: 500,000 Year 2: 500,000 Year 3: 500,000 Year 4: 500,000 Year 5: 500,000	Animals: Y Humans: Y Clinical Trial: N Current HS Code: Evaluative Info HESC: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
Nichole Klatt	University of Washington	PD/PI

Reference Letters

Redacted by agreement

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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

5. APPLICANT INFORMATION

Organizational DUNS*: 605799469

Legal Name*: University of Washington
Department: Office of Sponsored Programs
Division:
Street1*: 4333 Brooklyn Ave NE
Street2: Box 359472
City*: Seattle
County:
State*: WA: Washington
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 98195-9472

Person to be contacted on matters involving this application

Prefix: First Name*: Lynette Middle Name: Last Name*: Arias Suffix:
Position/Title: Director, Office of Sponsored Programs
Street1*: 4333 Brooklyn Ave NE
Street2: Box 359472
City*: Seattle
County:
State*: WA: Washington
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 98195-9472
Phone Number*: 206-543-4043 Fax Number: 206-685-1732 Email: fjrh@uw.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 91-6001537

7. TYPE OF APPLICANT* H: Public/State Controlled 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
93.279
TITLE: Drug Abuse and Addiction Research Programs

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Impact of Cannabis on Inflammation and Viral Persistence in Treated HIV/SIV

12. PROPOSED PROJECT

Start Date* 07/01/2014
Ending Date* 06/30/2019

13. CONGRESSIONAL DISTRICTS OF APPLICANT
WA-007

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Nichole Middle Name: R. Last Name*: Klatt Suffix:

Position/Title: Assistant Professor
Organization Name*: University of Washington
Department: Pharmaceutics
Division:
Street1*: 3000 Western Ave.
Street2: Box 357331
City*: Seattle
County:
State*: WA: Washington
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 98105-7331
Phone Number*: 206-221-0254 Fax Number: 206-543-1589 Email*: klattnr@uw.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$2,500,000.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$2,500,000.00
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*: Lynette Middle Name: Last Name*: Arias Suffix:

Position/Title*: Director, Office of Sponsored Programs
Organization Name*: University of Washington
Department: Office of Sponsored Programs
Division:
Street1*: 4333 Brooklyn Ave NE
Street2: Box 359472
City*: Seattle
County:
State*: WA: Washington
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 98195-9472
Phone Number*: 206-543-4043 Fax Number: 206-685-1732 Email*: fjrh@uw.edu

Signature of Authorized Representative*
Jane Heffernan

Date Signed*
11/04/2013

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:1240-AvGCoverLetter.pdf

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

OMB Number: 4040-0010
Expiration Date: 06/30/2016

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:	University of Washington
Duns Number:	6057994690000
Street1*:	3000 Western Ave.
Street2:	Box 357331
City*:	Seattle
County:	
State*:	WA: Washington
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	98105-7331
Project/Performance Site Congressional District*:	WA-007

Additional Location(s)

File Name

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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

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

IRB Approval Date:

Human Subject Assurance Number 00006878

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 A3464-01

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*	1236-ProjSummary_F.pdf
8. Project Narrative*	1237-ProjNarrative_F.pdf
9. Bibliography & References Cited	
10.Facilities & Other Resources	1238-Facilities_F.pdf
11.Equipment	
12. Other Attachments	1239-Other_accomplishment.pdf

With 35 million HIV-infected individuals worldwide, the challenge to improve health in these individuals is vast. Antiretroviral therapy (ART) suppresses HIV replication, which prevents AIDS and reduces overall mortality, however ART does not fully restore health. Indeed, despite sustained suppression of viremia, individuals cannot discontinue ART as residual HIV persists, and virus rebound is inevitable if ART is discontinued. This residual HIV reservoir is associated with ongoing inflammation. Cannabis is a widely used drug in the United States, and derivatives of cannabis such as cannabinoids are commonly used in treatment of nausea and cachexia in severe conditions such as cancer. Several studies have demonstrated that cannabinoids have the propensity to alter immune responses and decrease inflammation in vivo. We hypothesize that cannabis use in the context of ART-treated HIV infection may decrease inflammation and the persistent HIV reservoir. Here, we provocatively propose to test this hypothesis in humans by measuring inflammation, immunity, and the HIV reservoir from blood and gastrointestinal (GI) tissues from HIV-infected individuals who report using cannabis daily compared to those reporting no drug use. Furthermore, we will assess mechanisms of cannabinoid anti-inflammatory activity ex-vivo using cannabinoid receptor agonists in co-cultures. In addition, we will exploit the non-human primate model of SIV infection to test causality of this unconventional idea, by treating ART-treated, SIV infected macaques with cannabinoids to assess the effects on SIV reservoir and inflammation. With an outstanding team of researchers, we will assess the following: (i.) Global systems biology, including species-specific transcriptional analysis and bioinformatics; (ii.) The HIV and SIV reservoir using novel assays to measure integrated, total and inducible virus; (iii.) Inflammation and immunophenotype of immune cell subsets; (iv.) Systemic microbial translocation and GI tract barrier integrity; and (v.) drug levels and kinetics in blood and GI tract. We believe these proposed studies will be integral to better understanding facets associated with the HIV reservoir and may provide a novel therapeutic approach, exploiting a drug of abuse, towards development of an HIV cure.

There is no cure for HIV, thus despite suppression of HIV replication with antiretroviral therapy, residual HIV reservoir and inflammation still persist, leading to increased morbidity and mortality. We hypothesize that the anti-inflammatory properties of cannabis may decrease inflammation and thus reduce the latent HIV reservoir. Thus, here we propose an avant-garde translational approach to studies of HIV-infected humans who use cannabis, ex vivo mechanistic studies, and cannabinoid treatment in non-human primates.

FACILITIES & RESOURCES

Laboratories: The Primate Center has assigned to Dr. Klatt two laboratories at the Redacted by agreement one Redacted by agreement with additional access to a shared spaces and equipment already in place.

Biosafety Level Redacted by agreement and is a biological containment facility designed for the safe manipulation of human and simian immunodeficiency viruses (HIV and SIV), simian retroviruses (SRV). Access to the facility is restricted through a locked anteroom door and limited to trained personnel and escorted visitors (e.g., maintenance workers). This suite is maintained constantly under negative pressure and is monitored by an air pressure differential sensing alarm system. All exhaust air from the suite is HEPA filtered, and the ventilation system is monitored regularly by Environmental Health and Safety personnel in accordance with federal and state guidelines.
-The Redacted by agreement includes one anteroom (12) and three laboratories (11, 15 and 17):
Redacted by agreement room connects a hallway with the Redacted by agreement equipped with: Primus autoclave, shelves and cabinets with supplies and PPE, eye wash sink, shower, First Aid kit, Herpes B exposure kits, emergency instructions.
Redacted by agreement room is equipped with four four-foot-wide biosafety cabinets (BSC), six CO_2 incubators, sink; two benchtop centrifuges, Beckman; ; one luminescence counter Victor light; one shaker MaxMini 4450, one dissecting microscope; one fluorescent microscope; one UV irradiator; two microcentrifuges; one -80C freezer ThermoForma, one -20 C refrigerator/freezer; two waterbaths; inverted microscope; one networked computer; vortex; mixers;heating plate; balance and shelves and cabinets for storage of supplies.

Biosafety Level Redacted by agreement room is equipped with one 4-foot-wide BSC, one CO_2 incubator, two micro-centrifuges, one liquid nitrogen freezer, one -20C freezer, and one refrigerator 4C. ; one 17-color flow cytometer (Becton Dickinson LSRII); one ELISA reader w/printers and dedicated computers; one ELISA washer , two waterbaths; inverted microscope; vortex; one quantitative RT-PCR machine (Biorad); one benchtop centrifuge; balance;

Core Services: The Western laboratory occupies Redacted by agreement and is organized accordingly into four (4) major functional areas: tissue culture labs, general molecular biology labs, support/equipment rooms and office area. Dr. Klatt's laboratory space consists of Redacted by agreement for sample processing, and Redacted by agreement for PCR and Redacted by plot assays. In addition, several core services are available at Redacted by including cell sorting on a FACS Aria in Redacted by agreement this BD cell sorter is equipped with 4 lasers and can sort up to 4 cell populations at once. FACS analysis is also available on a FACS LSRII in Redacted by agreement also equipped with 4 lasers (up to 17 parameters0. In addition, 2 conference rooms are available for meetings. She has full access to shared administrative areas including meeting spaces at the Redacted by agreement facility. Tissue and pathology services (ACVP board certified Veterinary Pathologists), full histology services (including immunohistochemistry and frozen sectioning), and protocol development consultation are available.

Animal: The WaNPRC is fully equipped to acquire, house, and care for all experimental animals. Housing and care conditions for all WaNPRC facilities meet AAALAC accreditation standards for nonhuman primates and follow NIH guidelines for ABSL-2 containment facilities. ABSL-2 facilities located in the Redacted by agreement include Redacted by agreement indoor animal housing and Redacted by agreement animal quarter services. Animal support facilities include treatment rooms, clinical and microbiology laboratories, a quarantine service area, animal-holding areas, and surgical suites. Procedures utilized in all WaNPRC facilities are approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Washington and conform with recommendations in the NIH publication, *Guide for the Care and Use of Laboratory Animals* and biosafety standards for work with immunodeficiency viruses (including SIV, HIV-1, HIV-2, SHIV) as outlined by NIH, the CDC, and the UW Biosafety Committee. The WaNPRC has an extremely successful organizational structure in place to support implementation of research protocols at the facilities.

MOST SIGNIFICANT RESEARCH ACCOMPLISHMENT

One of my most significant research accomplishment is a study we published in 2013 in the *Journal of Clinical Investigation* entitled “Probiotic/prebiotic supplementation of antiretrovirals improves gastrointestinal immunity in SIV-infected macaques” (J Clin Invest. 2013 Feb 1;123(2):903-7). This study was a culmination of several years of studies and publications regarding mucosal immune dysfunction during HIV/SIV infection. Only recently it has become clear that HIV infection is truly a mucosal disease, with significant insults occurring at the level of the gastrointestinal (GI) tract, including breaches to the epithelial barrier, microbial translocation, and ensuing immune activation, all events being tightly associated with mortality during HIV infection, and establishment of the HIV reservoir. I spent several years studying mucosal immune dysfunction and damage to the barrier of the GI tract, and identified several mechanisms underlying lentiviral-induced GI dysfunction. These studies provided the basis for my passion for developing novel therapeutic interventions aimed at enhancing mucosal immunity and decreasing inflammation during HIV infection. For the study above, we investigated the benefits of synbiotic probiotic and prebiotic supplementation of antiretroviral therapy (ART) in SIV-infected Asian macaques. The role of the microbiome in gastrointestinal health and immunity has recently become a prevailing interest in mucosal immunology. Thus, we hypothesized that altering the microbiome using probiotics, which have a positive effect on mucosal health, would enhance GI immunity and decrease mucosal-associated inflammation. I led this intensive effort in which we treated SIV-infected macaques with suppressive ART, and one group received probiotics/prebiotics, and the other group was treated with ART alone. I was trained to approach science collaboratively, and I strongly believe in the increased success, innovation, and productivity that collaborative science generates. Indeed, with our collaboration here, we were able to collect extensive data from these studies including immunophenotyping, functionality and cell sorting by flow cytometry, transcriptional analysis via microarray, microbiome analysis via 454 sequencing, cell associated virus and residual SIV in tissues by qRT-PCR, histology analysis by immunohistochemistry, and ART tissue concentrations by mass spectrometry, all performed, analyzed, and sent for publication in less than two years. Overall, we found that this synbiotic probiotic/prebiotic treatment resulted in increased frequency and functionality of GI tract antigen presenting cells, enhanced reconstitution and functionality of $CD4^+$ T cells, and reduced fibrosis of lymphoid follicles in the colon, thus mitigating inflammatory sequelae and improving GI health. These studies suggest that probiotic/prebiotic treatment may be a useful approach to supplement ART therapy in HIV-infected individuals to mitigate residual GI inflammation and damage, thereby potentially beneficially impacting morbidity and mortality.

With the growing interest and optimism in HIV cure research, I am now translating my interventional studies to incorporate HIV/SIV reservoir eradication approaches. Indeed, while ameliorating mucosal immunity is an important step towards improved health in HIV-infected individuals, without targeting the HIV reservoir, individuals must still remain on ART permanently. Thus, I consider my mucosal intervention study I described above to be my most significant accomplishment thus far, as it has laid the groundwork for future studies in translational research focusing on decreasing mucosal and systemic inflammation, with the ultimate objective of eradicating the HIV reservoir.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Nichole Middle Name R. Last Name*: Klatt Suffix:

Position/Title*: Assistant Professor
Organization Name*: University of Washington
Department: Pharmaceutics
Division:
Street1*: 3000 Western Ave.
Street2: Box 357331
City*: Seattle
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Credential, e.g., agency login: eRA Commons User Name

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

Degree Type: PhD Degree Year: 2008

	File Name
Attach Biographical Sketch*:	1234-Updated_BioKlatt_2pg.pdf
Attach Current & Pending Support:	1235-OtherSupport_F.pdf

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Nichole R. Klatt, Ph.D.	Assistant Professor
eRA COMMONS USER NAME (credential, e.g., agency login) eRA Commons User Name	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Minnesota, Duluth, MN	B.S.	05/04	Biology, Chemistry
Emory University, Atlanta, GA, USA.	PhD	12/08	Immunology & Molecular Pathogenesis
University of Pennsylvania, Philadelphia, PA, USA.	Visiting PhD Student	06/06-12/08	Visiting PhD Molecular & Cellular Biology

B. Positions and Employment

01/2009-08/2010 IRTA Postdoctoral Fellow, Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD.
08/2010-10/2012 FTE Research Fellow, Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD.
11/1/12 – present Assistant Professor, Department of Pharmaceutics, Core Staff Scientist, Washington National Primate Research Center, University of Washington, Seattle, WA.

Honors

10/2013 Scientific Advisory Board, 30th Annual Symposium on NHP Models for AIDS, Atlanta, GA
05/2013 Early Career Faculty Travel Award, AAI 2013, Honolulu, HI
04/2012 NIAID/NIH Excellence in Service Award
03/2012 Young Investigator Award, CROI, Seattle, WA
10/2011 Young Investigator Award, 29th Annual Symposium on NHP Models for AIDS, Seattle, WA
01/2011 NIAID/NIH Excellence in Service Award
10/2010 Young Investigator Award, 28th Annual Symposium on NHP Models for AIDS, New Orleans, LA
09/2010 New Investigator Award, AIDS Vaccine 2010, Atlanta, GA

Ad Hoc Reviewer

AIDS Research and Human Retroviruses (Editorial Board), Journal of Virology, Journal of Infectious Diseases, Clinical and Experimental Immunology, PLoS ONE, Journal of Immunological Methods, Retrovirology, BMC Microbiology, BMC Infectious Diseases, Journal of Visualized Experiments, Faculty of 1000

C. Selected Peer-reviewed Publications (15 out of 35):

1. **Klatt NR,** Villinger F, Bostik P, Gordon SN, Pereira L, Engram JC, Mayne A, Dunham RM, Lawson B, Ratcliffe SJ, Sodora DL, Else J, Reimann K, Staprans SI, Haase AT, Estes JD, Silvestri G, Ansari AA. 2008 Availability of activated CD4+ T cells dictates the level of viremia in naturally SIV-infected sooty mangabeys. *J Clin Invest*. 118:2039-2049. PMCID: PMC2391063.
2. **Klatt NR,** Shudo E, Ortiz AM, Engram JC, Paiardini M, Lawson B, Miller MD, Else J, Pandrea I, Estes JD, Apetrei C, Schmitz JE, Ribeiro RM, Perelson AS, Silvestri G. 2010 CD8+ lymphocytes control viral replication in SIVmac239-infected rhesus macaques without decreasing the lifespan of productively infected cells. *PLoS Pathog.* Jan 29; 6(1):e1000747. PMCID: PMC2813271
3. **Klatt NR,** Brenchley JM. 2010 Th17 cell dynamics in HIV infection. *Curr Opin HIV AIDS.* Mar;5(2):135-40.PMCID: PMC2886291.
4. **Klatt NR,** Harris LD, Vinton CL, Sung H, Briant JA, Tabb B, Morcock D, McGinty JW, Lifson JD, Lafont BA, Martin MA, Levine AD, Estes JD, Brenchley JM. 2010 Compromised gastrointestinal integrity in pigtail macaques is associated with increased microbial translocation, immune activation, and IL-17 production in

the absence of SIV infection. *Mucosal Immunol.* Jul;3(4):387-98. PMCID: PMC2891168.

5. Estes JD, Harris LD, **Klatt NR,** Tabb B, Pittaluga S, Paiardini M, Barclay GR, Smedley J, Pung R, Oliveira KM, Hirsch VM, Silvestri G, Douek DC, Miller CJ, Haase AT, Lifson J, Brenchley JM. 2010 Damaged Intestinal Epithelial Integrity Linked to Microbial Translocation in Pathogenic Simian Immunodeficiency Virus Infections. *PLoS Pathog.* Aug 19; 6(8):e1001052. PMCID: PMC2924359.
6. Ortiz AM, **Klatt NR**, Carnathan P, Li B, Derdeyn C, Lawson B, Ryzhova E, Gonzalez-Scarano F, Paiardini M, Ratcliffe S, Else J, Brenchley JM, Silvestri G. 2011 Depletion of CD4+ T-cells abrogates post-peak decline of viremia in SIV-infected rhesus macaques. *J Clin Invest.* Nov 1;121(11):4433-45. PMCID: PMC3204830.
7. **Klatt NR**, Vinton CL, Lynch RM, Ho J, Darrah P, Estes JD, Seder RA, Moir SL, Brenchley JM. 2011 SIV infection of rhesus macaques results in dysfunctional T and B cell responses to neo and recall *Leishmania* vaccination. *Blood.* Nov 24;118(22):5803-12.PMCID: PMC3228497
8. **Klatt NR,** Canary LA, Vanderford TH, Vinton CL, Engram JC, Dunham RM, Swerczek J, Cronise-Santis H, Lafont B, Brenchley JM. 2012 Dynamics of SIVmac239 infection in pigtail macaques. *J Virol.* Jan;86(2):1203-13. PMCID: PMC3255820
9. **Klatt NR** and Silvestri G. 2012 CD4+ T Cells and HIV: A Paradoxical Pas de Deux. *Sci Transl Med.* Feb; 123(4): 123-4. PMID: 22378922
10. **Klatt NR**, Funderburg NT, Brenchley JM. Microbial translocation, immune activation, and HIV disease.*Trends Microbiol*. 2012 Oct 11. [Epub ahead of print] PMCID: PMC3534808
11. Bosinger SE, Jochems SP, Folkner KA, Hayes TL, **Klatt NR**, Silvestri G. 2013 Transcriptional profiling of experimental CD8+ lymphocyte depletion in rhesus macaques infected with SIVmac239. ***J Virol.*** *Jan; 87(1):433-43* Epub 2012 Oct 24. PMCID: PMC2786806
12. **Klatt NR,** Estes JD, Sun X, Harris LD, Ortiz AM, Cervasi B, Yokomizo LK, Vinton CL, Tabb B, Canary LA, Dang Q, Hirsch VM, Lifson JD, Alter G, Belkaid Y, Silvestri G, Milner JD, Paiardini M, Haddad EK, Brenchley JM. 2012 Loss of mucosal IL-17+ and IL-22+ lymphocytes after SIV infection is associated with loss of CD103+ DCs and damage to the mucosal barrier. *Mucosal Immunol.* Nov;5(6):646-57. PMCID: PMC3443541
13. **Klatt NR,** Canary LC, Sun X, Vinton CL, Funderburg NT, Deming C, Quinones M, Perkins M, Hazuda DJ, Miller MD, Lederman MM, Segre JA, Lifson JD, Haddad EK, Estes JD, Brenchley JM. Probiotic and prebiotic supplementation of antiretroviral therapy improves gastrointestinal immunity in SIV-infected macaques. *J Clin Invest.* 2013 Jan 16. doi:pii: 66227. PMCID: PMC3561826
14. Canary LA, Vinton CL, Morcock DR, Pierce J, Estes JD, Brenchley JM, **Klatt NR**. Rate of AIDS progression is associated with pre-existing microbial translocation in pigtail macaques. *Journal of Immunology.* J Immunol. 2013 Mar 15;190(6):2959-65. doi: 10.4049/jimmunol. PMCID: PMC3665608
15. **Klatt, N.R.,** Chomont, N., Douek, D.C., Deeks, S.G. Immune activation and persistence: Implications for curative approaches to HIV infection. Immunol Rev. 2013 Jul;254(1):326-42. PMID: 23886064

SELECT SEMINARS/PRESENTATIONS:

1. **Klatt, N.R.** Mucosal Immune Dysfunction during SIV Infection of Non-Human Primates. *Seattle BioMed, Program in Pathobiology, Seattle, WA, September 2013.*
2. **Klatt, N.R.** The Role of Microbial Communities and Mucosal Immunity in SIV Disease Progression. *Invited Symposium, International AIDS Society, Kuala Lumpur, Malaysia, May 2013.*
3. **Klatt, N.R.** Animal models: What can our furry friends teach us? *Rapporteur, AIDS Vaccine 2012. Boston, MA. September 2012***.**
4. **Klatt, N.R.** Therapeutic interventions aimed at improving mucosal immunity during SIV infection of pigtail macaques. *Department of Pharmaceutics, University of Washington. June 2012.*
5. **Klatt, N.R.** Probiotic treatment improves gastrointestinal immunity in SIV-infected macaques.*Department of Pediatrics, Emory University School of Medicine. June 2012.*
6. **Klatt, N.R.,** Canary, L.C., Sun, X., Vinton, C.L., Perkins, M., Hazuda, D.J., Lifson, J.D., Haddad, E.K., Estes, J.D., Brenchley, J.M. Probiotic supplementation of ARV treatment during SIV infection of pigtail macaques results in enhanced GI tract CD4+ T cell frequency and immunological function**.** *International Microbicides Conference, Sydney, Australia. April 2012.* Oral presentation.
7. **Klatt, N.R.,** Canary, L.C., Sun, X., Vinton, C.L., Perkins, M., Hazuda, D.J., Lifson, J.D., Haddad, E.K., Estes, J.D., Brenchley, J.M. Probiotic supplementation of ARV treatment during SIV infection of pigtail macaques results in enhanced GI tract CD4+ T cell frequency and immunological function**.** *19th Conference on Retroviruses and Opportunistic Infections, Seattle, WA. March 2012.* Oral presentation.

OTHER SUPPORT

KLATT, N.

CURRENT

1R21AI100782-01A1 (Sodora, D) 2/1/13 – 1/31/15 EFFORT
NIAID/SBRI $198,936 ADC
Inhibition of liver macrophage activation in SIV infected Macaques
Sub-project title: Modification of Liver Innate Immune cells in SIV infected pigtail macaques
Both HIV in humans and SIV in monkeys induce a general activation of the immune system and are associated with faster disease progression and a lack of an effective immune response. Our hypothesis is that Kupffer cells in the liver are the engine of this general immune activation. Therefore, we will test whether suppression of these cells reduces the systemic immune activation in SIV-infected monkeys and identify the potential for this approach to be utilized as a treatment for HIV-infected humans.

1K22AI098440-01 (Klatt, N) 2/4/13 – 1/31/15 EFFORT
NIH $150,000 ADC
Mucosal immune dysfunction after SIV infection
During HIV infection, there is massive dysfunction of the mucosal immune system that is associated with progression to AIDS, and the proposed studies are to determine the kinetics and mechanisms of this dysfunction after SIV infection. These studies will be essential to understand better how damage occurs in mucosal tissues during SIV infection in order to develop more effective therapeutic approaches aimed at treating HIV and AIDS.

PENDING

Pending Support

Pending Support

Pending Support

OVERLAP

Currently, there is no overlap.

If chosen to receive an award for this application, the applicant will commit a minimum of EFFORT **person months of her research effort to the project supported by the** Private Source Private Source

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:
First Name*: Nichole
Middle Name: R.
Last Name*: Klatt
Suffix:

2. Human Subjects

Clinical Trial? ● No ○ Yes
Agency-Defined Phase III Clinical Trial?* ○ No ○ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

● Yes ○ No

4. Program Income*

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

5. Human Embryonic Stem Cells

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

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, please check the box indicating that one from the registry will be used:

Cell Line(s): Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ○ Yes ○ No

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

Previously Reported*: ○ Yes ○ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Research Plan

Please attach applicable sections of the research plan, below.

OMB Number: 0925-0001

1. Introduction to Application (for RESUBMISSION or REVISION only)	
2. Specific Aims	
3. Research Strategy*	1241-ResearchPlan.pdf
4. Progress Report Publication List	
Human Subjects Sections	
5. Protection of Human Subjects	1242-ProtectionHS.pdf
6. Inclusion of Women and Minorities	1243-WomenMinor.pdf
7. Inclusion of Children	1244-Children.pdf
Other Research Plan Sections	
8. Vertebrate Animals	1245-VertAnimals_F.pdf
9. Select Agent Research	
10. Multiple PD/PI Leadership Plan	
11. Consortium/Contractual Arrangements	
12. Letters of Support	1246-LOS_NK_AvG.pdf
13. Resource Sharing Plan(s)	
Appendix (if applicable)	
14. Appendix	

IMPACT OF CANNABIS ON INFLAMMATION AND VIRAL PERSISTENCE IN TREATED HIV/SIV

PROJECT DESCRIPTION

Scientific Problem: With 35 million HIV-infected individuals worldwide, containment and eventual eradication of the AIDS pandemic remains a top priority in contemporary biomedical research. While antiretroviral therapy (ART) can improve health during HIV infection, a cure is not yet available, and despite suppression of viremia with ART, these individuals still have increased morbidity and mortality compared to uninfected individuals[1-3]. Indeed, HIV-infected subjects cannot discontinue ART, because residual HIV persists, and virus rebound is inevitable if ART is terminated[4]. The HIV reservoir is complex, and at least two mechanisms drive this latent pool of infected cells, including residual low levels of virus replication in anatomical sanctuaries (such as the gastrointestinal tract), and persistent proviral HIV DNA that is integrated into the host genome in long-lived cellular reservoirs[5,6]. Furthermore, HIV is closely associated with, and potentially driven by, immune activation and inflammation during HIV infection[4,6].

The pathology of disease caused by HIV infection is complex and multifaceted. In addition to high levels of systemic viral replication, HIV infection results in a vicious cycle of mucosal damage, chronic inflammation and overall immunological dysfunction, which are closely associated with disease (**Fig. 1A**)[4,7,8]. This chronic immune activation is strongly associated with gastrointestinal (GI) mucosal damage and microbial translocation, which do not resolve completely with ART (**Fig. 1B**)[1,2,4]. While there is a clear positive correlation between measures of immune activation and HIV persistence in ART-suppressed individuals[9,10], whether immune activation is a cause, a consequence or both a cause and a consequence of HIV persistence is unknown. Here, we propose a provocative approach to directly evaluate whether decreasing inflammation during ART-suppressed lentiviral infection results in a decreased HIV reservoir, using an extremely novel therapeutic concept.

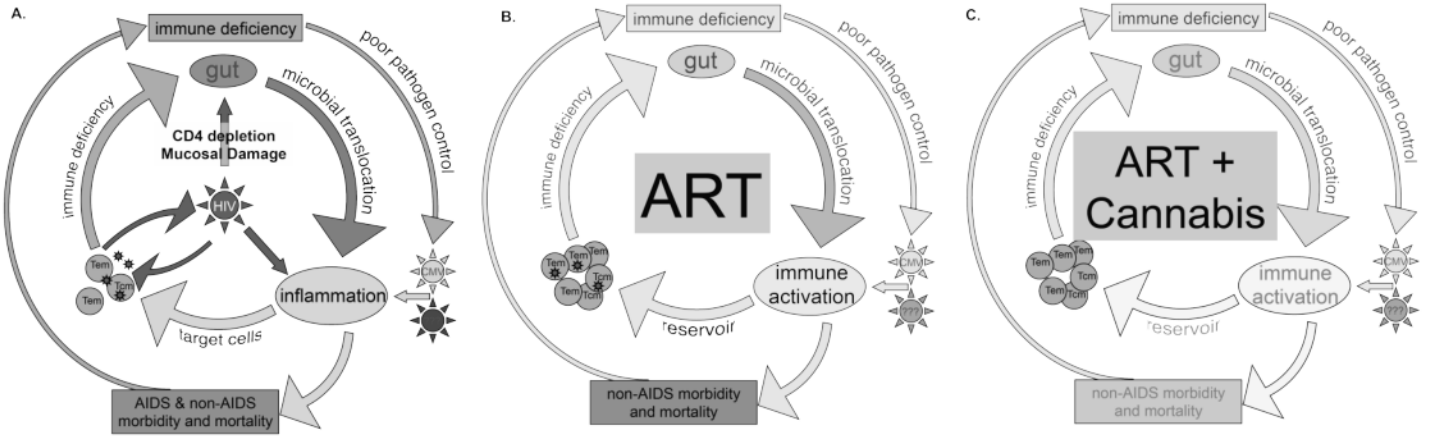

Figure 1. Hypothetical model of ART + cannabis treatment (A.) HIV initiates and sustains a vicious cycle of mucosal dysfunction and inflammation. (B.) ART suppresses HIV and slightly ameliorates microbial translocation and immune activation. (C.) ART + Cannabis decreases immune activation and the HIV reservoir, preserving health. (*Adapted from Klatt, N. et al., Immunological Reviews 2013*)

Cannabis is a widely used drug in the United States, and derivatives of cannabis such as cannabinoids are commonly used in treatment of nausea and cachexia in severe conditions such as cancer, pain and multiple sclerosis[11-13]. Several studies have demonstrated that cannabinoids have the propensity to alter immune responses and decrease inflammation. Indeed, treating immune cells with either Δ^9 tetrahydrocannabinol (Δ(9)-THC) or cannabidiol (CBD) decreases production of IL-8, MIP-1α, MIP-1β, RANTES, TNF, and IFN[11]. Importantly, these cytokines and chemokines are all associated with inflammation and chronic immune activation in HIV-infected individuals[4]. In addition, treatment of SIV-infected macaques with THC ameliorates SIV-induced AIDS progression[14]. Given that cannabis and/or cannabinoid use can decrease inflammatory factors and enhance gastrointestinal health, we hypothesize that HIV-infected individuals who use cannabis have decreased inflammation and biomarkers associated with mortality, resulting in a decreased HIV reservoir (**Fig. 1C**). While this cutting-edge hypothesis may be contrary to general belief that drugs of abuse are counter-indicated for health, we believe that the preliminary evidence that supports decreased inflammation in cannabis users or cannabinoid-treated non-human primates (NHPs) supports this innovative approach.

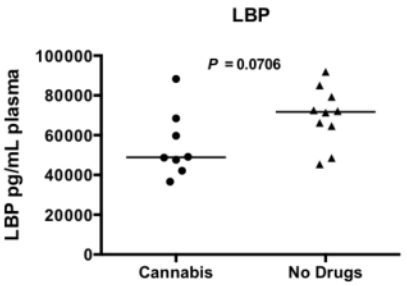

Figure 2. Microbial translocation is decreased in HIV-infected cannabis users

Preliminary Data: The basis for these studies comes from preliminary, provocative data that insinuates the potential ability to exploit the anti-inflammatory effects of cannabis in reduction of inflammation and the HIV reservoir in the context of ART-treated HIV/SIV infection. As discussed above, microbial translocation is tightly associated with inflammation, morbidity and mortality in HIV-infected individuals[4]. Given the known beneficial effects of cannabis on the GI tract, we therefore measured microbial translocation by expression of lipopolysachharide binding protein (LBP) from plasma of ART-treated (fully HIV suppressed), HIV-infected individuals who reported daily cannabis use, vs. individuals who reported no use of drugs of abuse. In our small group of subjects, we found a trend towards decreased microbial translocation (**Fig. 2**). In addition, transcriptional analysis of HIV-infected cocaine users demonstrated that individuals who used cannabis in addition to cocaine clustered separately. Furthermore, individuals who used cannabis in addition to cocaine had a distinct transcriptional signature, associated with decreased inflammation (**Fig. 3**). Thus, while our hypothesis that cannabis will decrease inflammation and HIV reservoir are avant-garde and unconventional, our preliminary data provide evidence that there is rationale for these innovative investigations.

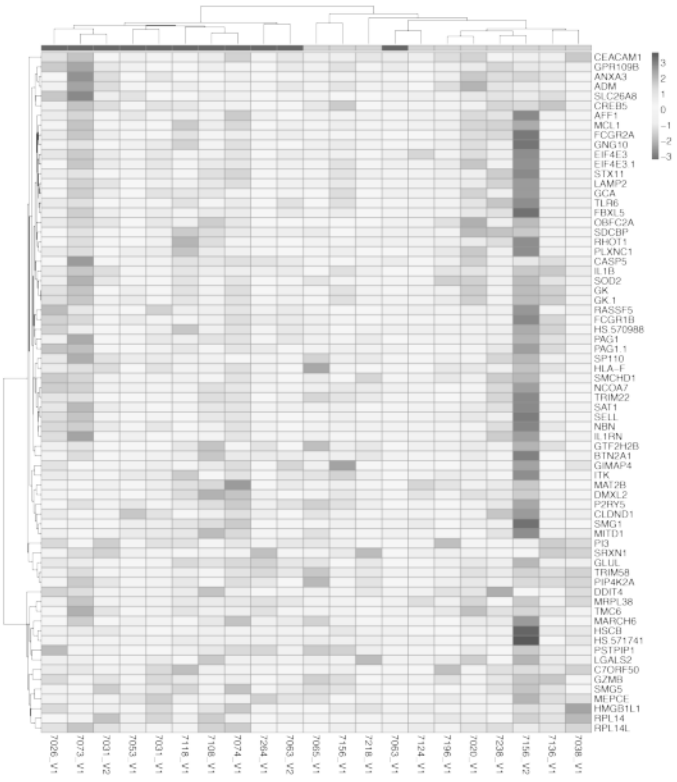

Fig. 3 Heatmap analysis demonstrating the top 70 differentially expressed genes (Fold change >1.3 and P value <0.05) between HIV-infected cocaine users (right, green) versus cocaine + cannabis users (left, blue).

Study Overview: Here we will test our innovative hypotheses that (i.) Cannabis use decreases inflammation and mucosal immune dysfunction during treated HIV infection; and (ii.) Cannabis-induced reduction of inflammation will decrease the HIV reservoir. We will use a comprehensive, multi-system translational approach to assess cannabis use and the effects on inflammation and HIV reservoir in HIV-infected, ART-suppressed individuals, as well as directly test these hypotheses by treating SIV-infected, ART-treated macaques with cannabinoids. We propose four key components to these studies: (i.) assessment of the HIV reservoir in banked PBMC samples from HIV-infected, ART-treated cannabis users versus controls; (ii.) assessment of the HIV reservoir in rectum and PBMCs from recruited patients who use cannabis compared to controls; (iii.) mechanistic studies of cannabinoid agonists ex vivo; and (iv.) assessment of causality in cannabinoid treatment of macaques infected with SIV.

- **Aim I**. Given the evidence for cannabis use decreasing inflammation, and the strong association between inflammation and the HIV reservoir, we hypothesize that HIV-infected, ART-treated individuals who regularly use cannabis will have decreased inflammation and residual HIV levels in peripheral blood. To evaluate our bold hypothesis that cannabis use decreases inflammation and thus the HIV reservoir, we will use existing samples from the UCSF SCOPE cohort to assess inflammation and levels of residual HIV from banked PBMC and plasma samples from HIV-infected, ART-suppressed patients who reported daily cannabis use at the time of sample collection, and also reported never having used other drugs of abuse (i.e. cocaine, heroin, or methamphetamines). We will compare these individuals to samples from HIV-infected, ART-suppressed patients who reported no drug of abuse use of any kind. These data will provide extensive information on how cannabis use effects inflammation and HIV reservoir compared to non-drug users, and provide a basis for approaching our future transformative studies outlined below.
- **Aim II.** While peripheral blood samples are readily attainable and will provide immense information regarding inflammation and HIV reservoir, the mucosal immune system is essential in these studies. Indeed, HIV infection causes massive dysfunction of the GI tract, resulting in the loss of mucosal integrity with

subsequent translocation of microbial products from the intestinal lumen to the systemic circulation (microbial translocation) and the establishment of a state of chronic, generalized immune activation, which is intimately associated with morbidity and mortality in both untreated and ART-treated individuals[1-3]. In addition, the GI tract is a major sanctuary for residual HIV. Given the role of cannabis in reducing GI dysfunction[12], we hypothesize that cannabis use will decrease mucosal dysfunction and inflammation, and reduce the reservoir in mucosal tissues. Thus, here we will recruit patients to the UCSF SCOPE cohort as described above, who either use cannabis daily or use no drugs of abuse, and will collect blood and rectal biopsies.

- **Aim III.** While cannabinoids are frequently used in studies of clinical disease and have demonstrated anti-inflammatory effects, the mechanisms underlying these effects in vivo are unclear[13]. Cannabinoids have differential pharmacological targets in vivo, and disparate agonist or inverse agonist activity may alter the anti-inflammatory effects we predict we will observe. One well studied cannabinoid, Δ(9)-THC, has psychoactivity and exerts pharmacological action via agonist activity to both cannabinoid receptor 1 (CB1), located mainly in cells of the central nervous system, as well as CB2, primarily located on immune cells[13]. In contrast, cannabidiol (CBD) lacks psychoactivity and its pharmacological activity is as an CB_2 inverse agonist[13]. There are also several synthetic cannabinoids available with differential CB activity. Thus, here we will perform a novel study whereby we will perform ex-vivo co-cultures with PBMCs and treat with Δ(9)-THC, CBD, and synthetic cannabinoids to dissect the mechanisms underlying anti-inflammatory effects of cannabinoids, and differential influence on HIV replication and infection of target cells.
- **Aim IV.** Given the association between maintenance of the HIV reservoir and immune dysfunction and inflammation[4,6,15], we hypothesize that decreasing persistent immune activation in combination with suppression of HIV by ART will decrease the HIV reservoir. To directly test this hypothesis and determine potential mechanisms for these actions, we will exploit the model of SIV infection of macaques to determine whether controlled cannabinoid treatment alters the SIV reservoir and inflammation *in vivo*. Here we will not only assess overall outcome and longitudinal effects of cannabis in multiple tissues, but we can also delineate the mechanisms of potential decreased inflammation and SIV reservoir by treating with cannabinoids that target different pathways, guided by capitalizing on mechanistic data from Aim III. In one group we will use ART + Δ(9)-THC to assess in vivo agonist activity against CB1 and CB2; In contrast, we will also treat a group of macaques with ART + CBD to assess inverse agonist pharmacological activity to CB2. We will treat an additional group with a synthetic cannabinoid (or combination of synthetic cannabinoids) based on data from Aim III. These groups will be compared to one another as well as to a control group of animals treated with ART alone. While both cannabinoids have been demonstrated to reduce clinical signs of inflammation[11,12], presumably these actions are through different pathways, thus use of the macaque model of SIV infection will allow us to determine mechanistic insight into the action of cannabinoids relative to inflammation and virus reservoir in an iterative process with our proposed human studies.

Klatt Lab	Human Samples (Hunt)	NHP Samples (Klatt)
Sample Processing	PBMC, Gut	PBMC, Gut
Flow Cytometry & Sorting	PBMC, Gut	PBMC, Gut
Luminex	Plasma	Plasma
ex Vivo Cannabinoid	PBMC	PBMC
GI Barrier Integrity		Gut
Microbial Translocation ELISAs	Plasma	Plasma, Gut
Residual SIV qRT-PCR		PBMC (CD4 subsets), Gut (Immune subsets)
Chomont Lab		
Total/integrated HIV DNA	PBMC, Gut (Immune Subsets)	
Inducible HIV msRNA	PBMC (CD4 subsets)	
Lifson/Piatak Lab		
Single copy SIV RNA		Plasma
Sekaly/Cameron Labs		
RNAseq	PBMC, Gut	PBMC, Gut
Fluidigm	PBMC, Gut	PBMC, Gut
Bioinformatics	PBMC, Gut	PBMC, Gut
Isoherranen Lab	PBMC, Gut	PBMC, Gut
Drug Level and Kinetics	PBMC, Gut	PBMC, Gut

Figure 4. Overview of Techniques

Experimental Approach

Here we will use an avant-garde approach to collaboratively assessing inflammation, HIV reservoir, and mechanisms underlying cannabinoid-induced effects (**Fig. 4**). We have put together an outstanding team of researchers for these studies, including Drs. Rafick Sekaly and Mark Cameron (VGTI-Florida) for expertise in transcriptional and bioinformatics studies; Dr. Peter Hunt (UCSF) for access to patient samples and expertise in inflammation, morbidities and mortalities in HIV infection; Dr. Nicolas Chomont (VGTI-Florida) for expertise in measurement of HIV reservoir; Drs. Jeffrey Lifson and Michael Piatak, Jr. (Frederick National Laboratory) for expertise in measurement of single copy SIV from plasma; Dr. Nina Isoherranen (UW) for expertise in cannabinoid and drug level analysis; and leading the study, Dr. Nichole Klatt (UW), with expertise in non-human primate models of SIV infection, flow cytometry, mucosal immunity, and inflammation. With this impressive group of scientists, we are certain that these high-caliber, translational studies will result in novel data and insights into both drugs of abuse and HIV-associated disease.

Brief Experimental Overview: Here we will use a systems biology approach to comprehensively identify the effects of cannabis in HIV/SIV disease and reservoir. To assess how cannabis alters cellular immunity and to isolate cells for HIV reservoir and RNA-seq based transcriptional analysis studies from our different models, we will use flow cytometric approaches to immunophenotype and sort cell subsets from peripheral blood and mucosal tissues. In blood, we will sort CD4+ T cell memory subsets into naïve (T_N), central memory (T_{CM}), effector memory (T_{EM}) and transitional memory (T_{TM}) for integrated HIV or SIV DNA analysis, inducible HIV reservoir (a state-of-the art technique developed by Dr. Chomont; **Fig. 5**), and inflammation. In mucosal tissues we will assess the integrated HIV or SIV DNA reservoir and inflammation from sorted lymphoid subsets, including bulk CD4+ T cells, dendritic cells, and monocytes/macrophages to determine which immune cell subsets harbor virus and how this is affected in vivo. Inflammation will be measured by a combination of flow cytometry, luminex, and species-specific transcriptomic analysis techniques. We will apply a meta-analysis bioinformatics approach to integrate global transcriptomic data with clinical, inflammation, and reservoir data collected to give a comprehensive oversight and clinical relevance to these transformative studies.

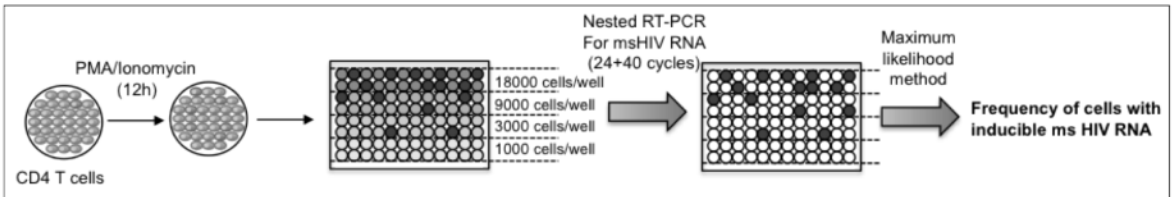

Fig. 5. Novel HIV RNA induction assay. Sorted CD4⁺ T cell subsets are stimulated and serial dilutions of the stimulated cells are distributed in a 96-well plate and digested. Multi-spliced HIV RNA are detected in a 2 step real time PCR. The maximum likelihood method is used to calculate the frequency of cells with inducible HIV RNA based on the number of positive well.

EVIDENCE OF INNOVATIVENESS

While ART has improved quality of life and decreased AIDS-associated mortality, it does not fully restore immune health, and ART-treated individuals still endure increased morbidities and mortality compared to uninfected individuals[4]. Thus, a common goal has surfaced to eradicate HIV by developing a cure to HIV. However, this is a major challenge given that HIV maintains a chronic reservoir of virus-infected cells, and thus discontinuation of ART results in a rapid rebound of HIV replication. Thus far, approaches to eradicate this HIV reservoir and/or decrease inflammation during ART therapy have been unsuccessful, and avant-garde approaches such as proposed here are necessary to better understand the reservoir and develop novel cure approaches.

Confounding the complexity of HIV infection, a large population of HIV-infected individuals report use marijuana, and thus authoritative information regarding health benefits (or injurious effect) should be highly prioritized. In addition, with the recent legalization of cannabis in select states, and the high probability of additional legalization in other states in the near future, a comprehensive understanding of how cannabis effects HIV infection is imperative. The studies proposed here provide not only an avant-garde approach to exploiting cannabis use to improve health, but will also provide a foundation for the role of cannabis in HIV-infected individuals.

Here we propose an extremely provocative study, whereby we aim to exploit cannabis to decrease inflammation and HIV reservoir using a systems biology approach. To our knowledge, these studies would be the first to address the effects of cannabis on inflammation and reservoir in HIV-infected patients, and to directly test how cannabis treatment affects inflammation and the SIV reservoir in macaques. The proposed cutting-edge studies here will provide a multi-parametric assessment of these factors by synergistically combining the expertise of several labs using innovative technology and a translational approach including mechanistic ex vivo co-culture studies, in vivo clinical human studies, and in vivo directed treatment of non-human primates. We have assembled a team of collaborators that is well poised to complete the detailed studies and will generate ample cutting-edge data that will provide essential information regarding our avant-garde question of how cannabis use effects inflammation and latent virus reservoir in ART-treated HIV/SIV infection. Indeed, these state-of-the-art techniques have been used and/or developed extensively by each lab as part of this outstanding team of interdisciplinary researchers. Taken together, we believe that this unorthodox approach to exploiting the potential beneficial effects of a drug of abuse, with the talented and innovative research team we have established, will provide a setting to better understand and develop treatments towards an HIV cure.

How the Planned Research Differs from my Previous Work

Our group has focused on mechanisms underlying mucosal dysfunction during HIV/SIV infection and novel therapeutic interventions to treat gastrointestinal dysfunction and inflammation in SIV-infected non-human primates. While these past studies have laid the groundwork for these pioneering efforts of HIV cure research, we have not yet focused on eradication of the HIV/SIV reservoir. Indeed, the global goal of developing an HIV cure has only just begun to be established. In addition, while our group has studied therapeutic strategies in HIV/SIV research, we have not performed research related to drugs of abuse. Thus, the proposed studies here to exploit cannabis as a novel approach to decrease inflammation and eradicate the HIV reservoir, with the ultimate goal to cure HIV, is transformative for our lab, but an evident translation of our expertise.

Compatibility with the goal of the Avant-Garde Award Program for HIV/AIDS Research

This proposal is uniquely suited for this Avant-Garde Award program as it is an extraordinarily innovative, transformative, potentially high-reward study, which would not be suitable for a standard R01 application, given the venturesome nature of the proposal. To our knowledge, this would be the first approach to exploit cannabis use to eradicate HIV. This cutting-edge concept comes with rational preliminary data to justify the attempt, but our approach of ex vivo, human and non-human primate studies provide a translational proposal to address clinical effects, mechanism, and causality. We believe these proposed studies will be integral to better understanding facets associated with the HIV reservoir and may provide a novel therapeutic approach towards development of an HIV cure.

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PROTECTION OF HUMAN SUBJECTS

A. Risks to Human Subjects

Human Subjects Involvement, Characteristics, and Design: This is an observational study involving human subjects. All subjects will be co-followed in the Clincal Core.

Recruitment and retention strategies: We have the capacity to conduct this study within the budget and time frame allotted due to our existing infrastructure through the UCSF SCOPE study and our long-standing experience in conducting intensive, single-center, pathogenesis-oriented studies. Advantages of using this established infrastructure include: (1) the ability to leverage existing infrastructure funds; (2) rapid identification of eligible subjects who have already consented to be contacted about related research projects, many of whom have a strong interest in supporting studies related to viral persistence; (3) reliance on an established infrastructure for patient retention, data collection, laboratory testing, and data management; (4) availability of data on a number of important co-variates, including HLA type; (5) access to stored longitudinal biologic specimens, including pre-treatment specimens for many patients; and (6) accurate, well-curated data on the duration and details of antiretroviral therapy.

UCSF SCOPE Cohort: The proposed research plan is made possible by the UCSF SCOPE and Options cohorts. SCOPE is directed by the PI and his colleague Dr. Jeffrey Martin. The cohort was initiated in 2000 and was established as a resource for collaborative translational research studies at UCSF. The cohort infrastrure is funded by the UCSF-Gladstone Institute of Virology and Immunology Center for AIDS Research (P30 MH59037), the Center for AIDS Prevention Studies (P30 MH62246), the Division of Experimental Medicine, and a number of individual grants by collaborating investigators. To date, SCOPE has enrolled over 1600 subjects, including over 800 subjects who have maintained virologic suppression on antiretroviral therapy. There is a diverse distribution of gender and racial/ethnic backgrounds among the subjects, which reflects the HIV epidemic in California; almost 50% of subjects are non-white and 15% are women. The distribution of exposure groups also closely mirrors the epidemic in San Francisco: 75% of subjects are men who have sex with men, and the remainder were infected through heterosexual contact or injection drug use.

Detailed interviews are conducted every 4 months, including questions regarding use of drugs of abuse, detailed and detailed information about cannabis usage, current medications, medication adherence, intercurrent illnesses, and hospitalizations. Plasma HIV-1 RNA levels and CD4+ T cell counts are measured at each visit. In addition, peripheral blood mononuclear cells (PBMCs) and plasma samples are obtained at each visit and stored at the UCSF AIDS Specimen Bank. All data are managed at the UCSF Data Coordinating Center through a state-of-the-art internet-based program that tracks participant recruitment and retention. The SCOPE study has a long track record of supporting investigator-initiated studies. Protocols are already in place for specimen collection and archiving, medication storage, safety monitoring, and data verification and management. The SCOPE team has also had extensive experience with all regulatory aspects of conducting single-center interventional clinical trials such as that outlined here.

Overall, the SCOPE cohort has had an excellent retention rate of 94% at 4 months, 88% at 1 year, 80% at 2 years, 76% at 3 years, 76% at 4 years, and 77% at 5 years. We believe the retention rate of the proposed study will be even higher with more intensive study monitoring, as reflected in our recently completed randomized, controlled trials with similar study durations, *all of which had 100% retention rates* (raltegravir intensification study, n=30, 24 week duration [81]; valganciclovir study, n=30, 8-week duration [122]; maraviroc intensification study, n=42, 24-week duration [155]). Moreover, many of these completed and ongoing (ClinicalTrials.gov NCT01025427) trials have included optional procedures (gut biopsy, lymph node biopsy, and/or leukapheresis) and we have been extremely successful at recruiting subjects for these more intensive substudies.

Sources of Materials

Demographic data: All sensitive information associated with this research study will be stored and secured in accordance with applicable UCSF policies and guidelines. At the screening visit we will obtain the following identifiers from study subjects: name, date of birth, address, phone number, and medical record number. We will also obtain the following medical information from study subjects: general medical history, current medications, date of HIV-1 infection, antiretroviral history, and all available CD4+ T cell count and plasma HIV RNA levels from previous 12 months. We will not disclose any personally identifying information to anyone

outside of the primary research team. Data will be coded using a four-digit study ID code and the data key will be kept separately and securely. Study charts will be kept in a locked file cabinet in a locked office. Electronic data will be protected with a password and kept on a secure network. All collaborators will receive specimens only identified by the four-digit study ID. All data are managed at the UCSF Data Coordinating Center through a state-of-the-art internet-based program that tracks participant recruitment and retention.

Blood collection: At the screening visit and each study visit, 20 cc of blood will be drawn through venipuncture for screening labs (serum pregnancy test, CMV serostatus, hepatitis B and C testing, coagulation panel, complete metabolic panel, complete blood count, CD4+ T cell count, plasma HIV RNA). Approximatley 120 cc of blood will be drawn at subsequent visits. These specimens will be used for the extensive immunologic assays outlined in the research plan. We will stay within Red Cross Guidelines of less than 500 mL every two months. Peripheral blood mononuclear cells (PBMCs) and plasma samples will be obtained at each visit and stored at the UCSF AIDS Specimen Bank.

Gut-associated lymphoid tissue (GALT) specimens: At the baseline visit subjects will have a colorectal biopsy performed to obtain GALT specimens. Thirty 3 mm mucosal biopsies will be taken. The procedure takes approximately 20 minutes. Subjects are instructed to self-administer two Fleet enemas two hours before the procedure and to take nothing by mouth after administering the enemas. Subjects are advised to not take any non-steroidal anti-inflammatory drugs (NSAIDs) or aspirin-containing compounds for five days before and after the procedure. The endoscopist introduces the sigmoidoscope into the anus and advances it to ~60 cm. Colorectal biopsies are obtained at the 10-30 cm level, circumferentially, using a disposable biopsy forceps with a 3.3 mm outside diameter. The sigmoidoscope is then withdrawn and the subject advised to avoid anal-receptive sex for at least one week. These GALT samples will then be analyzed for tissue HIV RNA and DNA, T cell activation, HIV Gag-specific T cell responses, collagen deposition by immunohistochemistry and quantitative image analysis, and fibrogenesis by heavy water labeling.

Potential Risks

Confidentiality: Participation in research may involve loss of privacy. Subject's records will be handled as confidentially as possible. All research records will be coded with a four-digit study ID code. Only the study investigators and their staff will have access to study records and test results. Study charts will be kept in a locked file cabinet in a locked office. Electronic data will be protected with a password and kept on a secure network. All collaborators will receive specimens only identified by the four-digit study ID. No individual identities will be used in any reports or publications resulting from this study.

Phlebotomy: Relevant personnel are trained and certified phlebotomists. Blood drawing may cause some discomfort, bleeding, or bruising where the needle enters the skin. Rarely, fainting or infection may occur. We have had no serious problems in any of our previous studies. We will stay within Red Cross Guidelines of less than 500 mL every two months. Risks of blood collection include anemia (low blood counts). Symptoms of anemia include tiredness, weakness and dizziness. Patients will be checked for anemia at each study visit. If the investigator feels that an individual is at significant risk for anemia, the amount of blood collected will be reduced. If the study participant's hemoglobin falls below 9 g/dl or hematocrit falls below 27%, we will draw a 5 mL (1 teaspoon) of blood drawn to check the hemoglobin and hematocrit. Other than the blood required to check the hemoglobin and hematocrit, the subject will not have more blood drawn until the hemoglobin rises above 9 g/dl or the hematocrit rises above 27%.

Colorectal biopsy: Sigmoidoscopy and biopsy will be performed by a gastroenterologist trained in this procedure. Colorectal biopsy itself is painless and any discomfort that may occur is associated with gas pain from the addition of air through the sigmoidoscope. There may be mild rectal irritation, urgency, and limited rectal bleeding for two to three days following the procedure. Subjects with abnormal clotting function will be excluded from the study. Subjects will also be advised to not take any non-steroidal anti-inflammatory drugs (NSAIDs) or aspirin-containing compounds for five days before and after the procedure. Bowel perforation is extremely rare and would require treatment with antibiotics and/or surgical repair. The risk of such complications is less than 1 in 1,000 each time the procedure is performed. The risk of bowel perforation and related complications during sigmoidoscopy was assessed in a large population-based cohort of Medicare beneficiaries (the Surveillance, Epidemiology, and End Results Program). From this registry, individuals who had undergone colonoscopy or sigmoidoscopy between 1991 and 1998 were identified (n=39,286 procedures). There were a total of 31 perforations during 35,298 sigmoidoscopies (incidence = 0.88/1000

procedures). The risk was even lower among those who were asymptomatic (0.54/1000) [156]. There is the additional risk of infection (peritonitis) and death as a result of bowel perforation. It is important to realize that the risk associated with multiple biopsies is not known. Hence there may be additional risks of the biopsy procedure which are unknown at this time. No more than 30 biopsies total will be obtained from any subject during each procedure. The risk related to the number of 3mm biopsies performed is not known to increase as long as the total is ≤30. Subjects will be monitored for symptoms and complications throughout and after the procedure.

Enemas: Subjects will be instructed to self-administer 2 enemas, approximately 2 hours prior to the colorectal biopsy. Enemas will likely cause subjects to have a large bowel movement or diarrhea prior to the procedure. This is expected. Occasionally, enemas can result in delayed diarrhea several hours later.

Alternative treatments and procedures: Prospective subjects may choose not to participate in the study.

B. Adequacy of Protection Against Risks

Institutional Review Board (IRB) review: The study protocol and informed consent form will receive approval from the Committee on Human Research, UCSF's institutional review board. The consent process is conducted in accordance with our division's SOP, which is based on GCP and HHS guidelines. All research staff have completed the NIH computer based-training on the Protection of Human Research Subjects. Certificates for this training are on file. In addition, all staff have completed the Human Subject Protections online course developed by UCSF.

HIPAA compliance: All sites are committed to ensuring that appropriate measures are taken to protect the privacy and confidentiality of all Personally-Identifiable Health Information (PHI) for which it is responsible. This includes compliance with all regulations set forth by the HIPAA Privacy Rule, as well as with existing State and Federal laws pertaining to PHI. All entering study volunteers will be given a copy of the Notice of Privacy Practices. Good faith efforts will be made to obtain each individual's written acknowledgement of receipt of the Notice(s). The rights mandated by the HIPAA Privacy Rule concerning an individual's ability to access, amend, and disclose certain sections of his or her own PHI are fully respected, and every effort will be made to assist patients who choose to exercise these rights. In accordance with HIPAA regulations, subjects will be identified by a four-digit study ID code (as is currently done in SCOPE).

Recruitment and Informed Consent:

Subject Recruitment: Potential subjects will be identified through Ward 86 at San Francisco General Hospital (SFGH), local advertisements, and local collaborators at SFGH, UCSF, San Francisco Veterans Affairs Medical Center, San Francisco Kaiser Permanente, the Women's Interagency Health Study, and the Community Consortium (which Dr. Deeks currently directs). Recruitment will also be greatly aided by an established prospective, clinic-based cohort study of HIV-infected adults (the SCOPE cohort, which is co-directed by the PI). SCOPE has enrolled over 1600 subjects, including over 800 subjects who have maintained virologic suppression on antiretroviral therapy. During the enrollment period all SCOPE patients who come in for regular cohort visits will be asked if they would like to learn more about this study.

Informed consent: Potentially eligible patients will be contacted and screened by the research coordinator. The risks and benefits of participating will be explained, study procedures will be outlined, study eligibility will be confirmed, and subjects will be enrolled into the study after obtaining written informed consent. Eligibility will be determined by the Principal Investigator, based upon medical history and screening test results after the screening visit. Prior to any study procedures, subjects will be consented by the Principal Investigator or research staff. The consent procedure will be performed in a private setting in the clinic. Subjects will be asked to provide authorization for release of personal health information and use of personally unidentified study data for research (HIPPA). Research staff will ensure that candidates understand all elements of the consent form by addressing questions posed during the consent procedure and by asking for verbal confirmation that the candidate has no additional questions and verbally understands the purpose of the study, study intervention, basic procedures, risks, and that participation is voluntary. Subjects will be given as much time as they need to read and understand the consent form. The subject will receive a signed/dated copy of the consent form to keep along with the UCSF Subject Bill of Rights and the HIPPA form to keep. Consent will

also be obtained for the blood draws, heavy water labeling, colorectal biopsy, and lymph node biopsy, as outlined above.

Protection against risks:

Clinical site monitoring and record availability: Organizations that may look at and/or copy records for research, quality assurance, and data analysis include representatives from the UCSF Committee on Human Research, National Institutes of Health, and the Food and Drug Administration (FDA) and other government regulatory agencies.

Confidentiality: Subject's records will be handled as confidentially as possible. Subjects will be assigned a unique four-digit study ID code that will appear on all specimens and in our database. All biologic specimens and clinical data obtained from this study will be linked to this code and not to personal identifying information (e.g., name, social security number, medical record number). A key which will link the four-digit code to the personal information will be maintained in a secure pass-word protected file maintained by the Principal Investigator. Lab personnel and database programmers will have access only to the coded number and the subject's date of birth; no other personal identification information will be available to them. Collaborators will receive specimens only identified by the four-digit study ID. No individual identities will be used in any reports or publications resulting from this study. However, the records may be reviewed under guidelines of the Federal Privacy Act by research personnel from the Positive Health Program at San Francisco General Hospital. The California AIDS Confidentiality ACT provides that subjects have a right to request a copy of their research records, which must be provided within 30 days of their written request.

Phlebotomy: Relevant personnel are trained and certified phlebotomists. We will stay within Red Cross Guidelines of less than 500 mL every two months. Patients will be checked for anemia at each study visit. If the investigator feels that an individual is at significant risk for anemia, the amount of blood collected will be reduced. If the study participant's hemoglobin falls below 9 g/dl or hematocrit falls below 27%, we will draw a 5 mL (1 teaspoon) of blood drawn to check the hemoglobin and hematocrit. Other than the blood required to check the hemoglobin and hematocrit, the subject will not have more blood drawn until the hemoglobin rises above 9 g/dl or the hematocrit rises above 27%.

Colorectal biopsy: Sigmoidoscopy and biopsy will be performed by a gastroenterologist trained in this procedure. Subjects with abnormal clotting function, inflammatory colitis (including Crohn's disease, ulcerative colitis), and/or any contraindications to sigmoidoscopy or colorectal biopsy such as peritonitis, active diverticulitis, or recent bowel surgery will be excluded from the study. Subjects will also be advised to not take any non-steroidal anti-inflammatory drugs (NSAIDs) or aspirin-containing compounds for five days before and after the procedure. Subjects will be monitored for symptoms and complications throughout and after the procedure.

C. Potential Benefits of the Proposed Research to Human Subjects and Others

There may be no direct benefit to subjects from participation in this study. It is possible, however, that knowledge gained from these individuals could bolster eradication efforts for future patients.

Subject compensation: Subjects will be reimbursed to cover costs related to the study (travel, loss of time). They will receive $25 at the completion of each study visit and $150 for any tissue biopsy visits.

D. Importance of the Knowledge to be Gained

With 35 million HIV-infected individuals worldwide, the challenge to improve health in these individuals is vast. Antiretroviral therapy (ART) suppresses HIV replication, which prevents AIDS and reduces overall mortality, however ART does not fully restore health. Indeed, despite sustained suppression of viremia, individuals cannot discontinue ART as residual HIV persists, and virus rebound is inevitable if ART is discontinued. This residual HIV reservoir is associated with ongoing inflammation. Cannabis is a widely used drug in the United States, and derivatives of cannabis such as cannabinoids are commonly used in treatment of nausea and cachexia in severe conditions such as cancer. Several studies have demonstrated that cannabinoids have the propensity to alter immune responses and decrease inflammation in vivo. We hypothesize that cannabis use

in the context of ART-treated HIV infection may decrease inflammation and the persistent HIV reservoir. Here, we provocatively propose to test this hypothesis in humans by measuring inflammation, immunity, and the HIV reservoir from blood and gastrointestinal (GI) tissues from HIV-infected individuals who report using cannabis daily compared to those reporting no drug use. We believe these proposed studies will be integral to better understanding facets associated with the HIV reservoir and may provide a novel therapeutic approach, exploiting a drug of abuse, towards development of an HIV cure.

E. Data and Safety Monitoring Plan

The primary monitoring responsibility will be done by the Principal Investigator, who will report adverse events and unanticipated problems to the Committee on Human Research (CHR), UCSF's institutional review board. All subjects will be followed for possible adverse events and unanticipated problems throughout their involvement in the study. At each study visit, research staff will elicit subject input as to discomforts or adverse experiences while taking the study medication. A complete blood count, complete metabolic panel, CD4+ T cell count, and plasma HIV-1 RNA level will be performed on most visits. Furthermore, detailed information regarding use of drugs of abuse will be collected. The Principal Investigator will obtain these safety data in "real-time" (i.e., within 1-10 days of a study visit) when laboratory values become available.

Study discontinuation: If it is determined by the principal investigator or the primary care provider that it is in the best interest for a subject to discontinue study drug, then the subject will be asked to drop out of the study.

F. ClinicalTrails.gov Requirements

Our group has routinely registered prospective studies such as the one outlined in this application with ClinicalTrials.gov.

V. INCLUSION OF WOMEN AND MINORITIES (see Targeted/Planned Enrollment Table, below)**:** There will be no exclusions based on gender or race. The demographics of HIV infection in most areas of the United States do not represent the demographics in the general population. The vast majority of HIV infected adults in San Francisco Bay Area are men. The investigators will actively recruit and perhaps oversample women to participate in the proposed study. Based on our prior experience with the SCOPE cohort, we expect that approximately 20% of our cohort will be women. Based on our prior experience with the SCOPE cohort, we expect that approximately 50% of our cohort to be non-Caucasian.

Inclusion of Women: We plan to perform a subgroup analysis for female gender. Every effort will be made to recruit and study women in this proposal. Females of childbearing potential must have a negative serum pregnancy test at screening and agree to use a double-barrier method of contraception throughout the study period.

Inclusion of Minorities: We plan to perform a subgroup analysis for ethnic minorities. Every effort will be made to study different ethnic minorities in this study proposal. The HIV-infected population at San Francisco General Hospital is linguistically, culturally and racially diverse. We anticipate participation from multiple racial and ethnic groups.

OMB Number: 0925-0002

Planned Enrollment Report

Study Title: Impact of Cannabis on Inflammation and Viral Persistence in Treated HIV/SIV

Domestic/Foreign: Domestic

Comments: We will use samples from HIV-infected individuals with >5 years of ART therapy, fully suppressed viremia. Individuals will be recruited who report daily cannabis use (71 individuals) versus individuals who report no use of drugs of abuse, including cocaine, crack, heroine, methamphetamines (142 individuals).

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	0	1	0	0	**1**
Asian	1	4	0	0	**5**
Native Hawaiian or Other Pacific Islander	0	1	0	0	**1**
Black or African American	16	62	0	0	**78**
White	20	78	3	16	**117**
More than One Race	1	6	2	2	**11**
Total	**38**	**152**	**5**	**18**	**213**

Study 1 of 1

Funding Opportunity Number: RFA-DA-14-008. Received Date: 2013-11-04T13:12:00.000-05:00

INCLUSION OF CHILDREN

Individuals <18 years of age will be excluded from the protocol because the mechanisms involved in viral persistence may be different in children compared to adults. Individuals 18-21 years of age are eligible for the study.

VERTEBRATE ANIMALS SUMMARY

(1) Provide a detailed description of the proposed use of the animals in the work outlined in the Research Design and Methods section. Identify the species, strains, ages, sex, and numbers of animals to be used in the proposed work.

For these studies, we propose to use rhesus macaques (RM, *Macaca mulatta*). RM are an ideal model for these studies, as the dynamics of SIV infection, progression to AIDS, mucosal immunology, and antiretroviral treatment during SIV infection have been extremely well characterized in this model. A total of 32 age-matched adult animals (male or female) will be used for these studies.

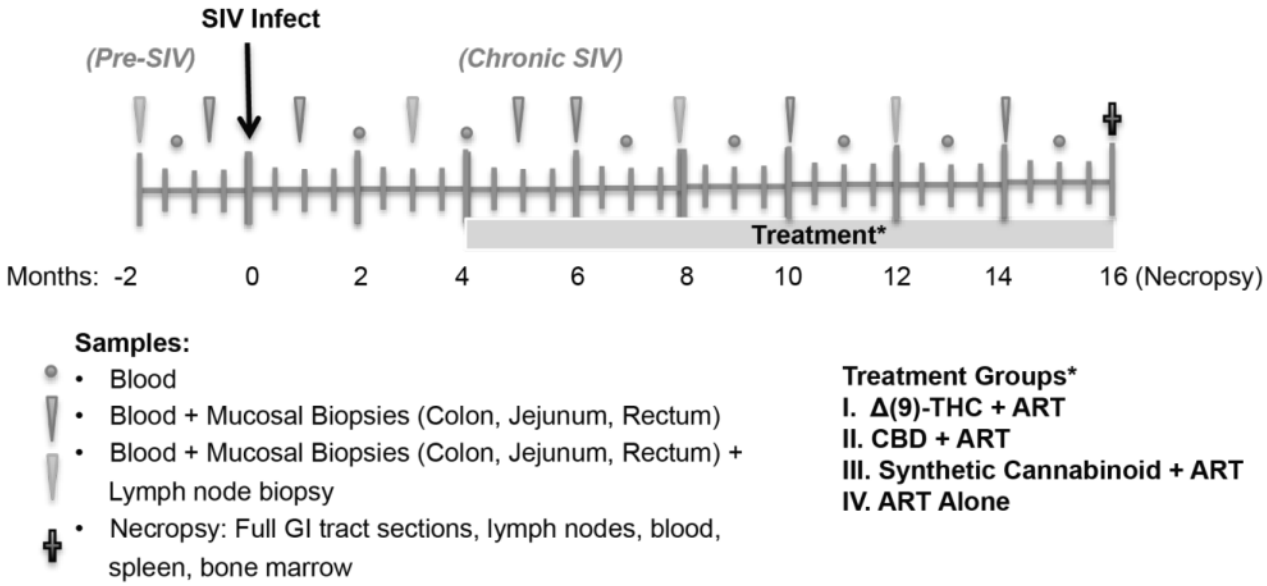
Treatment Plan

For these studies, we will have 4 treatment groups of 8 animals each: (i.) Δ^9 tetrahydrocannabinol (Δ(9)-THC) + ART; (ii.) cannabidiol (BD) + ART; (iii.) synthetic cannabinoid + ART; (iv.) ART alone (**see timeline figure below)**. Animals will be infected i.v. with 3000 TCID50 SIVmac239 (AIDS monitoring protocol established at the Washington National Primate Research Center (WaNPRC), described below). The ART regimen, which has previously successfully suppressed SIV in RM in our laboratory will be as follows: Tenofovir (PMPA, 20-40mg/kg subcutaneous daily), Emtricitabine (FTC, 30mg/kg subcutaneous daily) and Raltegravir (RAL, 100mg/kg oral BID). Δ(9)-THC will be administered 0.32 mg/kg i.m. BID. CBD will be administered 100mg/kg oral daily. Possible synthetic cannabinoids include SR141716A (1mg/kg oral daily), resiniferatoxin (18ug/kg s.c. daily), AM251 (0.3 mg/kg s.c. daily) or RU-38486 (10 mg/kg s.c. daily). All cannabinoid doses have been safely administered and shown effects in macaque and/or rodent models (Molina, P. et al., 2010; Winsauer, P. et al., 1999; Rosenkrantz, H., et al., 1980; Segev, A. et al., 2013).

Samples

We will longitudinally collect: blood alone, or blood + mucosal biopsies (colon, jejunum and rectum), or blood + mucosal biopsies (colon, jejunum and rectum) + lymph node biopsy, prior to SIV infection, after SIV infection, prior to treatment, and throughout the course of treatment. 24 pinch biopsies will be taken from each mucosal biopsy time point. 1-2 lymph nodes from axillary or inguinal sites will be removed at each biopsy. At necropsy the following tissues will be collected for analysis: gastrointestinal sections (jejunum (30cm), ileum (10cm), colon (30 cm), rectum (10cm)), mesenteric, inguinal and axillary lymph nodes (as many as available), spleen, bone marrow (10mL), blood (exsanguination, ~300mL). Samples will be analyzed immediately, or cryopreserved or fixed for later analysis.

***Example Timeline:* Chronic SIV Cannabinoid + ART Treatment**



The longitudinal sampling regimen proposed here has been approved by WaNPRC veterinary staff to be safe and feasible. Indeed, we have performed similar sampling regimens in rhesus macaques in past studies with great success.

AIDS-related Monitoring Protocol (WaNPRC)
Nonhuman primates involved in AIDS-related studies have the potential to develop acquired immune deficiency syndrome following infection with lentiviruses. It is therefore important to provide a comprehensive monitoring program to identify animals in early stages of illness to facilitate the clinical treatment and/or research decision process before end-stage disease.
All animals involved in AIDS-related projects are included in standard WaNPRC monitoring procedures. This includes at least twice-daily observation by the Animal Technician for basic husbandry parameters (e.g. food intake, activity, stool consistency, overall appearance) as well as daily observation by a Veterinary Technician and/or Veterinarian. WaNPRC Research Support personnel and Behavioral Management Services personnel also frequently monitor the health and clinical condition of each animal. If a clinical abnormality is noted, standard WaNPRC procedures will be followed to notify the veterinary staff for evaluation and determination for admission as a clinical case.
In addition to standard monitoring procedures, animals involved in AIDS-related studies will have enhanced evaluations throughout ongoing research protocols. As with all animal assignments, animals involved in immunization or challenge protocols will have a complete evaluation prior to initiation of the study including physical examination, complete blood count, and blood chemistry analysis. Routine exams following lentivirus inoculation during the course of the protocol will be performed at least monthly and will include:

a) Body weight
b) Temperature (rectal)
c) Complete blood count
d) Lymphocyte subsets (CD4+, CD8+)
e) Evaluation of major peripheral lymph nodes (axillary, inguinal)
f) Oral cavity examination
g) Stool observation
h) Blood chemistry panel as requested for clinical or research personnel
i) Bacteriological/parasitological analysis as requested for clinical or research personnel

WaNPRC veterinary staff may request additional exams at any time based on the clinical condition of any experimental animal. Monkeys infected with primate lentiviruses typically do not show overt signs of disease for variable periods of time despite laboratory evidence of infection. Therefore, the following criteria will be evaluated to determine the need for more intensive monitoring:
1) Weight loss of >15% baseline body weight.
2) Anemia (sustained hematocrit <15%)
3) CD4+ lymphocyte depletion (<200/µl)
4) Presence of opportunistic infection(s) or other clinical condition that is unresponsive to clinical intervention.

Animals which meet at least one of the criteria will be admitted as clinical cases and will have physical examinations performed by the veterinary staff in conjunction with the standard monthly monitoring protocol until the clinical condition resolves. If an animal meets two of the criteria, the Clinical Veterinarian will be notified for review of the case, and enhanced semi-monthly monitoring will be initiated. Complete examinations will be performed monthly, and at the intervening semi-monthly time points not covered by the standard monthly monitoring body weight, stool observation, and cage side examination findings will be recorded. Additional examinations may be performed at the request of the Veterinarian. If a minimum of three of the criteria are met, euthanasia will be elected to prevent unnecessary discomfort to the animal. It is also noted that a severe manifestation of any single condition may warrant euthanasia, therefore the Clinical or Attending Veterinarian will have authority to make the decision for euthanasia.

In the event of an unexpected death, the animal will have a necropsy performed for evaluation of the cause of death with final reports forwarded to WaNPRC personnel.

(2) Justify the use of animals, the choice of species, and the numbers to be used. If animals are in short supply, costly, or to be used in large numbers, provide an additional rationale for their selection and numbers.

Rhesus macaques will be employed as a highly relevant model for human HIV infection. The use of an animal model is essential to overcome the limitations in human studies as several longitudinal time points in a controlled setting of treatment interventions will be acquired, and the sampling protocol for the mucosal tissues required for these studies would be very difficult to achieve in human studies. However, the SIV model in non-human primates provides an excellent model for HIV in humans, and we will be able to safely and adequately test our hypotheses using the rhesus macaque model. Indeed, there is currently no alternative to nonhuman primate models (including in vitro tests and other non-primate animal models) that approximate the spectrum of mucosal and peripheral immune responses and the pathogenic responses that result from primate lentivirus infection. Macaca mulatta is the standard macaque species used for SIV/ SHIV research; human-like immune responses to these viruses and vaccine candidates against these viruses are well documented. These models have been used extensively at the Washington National Primate Research Center.

With 8 animals/group, the study can detect a difference of 6.7 units between groups in most of the inflammation outcomes with 80% power using a paired t-test. This estimate assumes an intra-animal correlation of 0.5 and a standard error of 4.75 (from preliminary data) at each time point. For the mucosal immunity and barrier studies, the study can detect a difference of 2 units between 2 times points with 80% power using a paired t-test, assuming an intra-animal correlation of 0.5 and a standard error of 1.5 (from preliminary data) at each time point. The primary analysis is likely to detect even smaller differences since linear regression with adjustment for intra-animal correlation will be used for analysis in addition to paired t-tests. Thus, we are confident we are sufficiently powered for these studies.

(3) Provide information on the veterinary care of the animals involved.

The nonhuman primates are housed and cared for under conditions that meet NIH standards as stated in the Guide for the Care and Use of Laboratory Animals (8th Edition, 2010), ILAR recommendations and AAALAC accreditation standards for animals of this species. The UW, including the NPRC, is fully accredited by AAALAC. The animals' home cages will be in a room with other similarly housed animals of the same species. Cages, racks, and accessories are sanitized in mechanical cage washers at least once every two weeks and waste pans are cleaned daily. Temperature in animal quarters is maintained at 72-82°F. Animals are fed a commercial monkey chow, supplemented daily with fruits and vegetables and drinking water is available at all times provided by automatic watering devices.

The WaNPRC employs a large number of full-time professional staff members to provide expertise in administration, animal husbandry, clinical medicine, psychological well-being, facilities maintenance, records, and comprehensive research support. Animal care personnel typically assist with research related matters as part of their daily activities and broad-based training sessions are regularly scheduled during biweekly staff meetings. All members of the professional veterinary staff participate in continuing education and take advantage of the rich academic environment at the University of Washington Medical Center to improve their clinical acumen.

(4) Describe the procedures for ensuring that discomfort, distress, pain, and injury will be limited to that which is unavoidable in the conduct of scientifically sound research. Describe the use of analgesic, anesthetic, and tranquilizing drugs and/or comfortable restraining devices, where appropriate, to minimize discomfort, distress, pain, and injury.

Anesthesia and Analgesia

The purpose of anesthesia is to render the animal unconscious and therefore insensate to handling, discomfort, or pain. General anesthesia is the state of unconsciousness produced by the controlled administration of a pharmacological agent. The selection of the anesthetic agents used is at the discretion and direction of a veterinarian and as indicated in approved IACUC protocols (where applicable). The following is a listing of anesthetics that are typically used and maintained in the WaNPRC veterinary pharmacies. Other agents may be acquired and used if indicated at the professional discretion of a veterinarian.

Atropine sulfate is not an anesthetic or analgesic but is administered to all animals receiving ketamine or Telezol or being prepared for general anesthesia to control salivary secretions, prevent vomiting and regurgitation, and strengthen cardiovascular function. This agent may be mixed in syringe with ketamine. DOSE: 0.04 mg/kg of body weight.

Anesthetics

Ketamine hydrochloride produces a dose-related response that ranges from mild sedation to profound unconsciousness. Ketamine is administered to all nonhuman primates that undergo manipulative procedures. It may be used as the single agent where mild to moderately painful procedures are anticipated, such as blood drawing, wound debridement, skin suturing and teeth scaling. Procedures that are likely to be more painful require the administration of another selective anesthetic or analgesic drug. DOSE: 10-15 mg/kg body weight, depending on desired depth and duration. May be administered IV or IM.

Tiletamine/Zoluzepam (Telazol) is a proprietary dissociative/benzodiazepine. Telazol can be used as a sedative for short procedures or to produce a light plane of anesthesia. Telazol is a good drug to be used on animals that have a higher tolerance to ketamine. Telazol must be reconstituted, held under refrigeration and used within 2 weeks. DOSE: 4-6 mg/kg body weight for sedation, 8-10 mg/kg body weight for light anesthesia.

Isoflurane is an inhalant anesthetic. Isoflurane allows for close control of the depth of anesthesia for extended periods of time. In addition to providing for surgical anesthesia, it is useful for imaging procedures which require absence of movement. DOSE: 1-2% in oxygen by inhalation.

Sevoflurane is a volatile liquid used in inhalation anesthesia. It is administered in a vaporized form through a cuffed endotracheal tube and generally follows a pre-induction dose of ketamine and atropine. Sevoflurane produces rapid induction, recovery and changes in anesthesia level. Because of its low solubility, sevoflurane acts more rapidly than isoflurane. Because it is less potent than isoflurane, higher concentrations must be used. A dose of 4.0-5.0% with oxygen produces a surgical level of anesthesia within 3-5 minutes. Surgical level anesthesia can be maintained with a dose of 2.0-2.8% sevoflurane with oxygen. Because it is less noxious than isoflurane, sevoflurane is the inhalant of choice for mask induction.

Analgesia

Analgesia is the reduction of pain without the loss of consciousness. A condition that would be considered painful to a human is presumed to be painful to animals, and appropriate analgesics are administered. Similarly, analgesics are provided for all post-surgical animals. The selection of the analgesic agents used is at the discretion and direction of a veterinarian and as indicated in approved IACUC protocols (where applicable). Following is a listing of analgesics that are typically used and maintained in the WaNPRC veterinary pharmacies. Other agents may be acquired and used if indicated at the professional discretion of a veterinarian.

Analgesics

Aspirin and *acetaminophen* is used for relief of mild pain due to minor trauma, skin laceration, chronic inflammatory problems, menstruation, and extraction of deciduous teeth. DOSE: *Aspirin*: 5-10 mg/kg body weight, T.I.D. PO., *acetaminophen:* 80 – 160 mg, PO.

Ketoprofen is a non-narcotic, non-steroidal anti-inflammatory agent in the same class as ibuprofen. Its analgesic and antipyretic properties make it an effective drug in controlling post-surgical, acute, or chronic pain and inflammation. For post-surgical analgesia, it is recommended that the initial dose be given prior to recovery from surgical anesthesia. Ketoprofen is not recommended for use in animals with gastritis or thrombocytopenia. DOSE: 5 mg/kg body weight IM or PO every 4-6 hours.

Butorphanol tartrate is used for relief of moderate to severe pain due to extensive trauma or wound. Peak analgesia occurs 30-50 min after IM injection. DOSE: 0.15 mg/kg body weight, IM or SQ, T.I.D.

Buprenorphine HCl is a parenteral opioid analgesic used for the relief of moderate to severe pain. It has a rapid onset (15-min) and persists for 6-8 hours. Peak activity is reached 60 min after IM injection. Use for relief of severe pain accompanying orthopedic repair, canine extraction, or extensive intra-abdominal surgery. DOSE: 0.015 mg/kg body weight IM, BID to TID.

Hydromorphone and *fentanyl* are potent opioid analgesics for the relief of moderate to severe pain. These agents can be given peri-operatively to supplement analgesia and/or as an analgesic for post-operative pain. DOSE: *Hydromorphone*: 0.1-0.2 mg/kg IM or IV, *fentanyl*: 5-10 mcg/kg IV, continuous infusion @ 10-25 mcg/kg/hr, or by dermal patch.

Lidocaine and bupivicaine are local anesthetics used perioperativel and postoperative to block sensation of pain. DOSE: *Lidocaine* and *bupivicaine* :1% or 2 % infiltrate local area prn.

(5) Describe any method of euthanasia to be used and the reasons for its selection. State whether this method is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. If not, present a justification for not following the recommendations.

Euthanasia is performed in accordance with guidelines as established by the 2013 American Veterinary Medical Association Guidelines on Euthanasia. An animal to be euthanized is first rendered unconscious by administration of ketamine hydrochloride, and then the animal is euthanized by one of the following methods:

- Intravenous administration of sodium pentobarbital, Beuthanasia® (or equivalent) while the animal is under deep anesthesia.
- Exsanguination while the animal is under deep anesthesia. This method is used only if a research protocol precludes the use of pentobarbital or euthanasia solution.

All of the following must be met for death to be declared:

- Absence of thoracic sounds by auscultation.
- Absence of respiratory effort.
- Dilation and immobility of pupils.

Euthanasia is assured by secondary means after use of the previously described anesthetic or euthanasia agents (usually bilateral pneumothorax associated with opening the thoracic cavity for tissue collection and necropsy).

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