PI: **Antar, Annukka Aida Rose**	Title: The HIV Latent Reservoir, Suboptimal Immune Response on Antiretroviral Therapy, and Exogenous Cytokine Therapies	
Received: 05/04/2018	Opportunity: PA-18-373 Clinical Trial:Not Allowed	Council: 10/2018
Competition ID: FORMS-E	FOA Title: Mentored Clinical Scientist Research Career Development Award (Parent K08 - No Independent Clinical Trials)	
1K08AI143391-01	Dual:	Accession Number: 4166773
IPF: 4134401	Organization: JOHNS HOPKINS UNIVERSITY	
Former Number:	Department: Medicine	
IRG/SRG: AIDS	AIDS: Y	Expedited: Y
Subtotal Direct Costs (excludes consortium F&A) Year 1: [redacted] Year 2: [redacted] Year 3: [redacted] Year 4: [redacted] Year 5: [redacted]	Animals: N Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: Early Stage Investigator:

Senior/Key Personnel:	*Organization:*	*Role Category:*
Annukka Antar	JOHNS HOPKINS UNIVERSITY	PD/PI
RICHARD MOORE	Johns Hopkins University	Other (Specify)-Co-mentor
JOEL BLANKSON	Johns Hopkins University	Other (Specify)-Co-mentor
STEVEN DEEKS	UCSF	Other (Specify)-Collaborator
ROBERT SILICIANO	Johns Hopkins University	Other (Specify)-Primary Mentor

OMB Number: 4040-0001
Expiration Date: 10/31/2019

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 112427

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION

Organizational DUNS*: [redacted]

Legal Name*: JOHNS HOPKINS UNIVERSITY
Department: Medicine
Division: Infectious Diseases
Street1*: [redacted]
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County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]

Person to be contacted on matters involving this application

Prefix: First Name*: Katrina Middle Name: Last Name*: Alston-Rodgers Suffix:

Position/Title: Senior Grants Associate
Street1*: [redacted]
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County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email: [redacted]

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** [redacted]

7. TYPE OF APPLICANT* O: Private Institution of Higher Education

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

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).

❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

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

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
The HIV Latent Reservoir, Suboptimal Immune Response on Antiretroviral Therapy, and Exogenous Cytokine Therapies

12. PROPOSED PROJECT

Start Date* Ending Date*
12/01/2018 11/30/2023

13. CONGRESSIONAL DISTRICTS OF APPLICANT
MD-007

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Annukka Middle Name: Aida Rose Last Name*: Antar Suffix:
Position/Title: Post-doctoral Fellow
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Division: Infectious Diseases
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City*: Baltimore
County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: 410-338-3000 Fax Number: Email*: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested* $[redacted]
b. Total Non-Federal Funds* $[redacted]
c. Total Federal & Non-Federal Funds* $[redacted]
d. Estimated Program Income* $[redacted]

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*: Katrina Middle Name: Last Name*: Alston-Rodgers Suffix:
Position/Title*: Senior Grants Associate
Organization Name*: Johns Hopkins University
Department: Medicine
Division: Research Administration
Street1*: [redacted]
Street2:
City*: Baltimore
County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

Signature of Authorized Representative*
[redacted]

Date Signed*
05/04/2018

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover_Letter_Antar-K08.pdf

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

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

Project/Performance Site Location(s)

Project/Performance Site Primary Location

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

Organization Name: JOHNS HOPKINS UNIVERSITY
Duns Number: [redacted]
Street1*: [redacted]
Street2: [redacted]
City*: BALTIMORE
County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: MD-007

Project/Performance Site Location 1

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

Organization Name: JOHNS HOPKINS UNIVERSITY
DUNS Number: [redacted]
Street1*: [redacted]
Street2:
City*: BALTIMORE
County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: MD-007

Additional Location(s) File Name:

Funding Opportunity Number: PA-18-373. Received Date: 2018-05-04T14:06:47.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

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

1.a. If YES to Human Subjects

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

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

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

IRB Approval Date:

Human Subject Assurance Number

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

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

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*	Project_Summary_Abstract.pdf
8. Project Narrative*	Project_Narrative-Antar-K08.pdf
9. Bibliography & References Cited	References_Antar-K08.pdf
10.Facilities & Other Resources	Facilities_and_Other_Resources.pdf
11.Equipment	Equipment.pdf

Funding Opportunity Number: PA-18-373. Received Date: 2018-05-04T14:06:47.000-04:00

Project Summary/Abstract

A significant percentage of HIV-positive individuals who start antiretroviral therapy (ART) with a low $CD4^+$ T cell count have CD4 counts that plateau at abnormally low levels despite years of virologic suppression on ART. The risk of death for these suboptimal immune responders (SoIRs) is 2-3 times higher than that of individuals whose CD4 count rises appropriately with ART, and this higher risk of death persists for a decade or more. The mechanisms underlying the suboptimal immune recovery and increased mortality rates in SoIRs remain poorly defined. One clear association has emerged: SoIRs have significantly higher levels of immune activation than other ART-treated individuals. We know that stimulation of HIV-infected CD4s through the T cell receptor results in HIV RNA and protein production and recognition by HIV-specific cytotoxic T lymphocytes. This suggests that the usual daily antigenic stimulation of CD4s could produce excess immune activation in individuals with a very large burden of latent HIV. Concordantly, a correlation between the frequency of infected $CD4^+$ T cells and low CD4 counts on ART has been reported several times. However, these studies did not control for CD4 nadir or time on ART, so it is not clear whether SoIRs have a higher burden of infected $CD4^+$ T cells. We hypothesize that increased induction from the HIV latent reservoir (LR), whether because of a larger LR size or increased inducibility from the LR, is correlated with suboptimal immune response. LR size and inducibility have never been simultaneously evaluated, but we will do so using efficient new assays that can discriminate intact from defective HIV proviruses. We will determine whether LR size and inducibility contribute to suboptimal immune response and whether cytokine therapies designed to increase CD4 counts also expand the LR. For Aim 1, we will determine whether the size of the HIV LR in blood and lymphoid tissue is positively correlated with suboptimal immune response using the new intact proviral DNA assay (IPDA), a droplet digital PCR assay that separately quantifies intact and defective proviruses, on samples from SoIRs and age- and nadir-matched controls identified from within the ACTG Longitudinal Linked Randomized Trials study and three large cohorts in Baltimore, San Francisco, and Cleveland. For Aim 2, we will determine whether infected $CD4^+$ T cells of SoIRs are more readily inducible from latency using a quantitative viral induction assay on blood samples from SoIRs and matched controls. For Aim 3, we will determine whether cytokine therapies that increase CD4 count also expand the HIV LR by using the IPDA to measure LR size in samples from clinical trials of exogenous IL-7, IL-15, and IL-2 in treated HIV. Through formal didactic training and structured mentorship from experts in HIV reservoirs, HIV immunology, clinical research, and biostatistics, the PI will develop a unique skillset in HIV latency techniques, immunological techniques and knowledge, statistics, and translational research. This training provides a pathway to an independent career as a translational investigator researching the contribution of viral factors to the pathogenesis of treated HIV.

Project Narrative

A significant percentage of HIV-positive individuals maintained on suppressive antiretroviral therapy have CD4 counts that plateau at abnormally low levels, and this population has a significantly higher rate of morbidity and mortality that persists for at least a decade after starting therapy. The mechanisms underlying this suboptimal immune recovery are poorly defined, but there is suggestive evidence that the induction of HIV from the latent reservoir is higher in this population. By determining whether LR size and inducibility contribute to suboptimal immune response and whether cytokine therapies designed to increase CD4 counts also expand the LR, this proposal will provide critical information that will aid in the development of therapeutics for this high-risk population.

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Facilities and Other Resources

Laboratory Facilities:

Siliciano Laboratory: The laboratory of Dr. Robert Siliciano is located on the [redacted] of the Miller Research Building at the Johns Hopkins University School of Medicine. The laboratory includes 2,200 square feet of laboratory space, a 670 square foot BSL-3 laboratory described below, a conference room, and an office suite. The main laboratory includes a BSL-2 tissue culture room equipped with 2 cell culture hoods, 2 humidified incubators, centrifuges and microscopes. There are two dedicated PCR setup rooms. One has four laminar flow hoods, and the other - specifically dedicated to droplet digital PCR setup - has two laminar flow hoods. The laboratory has a large, walk-in cold room as well as a dark room for film processing. Within the main laboratory space is one real-time PCR machine, one RainDrop digital droplet PCR System, more than 20 thermal cyclers capable of handling tubes and plates, two ELISA plate readers, three -80 freezers, two liquid nitrogen tanks, multiple refrigerators, one gel imaging system, floor and tabletop centrifuges, as well as standard molecular biology and tissue culture equipment. Dr. Antar has dedicated bench space, refrigerator space, and freezer space in the laboratory. The BSL-3 laboratory is equipped with five tissue culture hoods, six humidified incubators, one Sony SH800 Cell Sorter, four flow cytometers (BD FACS Calibur, BD FACS Canto II, Millipore Muse, and Intellicyte iQue Plus Screener), four -80 freezers, 5 centrifuges, 2 ultracentrifuges, a pass-through autoclave, large-scale cell separation magnets, automated cell separation workstation, automated RNA isolation workstation, as well as standard molecular biology and tissue culture equipment. The BSL-3 laboratory is equipped to process and isolate peripheral blood mononuclear cells from routine peripheral blood draws, leukaphereses, and cryopreserved samples. Glass-washing and autoclaving services are provided daily. All assays proposed in this application have been either developed in this laboratory or have been used regularly in this laboratory.

Core Laboratory Facilities: Dr. Antar will have access to the Bloomberg School of Public Health's Core Flow Cytometry facility which allows for live cell sorting of HIV infected cells and is a five minute walk from the Siliciano lab. Next generation sequencing (Illumina MiSeq and many others) is available to Dr. Antar via the Johns Hopkins Deep Sequencing and Microarray Core Facility. Sanger sequencing is available via GeneWiz, which maintains a daily order pickup site within the Siliciano lab.

Clinical Facilities:

HIV Clinic: The newly opened John G. Bartlett Specialty Practice is home to the clinics of the Johns Hopkins Division of Infectious Diseases and is within a five minute walk of Dr. Antar's office. The Barlett clinics are a state-of-the-art, 25 exam room clinic offering services for patients with HIV, viral hepatitis, infections after transplantation, and other infections. It is a multi-disciplinary clinic in which infectious diseases physicians practice alongside a wide range of non-infectious diseases specialists who focus on HIV including psychiatrists, cardiologists, endocrinologists, gynecologists, psychologists, nutritionists, case managers, and social workers. A pharmacy is located on-site, as are phlebotomists. Our HIV clinic has delivered care to over 7,000 patients since 1984. Dr. Antar will enrich her patient panel with suboptimal immune responders.

The Johns Hopkins Hospital: The Johns Hopkins Hospital is a 1000 bed general hospital with more than 30,000 patients discharged from therapeutic services annually. Johns Hopkins is an AIDS Clinical Trials Group site. The inpatient HIV service, the Polk Service, is located on the 4th floor of the Nelson building in the Johns Hopkins Hospital. It is run by four Osler medical residents and attending professors from the Division of Infectious Diseases dedicated to caring for HIV-infected people.

Clinical Samples: Four large cohorts have agreed to provide samples for this proposal.

The Johns Hopkins HIV Clinical Cohort: The JHHCC was founded by Dr. Antar's co-mentor Dr. Richard Moore in 1990 to provide comprehensive longitudinal data on adult patients receiving care at the Johns Hopkins HIV clinics. Approximately 9,000 adults have been enrolled and the cohort has been the source of over 300 manuscripts by over 140 investigators. Clinical data collection is comprehensive and yearly blood samples are cryopreserved and available for Dr. Antar's proposed studies. In addition, individuals can be brought back for extra sample collections if needed.

SCOPE Cohort: The Observational Study of the Consequences of the Protease Inhibitor Era Cohort was initiated in 2000 and is led by Dr. Antar's advisory committee member, Dr. Steven Deeks at UCSF. SCOPE

has enrolled over 2,000 adults. Each participant has clinical data and blood samples collected every 4 months and cryopreserved. Given Dr. Deeks' longstanding research interest in inflammation and immunological failure, the SCOPE cohort is enriched for suboptimal immune responders. Existing protocols have allowed the collection of > 250 research leukaphereses, > 1500 flexible sigmoidoscopies with several gut biopsies for each procedure, and > 100 research lymph node biopsies. Individuals can be brought back for extra sample collections if needed. Dr. Deeks has already provided multiple samples and has brought patients back for blood draws for Dr. Antar's previous studies. In addition, he has demonstrated his commitment to mentoring via frequent email contact, phone meetings, and several hours spent one-on-one advising Dr. Antar at the 2018 Conference on Retroviruses and Opportunistic Infections (CROI).

CLIF Cohort: The Cleveland Immune Failure Cohort was founded by Dr. Antar's collaborator, Dr. Michael Lederman in 2009. Its purpose is to examine the mechanisms underlying suboptimal immune responders' failure to achieve normal immunological function despite effective therapy. Clinical data and cryopreserved PBMCs are available for Dr. Antar's proposed studies, and sample identification is already underway.

ALLRT: The AIDS Clinical Trials Group Longitudinal Linked Randomized Trials study was a longitudinal cohort study of HIV-infected individuals prospectively randomized into selected clinical trials for treatment-naïve and treatment-experienced participants conducted by the AIDS Clinical Trials Group (ACTG). Data and biologic specimens were collected every 4 months for over 5,000 participants with follow-up up to 10 years. Dr. Antar's collaborator Dr. Ronald Bosch was the senior statistician for ALLRT and is facilitating identification of samples from ALLRT for Dr. Antar's proposed studies. Sample identification is already underway.

Office and Computing Resources:

Dr. Antar has an office next door to the offices of Drs. Robert and Janet Siliciano and directly across the hallway from the main Siliciano laboratory, which is on the main Johns Hopkins medical campus. Office resources include high-speed wireless internet, color laser printing, photocopying, scanning, and fax machines, a personal telephone, conference rooms with audiovisual and teleconferencing equipment, office supplies, and administrative support. The infectious diseases division has provided Dr. Antar with a Dell Latitude E7470 laptop computer with an Intel Core i5-6300U processor with 8 GB of RAM and 256 GB of memory. In addition, Dr. Siliciano has provided Dr. Antar with a Dell desktop computer with an Intel Core i5-3570U processor with 8 GB of RAM and 256 GB of memory for her office and access to a suite of Windows-compatible and Macintosh computers in a computing area just outside her office. She has access to Microsoft Office, CLC Genomics Workbench, Mega7, CodonCode Aligner, Anaconda, GPower, Matlab, Past, Adobe Illustrator, Graphpad Prism, RefWorks, and the Johns Hopkins electronic medical record. In addition, she has access to 80 GB of data storage on Johns Hopkins-maintained personal and laboratory network folders. Her co-mentors' office spaces and all classes, seminars, clinics, and meetings are within a five to ten minute walk.

Educational Resources:

A wide array of relevant research, clinical, and career-related conferences and workshops are available and in close proximity to Dr. Antar's office on the Johns Hopkins medical campus. In addition, advanced coursework is offered at the School of Medicine and the Bloomberg School of Public Health. Please see the Institutional Environment sections on the Johns Hopkins School of Medicine, Division of Infectious Diseases, CFAR, and Bloomberg School of Public Health, and see also the Plan for Career Development for specific planned coursework, conferences, and workshops.

Library Resources:

The William H. Welch Medical Library at Johns Hopkins collects medical and scientific literature in all fields of teaching and research represented within the Johns Hopkins Medical Institutions and is second only to the National Library of Medicine for size. A wide range of services are available to the PI including a trained informationist assigned to the infectious diseases division to help with systematic literature and genome searches, electronic access to hundreds of thousands of medical journals and books, and off-campus access to the entire online collection.

Institutional Investment in the Success of the PI:

The Johns Hopkins School of Medicine dedicates multiple resources to supporting junior investigators. The Division of Infectious Diseases has offered 80% protected time for research and career training activities once

Dr. Antar becomes an Assistant Professor, which is anticipated to be in July 2019 if not before, as is documented in the institutional support letter attached to this application. For the 2018-2019 academic year, Dr. Antar has 90% protected time. Tuition remission up to $[redacted] per year towards academic credit courses at Johns Hopkins University is available to Dr. Antar, and non-credit courses offered through continuing education units are free. The Johns Hopkins Center for AIDS Research (CFAR) offers several $[redacted] CFAR Scholar Grants for Faculty Development per year that are open only to junior investigators. [redacted] The Johns Hopkins CFAR provides biostatistical support and support for Institutional Review Board submissions. The Johns Hopkins CFAR has an active “K2R” club which organizes workshops throughout the year on grant writing topics for K awardees and it also provides internal grant reviews. The Johns Hopkins Institute for Clinical and Translational Research hosts an active “K-to-R” Transition Program that provides oversight, support, resources, networking opportunities, and workshops for K-level mentored awardees. [redacted]. Dr. Antar has access to grant support personnel from the Division of Infectious Diseases. The Johns Hopkins Department of Medicine’s Task Force on Women’s Academic Careers in Medicine provides programming in support of its mission to increase leadership development and opportunities for women and to support the career advancement of female faculty and fellows within the department. The Division of Infectious Diseases also has an active Women in ID networking group comprising female faculty and fellows that meets several times per year. These resources, together with the collegial, collaborative, and supportive atmosphere within the Division of Infectious Disease, ensure an excellent environment for Dr. Antar’s career development.

Equipment

- Flow cytometers – Siliciano Laboratory (4) and Johns Hopkins core facilities
- Sony SH800 Cell Sorter – Siliciano Laboratory (1) and Johns Hopkins core facilities
- Tissue culture hoods – Siliciano Laboratory (11)
- Tissue culture incubators – Siliciano Laboratory (8)
- Microscopes – Siliciano Laboratory (4), and Johns Hopkins core facilities
- Microplate readers – Siliciano Laboratory (2)
- ELISA plate washers – Siliciano Laboratory (1)
- Thermal cyclers – Siliciano Laboratory (20+)
- Ultracentrifuges – Siliciano Laboratory (2)
- Real-Time PCR Machines – Siliciano Laboratory (1) and Johns Hopkins core facilities
- Digital Droplet PCR System – Siliciano Laboratory (1) and Johns Hopkins core facilities
- Gel imaging system – Siliciano Laboratory (1)
- Automated liquid handling – Johns Hopkins HIT Center
- Intellicyt iQue Screener Plus - Siliciano Laboratory (1)
- Fume Hoods, microcentrifuges, pipettors, balances, water baths, microwave ovens - Siliciano Laboratory
- Millipore Synthesis nanopure water purification system - Siliciano Laboratory
- Nanodrop UV-Vis Spectrophometer - Siliciano Laboratory
- Alpha Imager 2200 gel documentation system - Siliciano Laboratory
- Liquid nitrogen tanks (2), -80C freezers (6), multiple refrigerators - Siliciano Laboratory
- Electrophoresis supplies, fraction collector, water baths - Siliciano Laboratory
- Dark room facilities, balances, and a cold room - Siliciano Laboratory
- Pass-through autoclave - Siliciano Laboratory

- Note: the numerous Johns Hopkins core facilities are equipped to meet a wide range of research needs.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Annukka Middle Name Aida Rose Last Name*: Antar Suffix:

Position/Title*: Post-doctoral Fellow
Organization Name*: JOHNS HOPKINS UNIVERSITY
Department: Medicine
Division: Infectious Diseases
Street1*: [redacted]
Street2:
City*: Baltimore
County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

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

Degree Type: MD,PHD Degree Year:

Attach Biographical Sketch*: File Name: Biosketch_Antar.pdf

Attach Current & Pending Support: File Name:

Tracking Number: GRANT12621368 Funding Opportunity Number: PA-18-373 . Received Date: 2018-05-04T14:06:47.000-04:00

PROFILE - Senior/Key Person

Prefix: First Name*: ROBERT Middle Name F Last Name*: SILICIANO Suffix:

Position/Title*: Professor
Organization Name*: Johns Hopkins University
Department: Medicine
Division: Infectious Diseases
Street1*: [redacted]
Street2:
City*: BALTIMORE
County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: [redacted]

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: Other (Specify) Other Project Role Category: Primary Mentor

Degree Type: MD,PHD,AB Degree Year: 1982,1983,1974

Attach Biographical Sketch*: File Name: Biosketch_Siliciano.pdf

Attach Current & Pending Support: File Name: OS_Siliciano.pdf

PROFILE - Senior/Key Person

Prefix: First Name*: RICHARD Middle Name Douglas Last Name*: MOORE Suffix:

Position/Title*: Professor
Organization Name*: Johns Hopkins University
Department: Medicine
Division: General Internal Medicine
Street1*: [redacted]
Street2:
City*: BALTIMORE
County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: [redacted]

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: Other (Specify) Other Project Role Category: Co-mentor

Degree Type: MD,MS,BA Degree Year: 1978,1988,1974

Attach Biographical Sketch*: File Name: Biosketch_Moore.pdf

Attach Current & Pending Support: File Name: OS_Moore.pdf

PROFILE - Senior/Key Person

Prefix: First Name*: JOEL Middle Name N Last Name*: BLANKSON Suffix:

Position/Title*: AssociateProfessor
Organization Name*: Johns Hopkins University
Department: Medicine
Division: Infectious Diseases
Street1*: [redacted]
Street2:
City*: BALTIMORE
County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: [redacted]

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: Other (Specify) Other Project Role Category: Co-mentor

Degree Type: MD,PHD,BA Degree Year: 1995,1994,1984

Attach Biographical Sketch*: File Name: Biosketch_Blankson.pdf

Attach Current & Pending Support: File Name: OS_Blankson.pdf

PROFILE - Senior/Key Person

Prefix: First Name*: STEVEN Middle Name Grant Last Name*: DEEKS Suffix:

Position/Title*: Professor
Organization Name*: UCSF
Department: Medicine
Division: Infectious Diseases
Street1*: [redacted]
Street2:
City*: SAN FRANCISCO
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: [redacted]

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: Other (Specify) Other Project Role Category: Collaborator

Degree Type: MD Degree Year: 1990

Attach Biographical Sketch*: File Name: Biosketch_Deeks.pdf

Attach Current & Pending Support: File Name:

BIOGRAPHICAL SKETCH

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

NAME: Annukka A. R. Antar

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Infectious Diseases Fellow

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Harvard University, Cambridge, MA	A.B.	06/2002	Chemistry
Vanderbilt University, Nashville, TN	Ph.D.	08/2008	Microbiology & Immunology
Vanderbilt University, Nashville, TN	M.D.	05/2010	Medicine
The Johns Hopkins Hospital, Baltimore, MD	Residency	06/2013	Internal Medicine
The Johns Hopkins Hospital, Baltimore, MD	Fellowship	06/2019 (expected)	Infectious Diseases

A. Personal Statement

I am an infectious diseases-trained physician-scientist, and my long-term research goal is to investigate the viral contributors to the pathogenesis of treated HIV. I have a long-standing commitment to HIV research, a track record of productivity, and a suite of prior research and clinical experiences that, together with the training proposed in this K08 application, will uniquely position me to use cutting-edge techniques to discern the contribution of persistent HIV to the excess morbidity and mortality seen in treated HIV. I developed foundational skills in basic viral pathogenesis during my PhD studies in the lab of Dr. Terence Dermody. While there, I elucidated the role of the mammalian reovirus receptor, Junctional Adhesion Molecule-A, in reovirus pathogenesis. [redacted]. I have a strong clinical background in internal medicine and infectious diseases, and I stayed productive while completing clinical training by publishing a chapter in an infectious diseases textbook, two opinion pieces, three case reports, and four online modules in the Johns Hopkins HIV Guide. My research training in fellowship is with Dr. Robert Siliciano (primary mentor), and during this time I have focused on characterizing the dynamics of the HIV provirus landscape over long periods of time on antiretroviral therapy. To obtain samples for this work, I have collaborated with Dr. Richard Moore (co-mentor) and Dr. Steven Deeks (advisory committee member). This work was presented at the 2017 Keystone Symposium on Viral Immunity. [redacted]. The work I have done in fellowship and my experiences taking care of HIV-positive people in clinic have motivated me to understand the contribution of HIV persistence to the underlying poor CD4 recovery and excess morbidity and mortality in suboptimal immune responders on antiretroviral therapy, a focus that builds on my research and clinical strengths and that of my mentorship team and is the subject of this K08 application. The basic pathogenesis skills I gained during my graduate studies and the intensive knowledge of the virologic factors important to HIV persistence in treated HIV gained during my fellowship studies provide a strong foundation for these studies, but I require more expertise in several areas to attain independence in my planned career. I propose training in viral latency techniques, immunological techniques and knowledge, biostatistics, practicalities of translational research, and scientific writing. An outstanding mentorship team, existing collaborations with large research cohorts and investigators, and a structured career development plan will make this goal attainable. With the benefit of a career development award, I will be able

to cultivate the necessary skills to launch a career as an independent investigator studying viral contributors to the pathogenesis of treated HIV.

1. Antar, AAR, Konopka, JL, Campbell, JA, Henry, RA, Perdigoto, AL, Carter, BD, Pozzi, A, Abel, TW, Dermody, TS. Junctional adhesion molecule-A is required for hematogenous dissemination of reovirus. Cell Host Microbe. 2009; 5:59-71. PubMed PMID: 19154988; PubMed Central PMCID: PMC2642927.

2. Kobayashi, T, Antar, AAR, Boehme, KW, Danthi, P, Eby, EA, Guglielmi, KM, Holm, GH, Johnson, EM, Maginnis, MS, Naik, S, Skelton, WB, Wetzel, JD, Wilson, GJ, Chappell, JD, Dermody, TS. A plasmid-based reverse genetics system for animal double-stranded RNA viruses. Cell Host Microbe. 2007; 1:147-57. PubMed PMID: 18005692; PubMed Central PMCID: PMC2034303.

3. Antar, AAR, Keruly, J, Moore, R, Siliciano J, Siliciano R, Ho, Y. The HIV-1 Provirus Landscape is Dynamic in Patients on ART. Keystone Symposium on Viral Immunity, Santa Fe, NM, 2017.

4. Bertagnolli, LN, White, JA, Beg, SA, Simonetti, FR, Lai, J, Tomescu, C, Murray, AJ, Antar, AAR, Zhang, H, Margolick, JB, Montaner, LJ, Siliciano, RF, Laird, GM, Siliciano, JD. Brief Communication Arising: Majority of HIV Reservoir is CD32 Negative. Accepted to Nature. 2018.

B. Positions and Honors

Positions:

2010-2013: Internal Medicine Residency, Johns Hopkins Hospital, Baltimore, MD
2013-2014: Instructor, Division of Hospital Medicine, University of Colorado School of Medicine, Aurora, CO
2014-2015: Hospitalist Physician, Northern Colorado Hospitalists, Fort Collins, CO
2015-present: Infectious Diseases Fellowship, Johns Hopkins Hospital, Baltimore, MD

Honors:

1998 Tandy Technology Scholar
1998-2002 National Merit Scholarship
1998-2002 Robert C. Byrd Scholarship
1998-2002 Aid Association for Lutherans Scholarship
1999-2001 Dean's List, Harvard College
2002 *Magna cum laude*, Chemistry, Harvard College
2008 Sidney P. Colowick Award for Graduate Research
2009 First-author manuscript selected as "Must Read" by Faculty of 1000
2010 Vanderbilt's Award for Excellence in Infectious Diseases
2010 Vanderbilt's Rudolph H. Kampmeier Prize in Clinical Medicine
2016-2018 NIH Loan Repayment Program Awardee
2018-2019 Pearl M. Stetler Research Fellowship

Clinical Licensures and Board Certifications:

2013 Board Certification in Internal Medicine, ABIM
2013-2015 Colorado Medical License
2015-current Maryland Medical License
2017 Board Certification in Infectious Diseases, ABIM

C. Contributions to Science

1. During my PhD studies, I elucidated the role of the mammalian reovirus receptor Junctional Adhesion Molecule-A (JAM-A) in reovirus pathogenesis. JAM-A, like many other virus receptors, is a member of the immunoglobulin superfamily, and it was known to localize to endothelial and epithelial cell tight junctions, which are smaller than the diameter of a reovirus virion. Prior to my work, the role of JAM-A in reovirus pathogenesis was unknown. Using a JAM-A-deficient mouse model and select primary tissues, I demonstrated that JAM-A is required for reovirus infection of endothelial cells and establishment of viremia, but it is dispensable for

replication in the brain and intestine, dissemination via nerves and lymphatics, and transmission to other individuals. The finding that a virus receptor expressed in a wide variety of tissues is responsible for a specific step in virus pathogenesis was novel and suggested that each step in the complex process of viral pathogenesis may be mediated by specific virus-host interactions. This work was selected as a “Must Read” by the Faculty of 1000. My work led to several further pathogenesis studies by others, including one that demonstrated that endothelial-expressed JAM-A was required for viremia and another that revealed that the Nogo receptor NgR1 mediates reovirus infection of nerve tissue. I also contributed significantly to the first report of a reverse genetics system for animal double stranded RNA viruses by testing the first engineered mutant viruses for virulence phenotypes in mice.

1. Antar, AAR, Konopka, JL, Campbell, JA, Henry, RA, Perdigoto, AL, Carter, BD, Pozzi, A, Abel, TW, Dermody, TS. Junctional adhesion molecule-A is required for hematogenous dissemination of reovirus. Cell Host Microbe. 2009; 5:59-71. PubMed PMID: 19154988; PubMed Central PMCID: PMC2642927.

2. Kobayashi, T, Antar, AAR, Boehme, KW, Danthi, P, Eby, EA, Guglielmi, KM, Holm, GH, Johnson, EM, Maginnis, MS, Naik, S, Skelton, WB, Wetzel, JD, Wilson, GJ, Chappell, JD, Dermody, TS. A plasmid-based reverse genetics system for animal double-stranded RNA viruses. Cell Host Microbe. 2007; 1:147-57. PubMed PMID: 18005692; PubMed Central PMCID: PMC2034303.

2. In my fellowship research to date, I have continued my studies of viral pathogenesis by using sequencing to characterize HIV persistence on antiretroviral therapy. I collaborated with Dr. Steve Deeks (advisory committee member) of UCSF and Dr. Richard Moore (co-mentor) of Johns Hopkins to find longitudinal samples from their research cohorts from HIV-positive people and elite controllers who had been suppressed on ART for 7-10 years. I then generated a large database of high-quality, full-length HIV provirus sequences from these longitudinal samples. [redacted] I have shown that the percentage of clonally proliferated cells harboring HIV proviruses increases over time on ART and that the HIV provirus landscape in elite controllers on ART is similar to that of chronic progressors. [redacted]. This work was presented at the 2017 Keystone Symposium on Viral Immunity. My work prior to joining the Siliciano laboratory resulted in a co-first author report describing the use of nanopore sequencing technology to elucidate bacterial pathogenesis in a patient infected with hypervirulent *Klebsiella pneumoniae*. I also contributed significantly via sequencing and PCR studies to the report that the majority of the HIV latent reservoir is CD32 negative.

1. Antar, AAR, Keruly, J, Moore, R, Siliciano J, Siliciano R, Ho, Y. The HIV-1 Provirus Landscape is Dynamic in Patients on ART. Keystone Symposium on Viral Immunity, Santa Fe, NM, 2017.

2. Simner, PJ, Antar, AAR (co-first author), Hao, S, Gurtowski, J, Tamma, PD, Rock, C, Opene BNA, Tekle, T, Carroll, KC, Schatz, MC, Timp, W. Antibiotic pressure on the acquisition and loss of antibiotic resistance genes in *Klebsiella pneumoniae*. J Antimicrob Chemother. 2018. Apr 10. Doi: 10.1093/jac/dky121. Epub ahead of print. Pubmed PMID: 29648629. No PMC ID available yet.

3. Bertagnolli, LN, White, JA, Beg, SA, Simonetti, FR, Lai, J, Tomescu, C, Murray, AJ, Antar, AAR, Zhang, H, Margolick, JB, Montaner, LJ, Siliciano, RF, Laird, GM, Siliciano, JD. Brief Communication Arising: Majority of HIV Reservoir is CD32 Negative. Accepted to Nature. 2018. No PMID or PMC ID available yet.

3. During my clinical training, I cared for patients who were affected by the sudden rise in price and restriction in access to the anti-toxoplasma medication pyrimethamine, also known as Daraprim. The cost of one pill rose from $[redacted] to $[redacted] overnight. One patient consented to have the use of her story published to illustrate the effects this marketing strategy was having on the health of individuals. I led the clinical team in writing an op-ed describing her story that was published in USA Today, and I contributed as second author to an opinion piece on antimicrobial access in the Annals of Internal Medicine. My other contributions during clinical training include one first-author and one last-author case report and an infectious diseases textbook chapter, not listed here.

1. Dickstein, L, Kruzan, R, Antar A. Real cost of outrageous drug prices. Dec 28, 2015. USA Today.

2. Shoham, S, Antar AA, Auwaerter PG, Durand CM, Sulkowski MS, Cotton DJ. Antimicrobial Access in the 21st Century: Delays and Critical Shortages. 2016. Ann Intern Med. 165(1):53-54.

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/1fSM4QQ5O8IQg/bibliography/50594701/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support
5T32AI007291-27
Cosgrove, Sara (PI)
07/01/16 – 06/30/18
Institutional Training Grant
I am supported by the Infectious Diseases Division's T32 grant in my fellowship research years. This supports my salary and some training-related expenses while I conduct research in Dr. Robert Siliciano's laboratory on the dynamics of the HIV-1 provirus landscape in individuals on long-term suppressive antiretroviral therapy.
Role: Fellow

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: JOHNS HOPKINS UNIVERSITY

Start Date*: 12-01-2018 **End Date*:** 11-30-2019 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Annukka	A.R.	Antar		PD/PI	[redacted]	9.6			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-18-373 . Received Date: 2018-05-04T14:06:47.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: JOHNS HOPKINS UNIVERSITY

Start Date*: 12-01-2018 **End Date*:** 11-30-2019 **Budget Period: 1**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12621368 Funding Opportunity Number: PA-18-373 . Received Date: 2018-05-04T14:06:47.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: JOHNS HOPKINS UNIVERSITY

Start Date*: 12-01-2018 **End Date*:** 11-30-2019 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	8.0	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification*	File Name: Budget_Justification.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: JOHNS HOPKINS UNIVERSITY

Start Date*: 12-01-2019 **End Date*:** 11-30-2020 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Annukka	A.R.	Antar		PD/PI	[redacted]	9.6			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**

Total Salary, Wages and Fringe Benefits (A+B) [redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-18-373 . Received Date: 2018-05-04T14:06:47.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 0019107770000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: JOHNS HOPKINS UNIVERSITY

Start Date*: 12-01-2019 **End Date*:** 11-30-2020 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $[redacted]

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12621368 Funding Opportunity Number: PA-18-373 . Received Date: 2018-05-04T14:06:47.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: JOHNS HOPKINS UNIVERSITY

Start Date*: 12-01-2019 **End Date*:** 11-30-2020 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	8.0	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification*	File Name: Budget_Justification.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: JOHNS HOPKINS UNIVERSITY

Start Date*: 12-01-2020 **End Date*:** 11-30-2021 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Annukka	A.R.	Antar		PD/PI	[redacted]	9.6			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**

Total Salary, Wages and Fringe Benefits (A+B) [redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-18-373 . Received Date: 2018-05-04T14:06:47.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: JOHNS HOPKINS UNIVERSITY

Start Date*: 12-01-2020 **End Date*:** 11-30-2021 **Budget Period: 3**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12621368 Funding Opportunity Number: PA-18-373 . Received Date: 2018-05-04T14:06:47.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: JOHNS HOPKINS UNIVERSITY

Start Date*: 12-01-2020 **End Date*:** 11-30-2021 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	8.0	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification*	File Name: Budget_Justification.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: JOHNS HOPKINS UNIVERSITY

Start Date*: 12-01-2021 **End Date*:** 11-30-2022 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Annukka	A.R.	Antar		PD/PI	[redacted]	9.6			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-18-373 . Received Date: 2018-05-04T14:06:47.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: JOHNS HOPKINS UNIVERSITY

Start Date*: 12-01-2021 **End Date*:** 11-30-2022 **Budget Period: 4**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12621368 Funding Opportunity Number: PA-18-373 . Received Date: 2018-05-04T14:06:47.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: JOHNS HOPKINS UNIVERSITY

Start Date*: 12-01-2021 **End Date*:** 11-30-2022 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	8.0	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification*	File Name: Budget_Justification.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: JOHNS HOPKINS UNIVERSITY

Start Date*: 12-01-2022 **End Date*:** 11-30-2023 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Annukka	A.R.	Antar		PD/PI	[redacted]	9.6			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**

Total Salary, Wages and Fringe Benefits (A+B) [redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-18-373 . Received Date: 2018-05-04T14:06:47.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: JOHNS HOPKINS UNIVERSITY

Start Date*: 12-01-2022 **End Date*:** 11-30-2023 **Budget Period: 5**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12621368 Funding Opportunity Number: PA-18-373 . Received Date: 2018-05-04T14:06:47.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: JOHNS HOPKINS UNIVERSITY

Start Date*: 12-01-2022 **End Date*:** 11-30-2023 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	8.0	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification*	File Name: Budget_Justification.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

PERSONNEL

Annukka A. R. Antar, MD, PhD (Principal Investigator)
As an Assistant Professor, Dr. Antar will devote 9.6 calendar months (80% effort) to mentored research and career development activities during the award period. The primary focus of her efforts throughout the career development program will be achieving the experimental aims of this proposal, attaining additional expertise in the topics outlined in the career development plan, and using this work to build towards an independent research platform. She will have primary responsibility for study design, data collection, analysis, interpretation, and presentation of results as outlined in this K08 application. We are requesting 5 years of salary and fringe benefits for Dr. Antar and propose to begin on December 1, 2018. The fringe benefit rate for personnel is 34% and comprises medical and dental insurance, short- and long-term disability insurance, life insurance, taxes, tuition remission, and 403(b) retirement plans.

No salary support is requested for other key personnel.

LABORATORY SUPPLIES AND COMPUTING

$ per year is requested for additional laboratory supplies, reagents, sample identification, and shipping costs. We are requesting additional funds totaling $ for costs pertaining to sample identification and shipping from off-site locations (SCOPE, ALLRT, CLIF, NIAID, ACTG, U. of Minnesota) for the duration of the award.

Funds are requested for Johns Hopkins IT management of Dr. Antar's laptop (required by the Department of Medicine at $/year), software purchases, and an upgrade of Dr. Antar's laptop computer in Year 4 of the award, at a total of $ over the course of the 5 year award.

EDUCATION, TRAVEL, AND PUBLICATION

Funds are requested for tuition expenses at the Johns Hopkins Bloomberg School of Public Health, the School of Medicine, and the Krieger School of Arts & Sciences (excluding those covered in the $/year tuition remission provided to Johns Hopkins faculty) estimated at $ total over the course of the 5 year award for the coursework listed in the career development plan.

Funds estimated at $/year x 5 years are requested for Dr. Antar to travel to related to HIV pathogenesis and immunity for presentation of results during the award period, as described in the career development plan. These costs will cover conference registration, airfare, accomodations, and daily costs incurred for attendance.

Funds estimated at $/year x 5 years are requested to cover funds for journal publication for Dr. Antar's first-author manuscripts and poster printing costs that arise from this award.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		[redacted]
Section B, Other Personnel		0.00
Total Number Other Personnel	0	
Total Salary, Wages and Fringe Benefits (A+B)		[redacted]
Section C, Equipment		0.00
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		[redacted]
1. Materials and Supplies	[redacted]	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		[redacted]
Section H, Indirect Costs		[redacted]
Section I, Total Direct and Indirect Costs (G + H)		[redacted]
Section J, Fee		0.00
Section K, Total Costs and Fee (I + J)		[redacted]

Funding Opportunity Number: PA-18-373 . Received Date: 2018-05-04T14:06:47.000-04:00

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OMB Number: 0925-0001

Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ○ Yes ● No

If "Yes" to euthanasia

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

○ Yes ○ No

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

2. *Program Income Section

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

○ Yes ● No

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

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

Tracking Number: GRANT12621368

Funding Opportunity Number: PA-18-373 . Received Date: 2018-05-04T14:06:47.000-04:00

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3. Human Embryonic Stem Cells Section

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

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

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

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ● No

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

*Previously Reported: ○ Yes ○ No

5. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

Tracking Number: GRANT12621368 Funding Opportunity Number: PA-18-373 . Received Date: 2018-05-04T14:06:47.000-04:00

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OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Candidate Section	
2. Candidate Information and Goals for Career Development	Candidate_Section_Antar-K08.pdf
Research Plan Section	
3. Specific Aims	Specific_Aims_K08.pdf
4. Research Strategy*	Research_Strategy_Antar-K08.pdf
5. Progress Report Publication List (for Renewal applications)	
6. Training in the Responsible Conduct of Research	Training_in_the_Responsible_Conduct_of_Research.pdf
Other Candidate Information Section	
7. Candidate's Plan to Provide Mentoring	
Mentor, Co-Mentor, Consultant, Collaborators Section	
8. Plans and Statements of Mentor and Co-Mentor(s)	Statement_of_Mentor_CoMentor.pdf
9. Letters of Support from Collaborators, Contributors, and Consultants	FINAL_LOSs_Collaborators_Contributors_Consultants.pdf
Environment and Institutional Commitment to Candidate Section	
10. Description of Institutional Environment	Description_of_the_Institutional_Environment_Antar-K08.pdf
11. Institutional Commitment to Candidate's Research Career Development	Institutional_Commitment_Antar.pdf
Other Research Plan Section	
12. Vertebrate Animals	
13. Select Agent Research	Select_Agent_Research.pdf
14. Consortium/Contractual Arrangements	
15. Resource Sharing	Resource_Sharing_Plan.pdf
16. Authentication of Key Biological and/or Chemical Resources	Authentication_of_Key_Biological_andor_Chemical_Resources.pdf
Appendix	
17. Appendix	

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Citizenship*:

18. U.S. Citizen or Non-Citizen National?* ● Yes ○ No

If no, select most appropriate Non-U.S. Citizen option

- ❑ With a Permanent U.S. Resident Visa
- ❑ With a Temporary U.S. Visa
- ❑ Not Residing in the U.S.

If you are a non-U.S. citizen with a temporary visa applying for an award that requires permanent residency status, and expect to be granted a permanent resident visa by the start date of the award, check here: ❑

Tracking Number: GRANT12621368 Funding Opportunity Number: PA-18-373. Received Date: 2018-05-04T14:06:47.000-04:00

CANDIDATE'S BACKGROUND

Introduction: I am a physician-scientist with specialty training in infectious diseases and a strong commitment to improving the lives of HIV-positive individuals worldwide through clinically impactful translational research. I first experienced the joy of scientific discovery from my parents, both physical scientists, who candidly discussed their scientific careers with me as I grew up. Since my undergraduate studies at Harvard, I have been motivated to pursue a career in HIV research and clinical care because of the global scale of the HIV pandemic and because HIV disproportionately affects the disadvantaged.

MD/PhD Training: During my first two years of medical school at Vanderbilt, I pursued my interest in HIV by working with physicians at Vanderbilt's multi-specialty HIV clinic. I also co-led a feasibility study using telemedicine to increase access to HIV specialty care for rural West Virginians. I prioritized excellent mentorship and meticulous science as I sought a graduate mentor, even if it meant that I did not directly study HIV in graduate school. Therefore, after my second year of medical school, I joined the lab of Dr. Terence Dermody, who himself had trained with Dr. Bernard Fields and whose lab focused on reovirus entry.

Dr. Dermody's lab had recently discovered that junctional-adhesion molecule A (JAM-A) served as a reovirus receptor. Many tight- and adherens-junction proteins like JAM-A were known to be viral receptors, but it was a mystery whether these proteins, which are localized to an intercellular space smaller than the diameter of a virion, played a significant role in host pathogenesis. I used a JAM-A-deficient mouse model and select primary tissues from fetal and neonatal mice to demonstrate that this tight-junction protein was required for infection of endothelial cells and spread through the bloodstream. Surprisingly, it had no role in viral infection at the primary site of replication - the intestine - nor did it have roles in fecal shedding or spread through nerves. My finding that a broadly expressed receptor mediates an exquisitely specific aspect of viral pathogenesis led to the model that virus-host interactions require multiple receptors that serve unique functions at each step of the disease process. This work led to a first-author manuscript in *Cell Host & Microbe* and prompted a search for the neural receptor for reovirus. During the course of this project, I acquired skills in basic viral pathogenesis and brought new techniques and collaborators to our lab. I also contributed as a second author to an exciting report of the first reverse genetics system for animal double-stranded RNA viruses. Soon after defending my dissertation, I was chosen by the faculty of the Microbiology & Immunology Department to receive the Sidney P. Colowick Award for Graduate Research, given each year to one graduate student. I focused on clinical training during my final two years of medical school and was awarded the Rudolph H. Kampmeier Prize in Clinical Medicine and the Award for Excellence in Infectious Diseases upon graduation.

Clinical Training: After completion of my MD/PhD in 2010, I joined the Osler internal medicine residency program at Johns Hopkins. During my brief elective stints, I wrote a chapter in an infectious diseases textbook and spent time on the infectious diseases (ID) consult service. [redacted] In 2015, I began ID fellowship at Johns Hopkins and furthered my manuscript and grant-writing skills by applying successfully for an NIH Loan Repayment Program award and publishing three case reports and two opinion pieces.

Fellowship Research: After finishing my clinical year of fellowship, I joined the lab of Dr. Robert Siliciano, a leader in the field of HIV latency. Dr. Ya-Chi Ho, a junior faculty in Dr. Siliciano's lab, had recently reported that the vast majority of HIV proviruses integrated into the genomes of latently-infected $CD4^+$ T cells are defective. She then demonstrated that stimulation of cells harboring certain types of defective HIV proviruses leads to HIV transcription and subsequent targeting by autologous HIV-specific cytotoxic T lymphocytes. I decided to look for *in vivo* evidence of this by characterizing the dynamics of the HIV provirus landscape over time on ART. Dr. Steve Deeks (advisory committee member) of UCSF and Dr. Richard Moore (co-mentor) helped me find longitudinal samples from their cohorts from chronic progressors and elite controllers who had been on ART for 7-10 years. I generated a large database of high-quality, full-length HIV provirus sequences from these samples. To optimize efficiency, I utilized next generation sequencing rather than the Sanger sequencing techniques traditionally used in our lab, and I developed an analysis pipeline for our lab. This work demonstrates that the percentage of clonally proliferated cells harboring HIV proviruses increases over time on ART and that the HIV provirus landscape in elite controllers on ART is similar to that of chronic progressors. [redacted] My collaborative

work within the laboratory has resulted in authorship on a brief communication accepted to *Nature*. During this time I also competed successfully for the Pearl M. Stetler Research Fellowship Award.

Proposed Work: In fellowship clinic, I cared for many patients with suboptimal CD4 rise despite antiretroviral therapy (ART). When I learned that their mortality rate was much higher than others, and that this increased mortality persisted for years, I became motivated to research why. Important work has been done in this population uncovering a strong correlation between suboptimal immune response and lymphocyte activation, but virologic persistence and its downstream effects have not been examined fully, in part because the HIV latent reservoir is technically challenging to study. New techniques recently developed in my primary mentor Dr. Siliciano's laboratory now allow us to answer fundamental questions about how the latent reservoir affects clinical outcomes such as suboptimal immune response.

I currently possess skills in basic viral pathogenesis from my graduate studies and intensive knowledge of the virologic factors important for HIV persistence from my fellowship studies. I envision for myself a career as an independent translational investigator studying the contribution of viral persistence to the excess morbidity and mortality in treated HIV. To attain this goal, I still need training in several areas: viral latency techniques, immunological techniques and knowledge, statistics, practicalities of translational research, scientific writing, leadership, mentorship, and pedagogy. The mentoring team I have assembled and the career development plan we have created will fill these training gaps and position me for independence.

CAREER GOALS & OBJECTIVES

LONG-TERM GOALS: (1) to become an independent translational investigator researching the viral contributors to the pathogenesis of treated HIV, **(2)** to provide excellent clinical care for people living with HIV, and **(3)** to provide warm and joyful mentorship for more-junior scientists and physicians.

ADDITIONAL EXPERTISE NEEDED:

Objective 1: Viral Latency Techniques. I currently have expertise in sequencing HIV proviruses and in standard quantitative PCR-based latency assays. To achieve my career goals, I must master the other important methods used in the field: [redacted]

Objective 2: Immunological Techniques and Knowledge. Because inflammation and legacy immune damage likely play a role in the pathogenesis of treated HIV, I require training and mentorship in immunology and immunological techniques beyond the basics gleaned from my graduate training. [redacted]

Mentors: My co-mentor Dr. Joel Blankson and my advisory committee members Drs. Steven Deeks and Peter Hunt possess significant expertise in inflammation and immunity in HIV. All three as well as Dr. Robert Siliciano will provide guidance as I master basic immunological techniques such as flow cytometry.

Objective 3: Statistics and Translational Research. In order to launch a career as an independent investigator, I need further training in statistics and the practicalities of translational research. All researchers need a firm grounding in statistics in order to implement research that is rigorous and reproducible, even when including biostatisticians as key personnel on the research team. [redacted] My co-mentor Dr. Richard Moore will provide expertise in the practicalities of working with clinical cohorts, and my co-mentor Dr. Joel Blankson will advise me in the practicalities of translational research in HIV.

Objective 4: Scientific Writing, Team Leadership, Mentorship, and Pedagogy. I propose specific training activities, described below, to further my skills in grant writing, team dynamics and leadership, mentorship, and pedagogy. Each of these skills is required for a successful career as an academic physician-scientist. Mentors: Each member of my mentorship team models individual successful practices in these areas and will provide guidance as opportunities arise.

Objective 5: Clinical Infectious Diseases. To continually hone and improve my clinical skills, I will practice in the HIV clinic, on the ID consult service, [redacted]

[redacted] below. In my proposed work, which underlies my career development plan, I will determine whether HIV persistence contributes to suboptimal immune response and whether cytokine-based therapies amplify the latent reservoir of HIV. I will dedicate a minimum of 80% FTE to the training and research proposed here and 20% to clinical practice. Mastering the competencies outlined above and completing the studies proposed here will be foundational to my success in obtaining an R01 grant and will help launch my career as an independent physician-scientist.

PLAN FOR CAREER DEVELOPMENT/TRAINING ACTIVITIES

[redacted] This plan, guided by the mentorship team outlined below, will lead to my developing expertise in the following areas: viral latency techniques, immunological techniques & knowledge, statistics & translational research, scientific writing, team leadership, mentorship, pedagogy, and clinical infectious diseases.

MENTORS:

Robert Siliciano MD, PhD (primary mentor). Professor of Medicine at Johns Hopkins. Expertise: He originally described the latent reservoir of HIV and has remained a leader in the field of HIV latency for nearly two decades. Contribution to K08: He will directly supervise and oversee all aspects of this project, and he will direct my independent reading during the early years of the award. [redacted]

Richard Moore, MD (co-mentor). Professor of Medicine at Johns Hopkins. Expertise: He established the Johns Hopkins HIV Clinical Cohort (JHHCC) and the North American AIDS Cohorts Collaboration on Research and Design (NA-ACCORD), and he has performed foundational research describing the natural history of HIV and its comorbidities. [redacted]

Joel Blankson, MD, PhD (co-mentor). Associate Professor at Johns Hopkins. Expertise: He has performed foundational research on the pathogenesis of elite control in HIV. [redacted]

ADVISORY COMMITTEE:

Steven Deeks, MD (advisory committee). Professor of Medicine at UCSF. Expertise: He defined the association between inflammation, immune dysfunction, viral persistence, and disease in HIV. He leads the SCOPE cohort and is the PI of the DARE UM1 Martin Delaney Collaboratory. [redacted]

Peter Hunt, MD (advisory committee). Associate Professor of Medicine at UCSF. Expertise: He characterized the immune activation that persists in treated HIV infection and found that it is associated with suboptimal immune response. He has also led randomized clinical trials of immune interventions in suboptimal immune responders (SoIRs). [redacted]

Janet Siliciano, PhD (advisory committee). Associate Professor of Medicine at Johns Hopkins. Expertise: She helped design and refine the gold-standard HIV latent reservoir assay and has deep knowledge of all latency assays. [redacted]

Bareng Aletta Nonyane, PhD, MSc (advisory committee). Assistant Scientist at Johns Hopkins. Expertise: She is a biostatistician with expertise in evaluation of biologic markers for disease diagnosis and analysis of clustered data with applications in HIV research. Contribution to K08: Her statistical expertise will help ensure my research design is rigorous and she will guide my acquisition of knowledge in statistics. In our bi-monthly meetings, we will discuss research design, data analysis, and plan future studies.

COLLABORATORS:

Irini Sereti, MD. Chief, HIV Pathogenesis Section, NIAID. She has expertise in CD4 lymphopenic states, and her contribution to the K08 is to provide samples from IL-2 treated individuals for Aim 3.

Michael Lederman, MD. Professor at Case Western. He has expertise in suboptimal immune responders, and his contribution to the K08 is to provide samples from the Cleveland Immune Failure cohort for Aims 1 and 2.

Timothy Schacker, MD. Professor at the University of Minnesota. He reported the lymphoid fibrosis seen in many suboptimal immune responders and has expertise in HIV pathogenesis in lymphoid tissues. His contribution to the K08 is to provide samples from IL-15 treated individuals for Aim 3.
Ronald Bosch, PhD. Principal Research Scientist at Harvard. He is a biostatistician with expertise in data synthesis to understand disease pathogenesis in HIV. His contribution to the K08 is to help provide samples from the AIDS Clinical Trials Longitudinal Linked Randomized Trials (ALLRT) cohort for Aims 1 and 2.

CAREER DEVELOPMENT PLAN:

Objective 1: Viral Latency Techniques. I will take the graduate *HIV Biology* course taught by Dr. Robert Siliciano, and I will continue my independent reading of manuscripts relevant to HIV persistence and pathology, overseen by Dr. Robert Siliciano in our weekly meetings. pathogenesis.

Objective 2: Immunological Techniques and Knowledge. I will employ hands-on training in the laboratory and discuss immunity-related research directions regularly with Drs. Siliciano, Blankson, Deeks, and Hunt.

Objective 3:

Planned Coursework

Course	Course	Award Yr /Term	Topics covered

Objective 4: Scientific Writing, Team Leadership, Mentorship, and Pedagogy. **Scientific Writing**. I will hone my grant writing skills by applying for the NIH Center for Scientific Review's Early Career Reviewer Program. I will attend the rich grant writing programming offered by the Johns Hopkins CFAR K2R Club and the JHU Institute for Clinical and Translational Research's "K-to-R" Transition Program. Each offers 2-4 activities per year aimed at strengthening participants' grant and scientific writing skills. **Team Leadership**. To prepare myself for a career leading a laboratory team, I will attend the Johns Hopkins School of Medicine's Junior

Faculty Leadership Program, consisting of 14 hours of leadership training in the 3rd award year. **Mentorship**. To achieve my goal of providing warm and joyful mentorship to trainees, I will engage with the National Research Mentoring Network (NRMN), take their 2-hour online course "Optimizing the Practice of Mentoring", and complete the requirements to be a certified NRMN Mentor in years 4 and 5 of the award. I will employ formal two-way feedback with my mentees every 6 months to ensure a healthy mentoring relationship. [redacted]

Objective 5: Clinical Infectious Diseases. I will continue to hone my clinical skills while learning to balance clinical and research responsibilities by devoting no more than **20% FTE** to clinical practice and clinical conferences. I will attend on the ID consult service for 4 weeks per year and maintain a weekly half-day HIV continuity clinic, into which I will recruit suboptimal immune responders. [redacted] My advisory committee includes several physician-scientist mentors that model the ability to maintain successful research careers while continuing to provide excellent clinical care.

[redacted]

Career Milestones: [redacted]

Timeline of Activities (RS- Robert Siliciano; RM- Richard Moore; JB-Joel Blankson; SD-Steven Deeks; PH- Peter Hunt; JS- Janet Siliciano; AN- Aletta Nonyane.)

	Year 1	Year 2	Year 3	Year 4	Year 5
Objective 1: Viral Latency Techniques					
[redacted]					

A significant percentage of HIV-positive individuals who start antiretroviral therapy (ART) with a low $CD4^+$ T cell count have CD4 counts that plateau at abnormally low levels (<350 cells/μL) despite years of virologic suppression on ART (suboptimal immune responders or SoIRs)[1]. Several studies have shown that the risk of death for SoIRs is 2-3 times higher than that of individuals whose CD4 count rises appropriately with ART[2,3]. We estimate conservatively that over one million people globally are SoIRs today, and their numbers will rise with the global effort to increase access to ART[2,4-7]. Appropriate therapeutics for SoIRs can only be designed once the contributors to suboptimal immune response are understood. **Knowledge gap:** The mechanisms underlying suboptimal immune response and increased mortality among SoIRs remain poorly defined.

One clear association has emerged: SoIRs have higher levels of immune activation than other ART-treated individuals[8-15]. Recent work from our lab demonstrated that if $CD4^+$ T cells harboring intact and certain defective latent HIV proviruses are stimulated through their T cell receptor, HIV RNA and protein production are induced and results in recognition by HIV-specific cytotoxic T lymphocytes[16]. This suggests that the usual daily antigenic stimulation of $CD4^+$ T cells could produce excess immune activation in individuals with a very large burden of latent HIV. Given that there are reports suggesting that SoIRs have increased frequencies of cell-associated HIV DNA and RNA, we hypothesize that increased HIV induction from the latent reservoir (LR), whether because of a larger LR size or increased inducibility from the LR or both, is correlated with suboptimal immune response[15,17-19]. LR size and inducibility have never been simultaneously evaluated, but we will do so accurately and efficiently using two exciting new assays developed in the lab of my primary mentor, Dr. Robert Siliciano. Because these assays require many fewer input cells and less labor than prior LR assays, we can assess whether LR characteristics correlate with clinical outcomes in cohort studies. For the first time, we will be able to determine whether LR size is a clinically relevant biomarker. Together with a dedicated mentorship team in HIV virology and immunology, **we propose to examine whether LR size and inducibility in blood and lymphoid tissue contribute to suboptimal immune response and whether cytokine therapies designed to increase CD4 counts also expand the LR.**

AIM 1. Determine whether the size of the HIV latent reservoir (LR) in blood and lymphoid tissue is positively correlated with suboptimal immune response.
Hypothesis 1: LR size in blood and lymphoid tissue is positively correlated with suboptimal immune response. Background: Several independent groups have demonstrated a correlation between total HIV DNA frequency in CD4s and low CD4 counts despite ART[15,17-19]. However, because defective HIV proviruses greatly outnumber intact HIV proviruses, total HIV DNA measurements do not give an accurate measure of the LR[21-23]. Methods: Using the intact proviral DNA assay (IPDA), a duplex droplet digital PCR assay designed to separately quantify intact and defective HIV proviruses, we will assess whether the size of the LR in blood and lymphoid tissue correlates with suboptimal immune response in SoIRs and age-, sex-, race- and nadir-matched controls. Blood and lymphoid tissue samples will be identified from four collaborating cohorts[24].

AIM 2. Assess whether infected $CD4^+$ T cells of suboptimal immune responders (SoIRs) are more readily inducible from latency.
Hypothesis 2: Infected T cells of SoIRs have increased HIV induction from latency when normalized for LR size. Methods: We will test whether increased *in vivo* or *ex vivo* inducibility of HIV from latency correlates with suboptimal immune response in SoIRs and matched controls. We will do so by measuring *in vivo* cell-associated HIV RNA from participants or *ex vivo* HIV RNA induction after maximal T cell activation. Each of these will be normalized to the frequency of intact HIV DNA to calculate HIV inducibility indices. If either LR size or inducibility are correlated with suboptimal immune response, shock-and-kill strategies and therapeutic vaccines may decrease morbidity and mortality in SoIRs even if they do not produce an HIV cure.

AIM 3. Determine whether cytokine therapies that increase CD4 count also expand the LR.
Hypothesis 3: Exogenous IL-7 and IL-15 lead to an increase in the LR size by favoring proliferation of CD4s over latency reactivation, whereas exogenous IL-2 leads to a decrease in LR size via latency-inducing effects. Background: Cytokine-based immunotherapies have been trialed as a means of boosting CD4 counts and inducing latency reactivation. However, the effect that exogenous cytokines have on the intact, inducible HIV LR *in vivo* remains unknown. Methods: We will measure the size of the LR via IPDA in blood samples from clinical trials of exogenous IL-7, IL-15, and IL-2 at timepoints before and after cytokine administration.

IMPACT: These studies will provide mechanistic insight into suboptimal immune response on ART and will inform efforts to avert the tens of thousands of excess deaths per year in SoIRs. The proposed work and training, together with my graduate and fellowship research training, uniquely position me to launch a career as an independent investigator studying the contribution of HIV persistence to the pathogenesis of treated HIV.

A. SIGNIFICANCE

Over the next decade there will be vast increases in the number of people living with HIV who are on antiretroviral therapy (ART). Since the recognition of the HIV pandemic in the early 1980s, astounding progress has been made. As of 2017, 21 of the 37 million people globally living with HIV were accessing ART[24]. The Joint United Nations Programme on HIV/AIDS has articulated a clear agenda for rapid scale-up of HIV treatment with its "90-90-90" target[6]. The goal is that 90% of all people living with HIV will know their status, 90% of those diagnosed will receive ART, and 90% of those treated will be virally suppressed. Even if this goal is not reached by 2020, it has inspired a sustained global coordinated effort that will ensure that millions of untreated HIV-positive people gain access to ART.

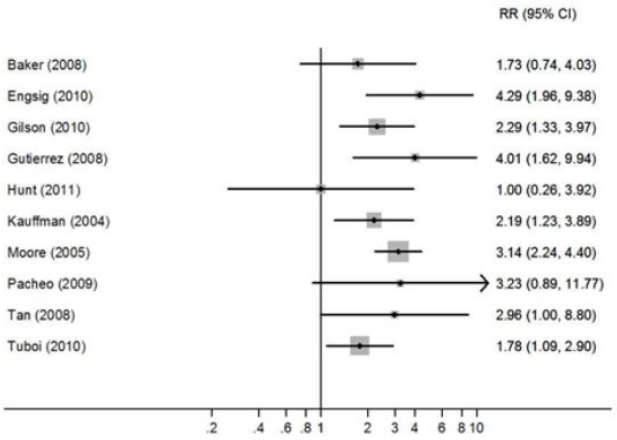


Figure 1. Forest plot from a 2016 review showing risk of mortality for SoIRs vs immune responders in 10 cohort studies[3].

A subpopulation of people on ART – suboptimal immune responders (SoIRs) - have persistently high morbidity and mortality rates despite years of viral suppression. Once on ART, people who achieve absolute $CD4^+$ T cell counts > 500 cells/μL have life expectancies similar to the general population[25,26]. However, well over one-third of people starting ART with CD4 < 200 cells/μL do not achieve a CD4 count > 500, even after 5-10 years of viral suppression[1,27-29]. People whose CD4 counts plateau at abnormally low levels despite ART, termed suboptimal immune responders (SoIRs), have a significantly higher morbidity and mortality rate than people whose CD4 count rises appropriately with treatment, and this mortality effect persists for at least 7-10 years[2,27,28]. Although the definition of SoIR has varied, studies from high-income and low-income countries show a consistent 2-3 fold higher mortality rate in this population compared to other people on ART (**Fig. 1**)[2,3].

An estimated 1.1 million people globally are SoIRs. A recent epidemiological study of close to one million HIV-positive people living in 55 countries found that 55% of all people starting ART for the first time between 2002-2015 had a CD4 count < 200[7]. Even in high-income countries in 2015, 29% of people starting ART had a CD4 < 200[7]. Approximately 10-15% of those starting ART with a CD4 < 200 will not achieve a CD4 > 200 after 3-5 years of suppressive ART[2,27,28]. Using the numbers cited here (21 million on ART, 55% started ART with CD4 < 200, 10% remain with CD4 < 200 after 3-5 years of ART), we estimate that at least 1.1 million people are SoIRs. Given their higher mortality rates, this means that tens of thousands of excess deaths occur per year in SoIRs. So while it is of the utmost importance to find and treat HIV-positive people as soon as possible after infection, it is also vitally important to understand and reduce the excess morbidity and mortality seen in SoIRs. The majority of deaths in SoIRs are from non-AIDS-related causes such as non-AIDS-associated cancer, cardiovascular disease, and liver disease, but AIDS-related death rates are also higher in SoIRs[2,3,30]. The strongest risk factor for suboptimal immune response is lowest recorded or nadir CD4 count, but several other risk factors are known: age > 40, greater time with CD4 count < 200 before the start of ART, intravenous drug use, and male sex[2,9,31,32].

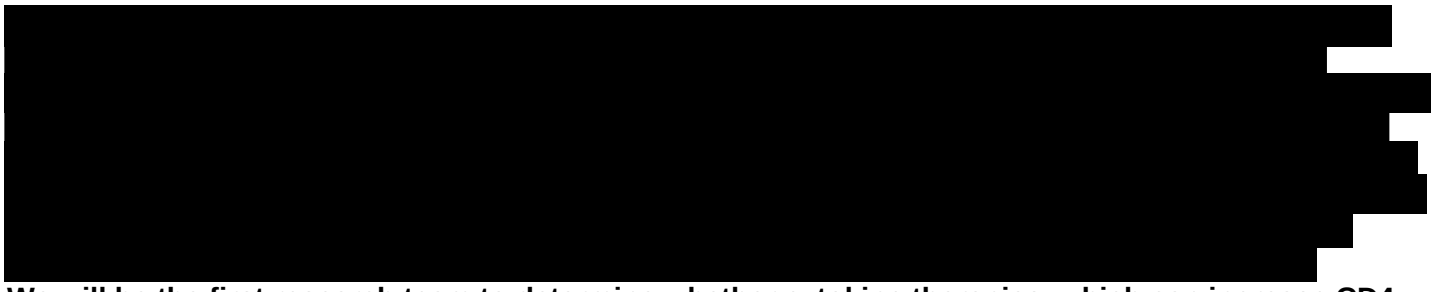
The basic mechanisms underlying the poor immune recovery and the increased morbidity and mortality rates in SoIRs are not well understood. However, intriguing associations have been reported. Higher levels of $CD8^+$ and $CD4^+$ T cell activation have consistently been observed in SoIRs versus immune responders, both less than in viremic individuals[8-15]. It is unclear what is driving persistent T cell activation, but products of microbial translocation probably contribute and have also been correlated with suboptimal immune response in most reports[13,33-38]. SoIRs appear to have greater turnover and impaired regeneration of their $CD4^+$ T cell compartment, as evidenced by increased markers of apoptosis and fewer thymic emigrants and naïve cells seen among CD4s from SoIRs[15,35,39-48]. In addition, SoIRs may be deficient in IL-7-receptor mediated T cell proliferation and survival, as suggested by associations between suboptimal immune response, increased serum IL-7, and downregulation of CD127 (IL-7 receptor α) on T cells, although not all reports corroborate this[15,36,42,46,49-52]. There is good evidence that immune activation can lead to collagen deposition in lymphatic tissues, which restricts access to IL-7 and thus impairs peripheral proliferation of $CD4^+$ T cells, although there are conflicting reports of whether there is more collagen deposition in SoIRs than others[53-58]. Also, there are recent reports of increased expression of caspase-1 (which mediates pyroptosis) in lymphocytes of SoIRs; increased anti-CD4 IgG in the serum of SoIRs; and impairment in differentiation of $CD34^+$ hematopoietic progenitor cells of SoIRs into T cell progenitors[34,40,41].

Several studies have found a correlation between the frequency of infected CD4$^+$ T cells and low CD4 counts on ART[15,17-19]. This has led to our hypothesis that SoIRs have a greater HIV latent reservoir (LR) size or increased LR inducibility compared to immune responders. This makes biologic sense given that the larger the size or the inducibility of the LR, the more likely it is that HIV will be induced from latency during daily antigenic stimulation of T cells. HIV induction from latency may lead to increased immune activation and loss of CD4s without ongoing cycles of replication during ART via many of the same mechanisms pertinent in viremic individuals: apoptosis via HIV cytopathic effects, activation of HIV-specific CD8$^+$ T cells, binding of innate pattern recognition receptors, cytokine release after adaptive or innate recognition, and pyroptotic cell death upon sensing abortive HIV replication intermediates[59-61]. We will conduct a definitive study to determine whether HIV latent reservoir size or inducibility correlate with suboptimal immune recovery. This type of study has not been done because the vast number of defective HIV proviruses makes studying the HIV LR technically challenging[21,22]. However, we will be able to do so using two exciting new assays developed and validated in the laboratory of my primary mentor, Dr. Robert Siliciano, that afford the first opportunity to directly measure the HIV LR and its inducibility in an efficient manner on banked patient samples.

IMPACT: For the first time, we can ask in a rigorous way whether the HIV latent reservoir (LR) serves as a clinically meaningful biomarker like HIV viral load and CD4 count. This is because our new intact proviral DNA assay (IPDA) requires substantially fewer input cells and less time than prior methods, and it can thus be used to measure the LR from a large number of samples and also from lymphoid tissue samples[20,23]. In addition, the IPDA can accurately quantify and discriminate intact and defective proviruses, so the relative contribution of defective and intact proviruses to clinical outcomes can be examined. If LR size is found to predict suboptimal immune recovery, this assay could be performed within the first year of ART and those predicted to have suboptimal immune response could be identified early on and offered more intensive follow-up and screening for cancer, atherosclerosis, or liver disease to prevent morbidity and mortality. Further, if either LR size or inducibility are associated with suboptimal immune response, then latency-reversing agents or therapeutic vaccines that are currently under investigation may decrease morbidity and mortality in SoIRs, even if they do not produce a sterilizing cure of HIV. Finally, by accurately measuring the changes in the LR for the first time in HIV-positive people on ART given cytokine therapy that stimulates CD4 proliferation, we will be able to inform trials of cytokine therapies in HIV and provide mechanistic insights into HIV persistence.

B. INNOVATION

Using an innovative new assay developed and validated in our lab, we will perform one of the first studies to determine whether the size of the HIV latent reservoir (LR) correlates with measurable clinical outcomes in people on ART (Aim 1). The intact proviral DNA assay (IPDA) separately quantifies the frequency of intact and defective HIV proviruses in a population of cells via duplex droplet digital PCR technology[23]. The IPDA requires 10 times fewer cells as input than the previous gold standard LR assay, the quantitative viral outgrowth assay (QVOA), and it can be run in one day rather than over 2-3 weeks[20,23,62]. With the IPDA, we can now accurately measure the LR in large numbers of samples, cryopreserved samples, and in lymphoid tissue. By correlating LR size with specific clinical outcomes such as suboptimal immune response, we can determine whether the size of the LR is a clinically meaningful biomarker.



We will be the first research team to determine whether cytokine therapies, which can increase CD4 counts, also expand the LR *in vivo* (Aim 3). We will do so using the IPDA on samples from clinical trials. IL-15 is currently in trials as an adjuvant for therapeutic vaccines in HIV-positive people, but its effect on the HIV LR *in vivo* is unknown. IL-15 has a weak latency inducing effect, but its proliferative effect on infected CD4+ T cells may negate this. An understanding of its impact on the LR is critical knowledge that will inform future trials.

C. APPROACH

AIM 1. Determine whether the size of the HIV latent reservoir (LR) in blood and lymphoid tissue is positively correlated with suboptimal immune response.

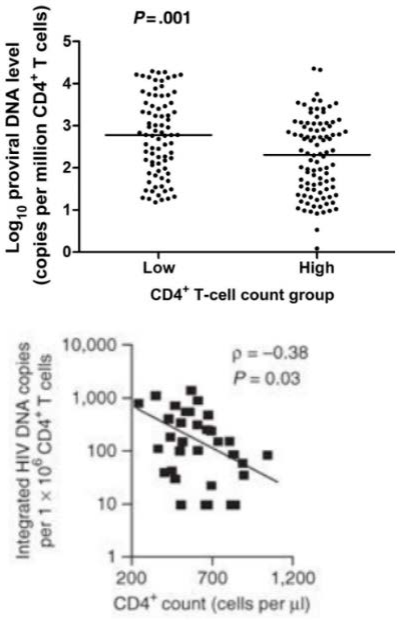


Figure 2. (Top) A low (< 350) $CD4^+$ T cell count is associated with a higher frequency of total HIV DNA per 1M $CD4^+$ T cells in 190 people on suppressive ART[17]. (Bottom) $CD4^+$ T cell count negatively correlates with the frequency of integrated total HIV DNA copies per 1M $CD4^+$ T cells from 33 people on suppressive ART[19].

Background & Rationale. Several studies have demonstrated a correlation between the frequency of infected $CD4^+$ T cells and low CD4 counts on ART (Fig. 2)[15,17-19]. This suggests that there is a larger HIV latent reservoir (LR) in SoIRs. However, this does not establish a correlation between suboptimal immune response and a large LR because in people on suppressive ART, defective proviruses greatly outnumber intact proviruses, and these assays simply measured total HIV DNA[20]. In addition, these studies did not control for CD4 nadir or time on ART[19,61,68].

Recently, the Siliciano lab developed and validated an innovative new assay, the intact proviral DNA assay (IPDA), to quickly and accurately measure the HIV LR. The IPDA separately measures the frequency of intact and defective HIV-1 subtype B proviruses in a cell population and thus provides a definitive maximal estimate of the LR[23]. The HIV LR has been technically difficult to quantify because of the low frequency of latently infected cells and the high ratio of defective proviruses to intact proviruses[20]. In the late 1990s, the Siliciano lab developed the precursor to the current gold-standard LR assay, the quantitative viral outgrowth assay (QVOA), a limiting-dilution co-culture assay that provides a definitive minimal estimate of the LR[62,69]. The QVOA has only been used for research purposes because it is labor-intensive, requires a large number of cells, and takes 2-3 weeks to complete[62]. Conversely, the IPDA takes 1-2 days to complete and requires 10 times fewer input cells than the QVOA and thus can be used on cryopreserved samples and lymphoid tissue cells. This affords a new opportunity to measure the HIV LR in cohort samples and lymphoid tissue, which harbors a large fraction of the LR[70,71]. An additional advantage is that the IPDA quantifies all intact proviruses, not just those induced by a single round of T cell activation. This is important because not all cells of the LR are induced to produce virus after a single round of T cell activation[21].

The HIV LR is depleted of fast-decaying populations and is stable after approximately 12 months on suppressive ART (Fig. 3)[61,72]. For this reason, 1 year after starting ART is the earliest timepoint that accurately represents the stable, persistent LR. We will measure the LR by IPDA 1-2 years after the start of ART in cryopreserved cohort blood and tissue samples in SoIRs and age-, sex-, race-, and nadir-matched controls to determine if a larger LR size is correlated with suboptimal immune response. If there appears to be a correlation, this study will have immediate translational impact by demonstrating that the LR is a useful clinical biomarker that can predict immune recovery in people on ART.

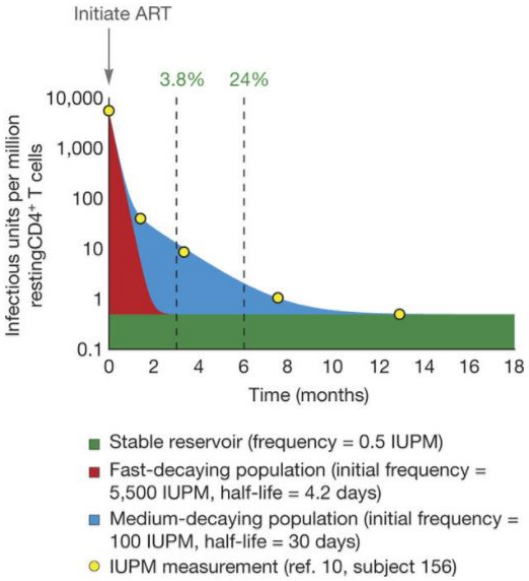


Figure 3. Mathematical model of three populations decaying at different rates after the start of suppressive ART. This model is based on longitudinal QVOA measurements of 7 early-treated individuals[61].

Hypothesis 1. The size of the LR measured in blood and lymphoid tissue is positively correlated with suboptimal immune response.

Study Population. Suboptimal immune responders (SoIRs) are defined here as HIV-1-infected individuals who began a period of viral suppression with an absolute CD4 count < 200 cells/µL and had a CD4 count < 350 after 5 years of viral suppression, during which viral loads (VLs) were measured to be < 200 copies/mL or < limit of detection (LOD) at least every 12 months. This definition was chosen to mirror the definition in published morbidity/mortality studies and to maximize participant-finding in our collaborating cohorts[73,74]. To exclude the possibility of virologic failure as a cause of suboptimal immune recovery, we define viral load

suppression as < 200 copies/mL in keeping with AIDS Clinical Trials Group definitions of virologic failure[75,76].

Controls are HIV-1-infected individuals who began a period of viral suppression with CD4 < 200 and had a CD4 count > 500 after 5 years of viral suppression, during which viral loads were measured to be < 200 copies/mL or < LOD at least every 12 months. Controls will be matched to identified SoIRs by age (within 7 yrs), sex, race, and nadir CD4 count (within 50 cells/μL), and where possible, also by HCV and CMV co-infection status and ever/never intravenous drug use.

SoIRs and controls for Aims 1 and 2 will be identified from within our 4 collaborating clinical cohorts. (1) The Observational Study of the Consequences of the Protease Inhibitor Era (SCOPE) Cohort led by Dr. Steven Deeks (advisory committee member) at UCSF, which has >2,000 participants and collects cryopreserved PBMCs, leukaphereses, gut biopsies, and lymph node biopsies. (2) The Johns Hopkins HIV Clinical Cohort (JHHCC) led by Dr. Richard Moore (co-mentor) which has > 9,000 participants and collects clinical data and PBMCs.(3) The Cleveland Immune Failure (CLIF) cohort led by Dr. Michael Lederman (collaborator) which collects clinical data and PBMCs. (4) AIDS Clinical Trials Group Longitudinal Linked Randomized Trials (ALLRT) which collects clinical data and PBMCs.

[redacted]

Rigor & Reproducibility:

Methods & Preliminary Data. To accomplish this aim, we will isolate resting CD4⁺ T cells from viably stored PBMCs and lymphatic tissue as previously described and perform the IPDA (**Fig. 4**)[23,62]. [redacted]

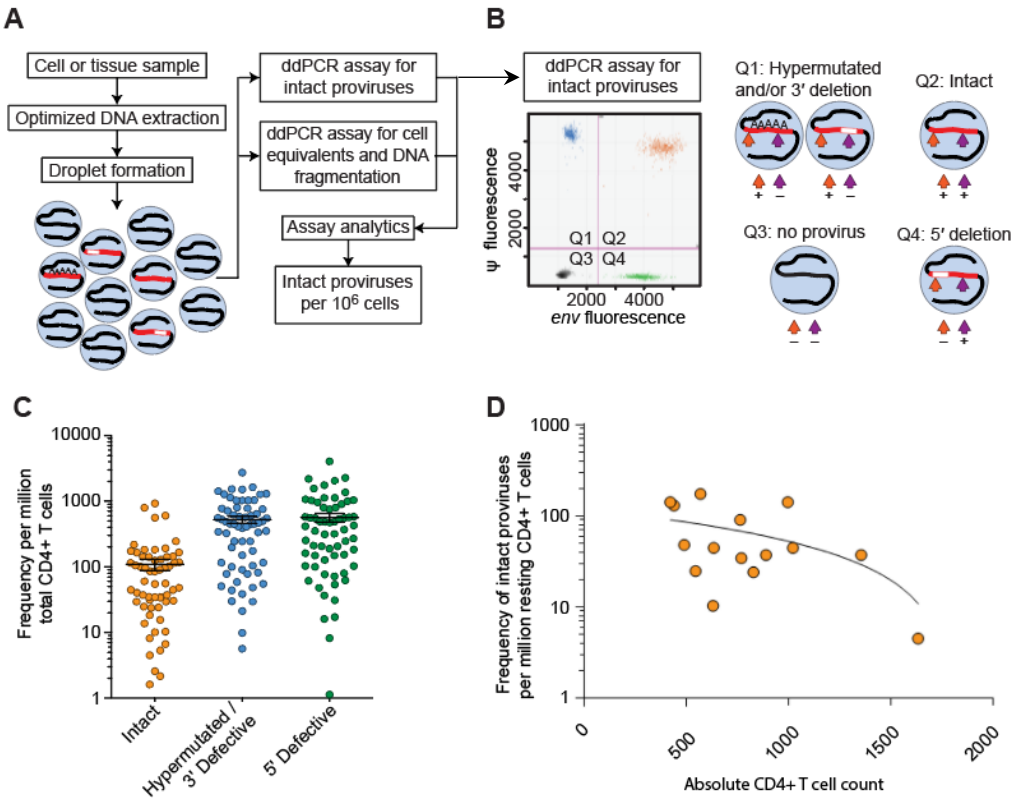


Figure 4. Measurement of the LR by IPDA[23]. (A) Schematic of the IPDA. (B) Representative control experiment using previously described proviral constructs with a 5' deletion, a 3' deletion, or no defects[16]. Types of proviruses appearing in different quadrants are shown on the right. (C) IPDA measurements of LR in 52 HIV-infected individuals on suppressive ART (mean ± SEM). (D) Intact proviruses per million resting CD4+ T cells plotted based on absolute CD4+ T cell count measured in each individual. This represents the subset of individuals who had IPDA run on resting CD4+ T cells, in which the latent reservoir resides. Plotted line represents linear regression.

The IPDA uses duplex droplet digital PCR technology to estimate the frequency of cells harboring intact HIV proviruses in a population of cells (Fig. 4A). I and others in the laboratory generated hundreds of near full-length HIV provirus sequences over the past few years[21,22]. This sequence data was mined to model the discriminatory capacity of dual primer/probe sets, and two primer/probe sets were selected that maximize the likelihood of capturing intact proviruses while also quantifying proviruses harboring deletions in the 5' or 3' regions or proviruses with APOBEC-mediated hypermutation (Fig. 4B). This assay signifies a major improvement over single primer/probe ddPCR or qPCR HIV DNA detection methods because it can separately quantify intact and defective HIV proviruses (Fig. 4C). [redacted]

Experimental Design. Using the IPDA, we will assess whether the size of the LR in blood and lymphoid tissue correlates with CD4 recovery in SoIRs and matched controls as defined above. Viably-frozen PBMCs from SoIRs and controls at a timepoint 1-2 years after starting suppressive ART will be obtained from the 4 cohorts described above. Lymph node tissue and gut biopsy tissue from a subset of SoIRs and controls will be obtained from the SCOPE cohort. Samples with > 10M resting CD4s will also be tested by QVOA. We will measure the LR from blood samples at 1-2 years after the start of ART because we wish to determine whether LR size predicts immune recovery, and this is the earliest timepoint at which LR measurements reflect the stable LR (Fig. 4)[61].

Using the frequency of infected CD4s from the published data shown in Fig 2 and the relative group effect method, we show the estimated required sample size per group to detect a difference in LR size for various measures of the coefficient of variation (standard deviation over mean) denoted k in **Table 1**[17,19,77]. These numbers will be sufficient to determine whether a difference in LR size exists between a subpopulation of SoIRs and controls with 80% power at 0.05 level of significance. We denote varying ks given the variance in IPDA measurements of immune responders seen in Fig. 4C and the unknown variance of SoIRs.

Table 1. Sample size per group

k	Required sample size
1	30
1.5	52
2	70

Anticipated Results. We expect to see a positive correlation between the size of the LR in blood and tissue and subsequent suboptimal immune response, similar to the published correlation seen between total HIV DNA and low CD4 count (Fig. 2). Because suboptimal immune response has a few disparate risk factors, reviewed above, we have powered our study to detect this correlation within a subpopulation of SoIRs. If this correlation exists, this work would be foundational to an R01 grant aimed at understanding mechanistically why large LR size is linked to poor immune reconstitution and worse clinical outcomes.

Potential Problems & Alternative Strategies. If more SoIRs and control samples are needed than can be found in our 4 collaborating cohorts, we will expand our search to the Multicenter AIDS Cohort Study (MACS), co-led by Dr. Joseph Margolick of Johns Hopkins, or the Center for AIDS Research Network of Integrated Clinical Systems (CNICS), led by my co-mentor Dr. Moore. [redacted]

AIM 2. Assess whether infected CD4$^+$ T cells of suboptimal immune responders (SoIRs) are more readily inducible from latency.

Background & Rationale. The frequency of cell-associated HIV RNA in virally suppressed individuals with low CD4 counts is higher than that in individuals with high CD4 counts, as shown by members of my advisory committee (**Fig. 5**)[17]. The frequency of cell-associated HIV RNA is a function of both size and inducibility of the HIV latent reservoir[78]. Therefore this association could be seen because of one or both of two possibilities: 1) the LR of intact, inducible DNA proviruses is larger in people with low CD4 counts or 2) the induction rate of HIV transcription from intact proviruses is higher in those with low CD4 counts. [redacted] urther, because the study shown in Fig. 5 didn't control for nadir CD4 or time on ART, it is unknown whether those with low CD4 counts in this study truly are suboptimal immune responders. We will control for this by comparing SoIRs with age- and nadir-matched controls.

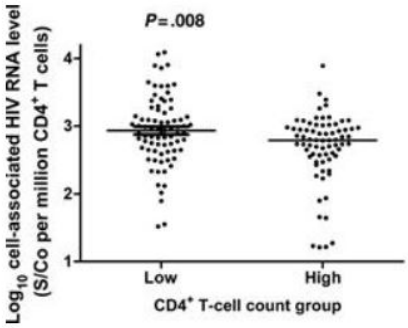


Figure 5. A higher frequency of cell-associated RNA is seen in people with lower (< 350) CD4$^+$ T cell count vs those with higher CD4 count (≥ 350)[17].

Our lab has shown that inducibility from the LR is an important factor in reactivation from latency by demonstrating that many cells harboring intact proviruses do not produce viral outgrowth after one round of maximal T cell activation[21]. In some people, a very small proportion of intact proviruses produce viral outgrowth after a single round of T cell activation, while in others, up to 1/3 do[21]. In Aim 2, we will measure HIV induction from latency normalized to the frequency of intact proviruses by calculating an "inducibility index." The inducibility index is the frequency of intact induced HIV RNA in CD4s over the frequency of intact HIV DNA in the same sample. This work will clarify whether increased HIV induction from latency is associated with suboptimal immune response. This is important because if so, then therapeutic vaccines and shock-and-kill strategies may alleviate mortality in SoIRs.

Hypothesis 2. Infected CD4$^+$ T cells of SoIRs have increased HIV induction from latency when normalized for LR size.

Methods. Samples from SoIRs and controls will be identified from the 4 cohorts described in Aim 1. The frequency of intact HIV DNA in CD4s will be measured by IPDA as described in Aim 1. The frequency of *in vivo* HIV induction from latency will be measured by quantifying cell-associated RNA in CD4s as described previously[17]. The frequency of *ex vivo* HIV induction from latency will be measured by the quantitative viral induction assay (QVIA), which was recently developed and validated in our laboratory[67]. The QVIA has the potential to distinguish between transcription from intact HIV proviruses and transcription from transcription-competent but defective proviruses, unlike the TILDA (Tat/rev Induced Limiting Dilution Assay)[67]. This is important given that our lab and others have shown that defective proviruses can be induced to transcribe and splice HIV RNA both *in vitro* and *ex vivo*[16,63-66]. Like the TILDA, the QVIA is a limiting dilution assay that requires many times fewer input cells than the QVOA and takes two days to complete[67]. The protocol and dynamic range of the QVIA are shown in **Fig. 6**[67].

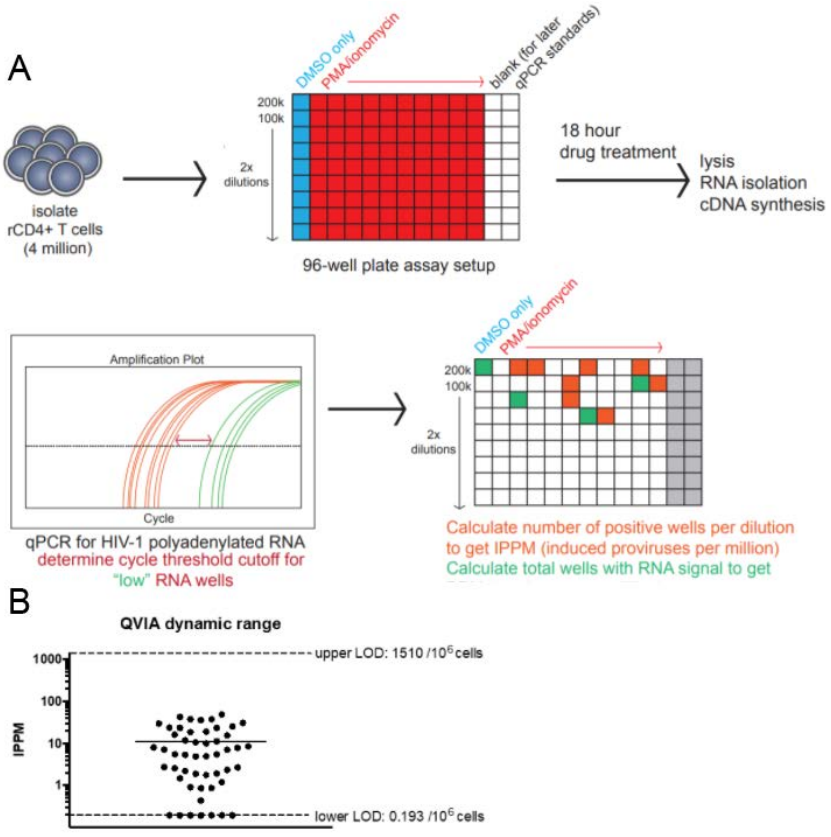


Figure 6. (A) Schematic of the QVIA[67]. CD4$^+$ T cells are plated at limiting dilution, undergo maximal T cell activation via 18 hours of treatment with phorbol 12-myristate 13-acetate (PMA) and ionomycin, and then undergo in-well lysis, RNA isolation, and cDNA synthesis. qPCR determines quantity of polyadenylated HIV RNA transcript, and a cutoff for low RNA wells (defective proviruses) is established. The frequency of intact induced proviruses per million is then calculated. (B) Dynamic range of the QVIA[67].

Experimental Design. To measure *in vivo* HIV induction from latency, we will quantify the frequency of cell-associated HIV RNA in CD4$^+$ T cells and divide it by the frequency of intact HIV DNA proviruses from IPDA (the *in vivo* inducibility index) in individuals who are SoIRs and from their age-, sex-, race-, and nadir-matched controls. To measure *ex vivo* HIV induction from latency, we will measure the frequency of *ex vivo* induction from latency in CD4s by QVIA and divide it by the frequency of intact HIV DNA proviruses from IPDA (the *ex vivo* inducibility index) in SoIRs and matched controls. We will choose timepoints after one year of suppressive ART to avoid measuring HIV RNA and DNA from fast-decaying populations of the LR (Fig. 3). With a sample size of 30 per group, we can detect a relative index as low as 1.12 between controls and SoIRs in the case of variation k = 1. If k = 1.5, we can detect a relative index equal to 1.67 with the same sample size.

Anticipated Results. We expect higher *in vivo* and *ex vivo* inducibility indices in SoIRs compared to controls. If this is the case, this would lead to an R01 proposal aimed at understanding the mechanisms that account for the difference, e.g. via RNAseq. Regardless of the outcome, defining typical values and ranges of ART-suppressed individuals' inducibility indices will still be important new knowledge that will inform attempts to find suitable latency reactivating agents for HIV cure.

Potential Problems & Alternative Strategies. Running the IPDA and the QVIA simultaneously requires approximately 5 million CD4$^+$ T cells, which in some SoIRs with very low CD4 counts may require a separate blood draw. This is feasible given that individuals in SCOPE, JHHCC, and CLIF can be brought back by the cohort team to provide extra samples. If obtaining enough cells for QVIA proves difficult, then we will use the TILDA instead, which requires only 1 million resting CD4s as input. Because SoIRs have a higher percentage of activated T cells, we will measure the *ex vivo* inducibility index using resting CD4s to ensure we are quantifying the potential for induction and not simply measuring induction in cells that are already activated[8-15].

AIM 3. Determine whether cytokine therapies that increase CD4$^+$ count also expand the HIV LR.

Background & Rationale. Cytokine-based therapies for HIV have been trialed several times over the past 3 decades and ongoing trials are assessing their role in treated HIV, but little is known about their effect on the HIV LR. Most recently, cytokine-based therapies have been trialed to enhance immune reconstitution, reactivate HIV from latency, and expand NK cells and HIV-specific cytotoxic T lymphocytes[79]. **IL-2:** Interleukin-2 (IL-2) administration results in virus reactivation from latency and proliferation of T cells. However, the SILCAAT and ESPRIT studies showed that while exogenous IL-2 increases CD4 counts, it does not lead to long-term clinical improvements[80]. Reports using standard qPCR-based latency assays were mixed on whether IL-2 affected the size of the LR[81-85]. **IL-7:** Exogenous IL-7 can increase CD4 count and reactivate latent HIV without the toxic effects of global T cell activation, and thus was a promising therapy[86,87]. Several small trials evaluated the impact of IL-7: ACTG 5214, ERAMUNE-01, and the INSPIRE trials[88-91]. The frequency of infected CD4$^+$ T cells as measured by total HIV DNA increased along with the proliferation of CD4s, and interest in IL-7-based therapy waned[89,90]. However, this does not provide insight into LR effects, given the high ratio of defective to intact HIV proviruses[21,22]. **IL-15:** IL-15 is an exciting potential therapy because of its ability to act as an adjuvant for HIV therapeutic vaccines by enhancing cell-mediated immunity[92-97]. Using the IPDA on clinical trial samples, we will for the first time be able to directly measure and compare the effects of IL-2, IL-7, and IL-15 on intact and defective latent HIV in ART-treated individuals.

This study has important implications in the field of HIV cure. Many studies have shown that proliferation of infected cells contributes to the persistence of HIV in people on ART[19,98-106]. Proliferation of CD4$^+$ T cells latently infected with HIV may be driven by homeostatic proliferation of T cells, antigen-driven proliferation of T cells, or integration site-specific effects on proliferation. There is evidence suggesting that all three mechanisms play a role in driving HIV persistence in people on ART, although the relative contribution of each remains unknown[19,98-100,107]. Our study provides a unique way to observe how the LR responds *in vivo* to IL-7 and IL-15-mediated homeostatic proliferation and enhanced survival of T cells. In addition, we will determine whether IL-7-stimulated proliferation favors cells harboring defective, potentially less cytopathic, proviruses.

Hypothesis 3. Exogenous IL-7 and IL-15 lead to an increase in the LR size by favoring proliferation of CD4$^+$ T cells over latency reactivation. Exogenous IL-2 leads to a decrease in the LR size because of its stronger latency-inducing effects.

Experimental Design & Methods. Dr. Sereti, Dr. Schacker, and the ACTG leadership have agreed to supply us with cryopreserved PBMCs from timepoints before and after cytokine administration from clinical trials of IL-2, IL-15, and IL-7 respectively (**Table 2**)[80,90,91,108,109]. We will measure the frequency of intact and defective HIV proviruses in CD4s isolated from cryopreserved PBMCs via the IPDA.

Table 2. [redacted]

Potential Problems & Alternative Strategies. This study will be conducted on banked samples from clinical trials and because of this we cannot vary the sample size. [redacted]

Anticipated Results. We anticipate that our results from the trials of IL-7 and IL-15 will demonstrate that the frequency of intact HIV proviruses in CD4s increases after cytokine administration and stays elevated with time. [redacted] We anticipate that our results from the trial of IL-2 will demonstrate a reduction in the LR. If our hypotheses are supported, this work may temper enthusiasm for IL-15-based therapies.

Table 3. Timeline for proposed project

Activity	Year 1		Year 2		Year 3		Year 4		Year 5	
	Q1-Q2	Q3-Q4	Q1-Q2	Q3-Q4	Q1-Q2	Q3-Q4	Q1-Q2	Q3-Q4	Q1-Q2	Q3-Q4
Aim 1: Sample/Data analysis										
Manuscript writing										
Aim 2: Sample/Data analysis										
Manuscript writing										
Aim 1: Sample/Data analysis										
Manuscript writing										
R01 preparation/submission										

Training in the Responsible Conduct of Research

The Johns Hopkins School of Medicine has established a Responsible Conduct of Research (RCR) program that uses a variety of educational methods to ensure that its faculty, fellows, and staff conduct research with integrity. As a fellow, I completed in July of 2017 its RCR Collaborative Institutional Training Initiative (CITI) course, which is a 5.5 hour online course that provides a comprehensive overview of the essential elements of RCR, including research misconduct, data acquisition and management, responsible authorship, peer review, mentoring, conflicts of interest and commitment, and collaborative research. I will retake this course once every four years. In July of 2016, I completed online training in conflict of interest and commitment, HIPAA for research, and basic human subjects. I attended a 90 minute in-person discussion on mentor/mentee responsibilities and relationships led by School of Medicine faculty in February of 2018. In addition, I have engaged in informal instruction and conversations on the responsible conduct of research with my primary mentor Dr. Robert Siliciano over the past two years. In the next year I will attend at least two Research Integrity Colloquia, which are monthly face-to-face interactive presentations by School of Medicine faculty who discuss their perspectives on conducting research with integrity. These presentations cover a wide spectrum of RCR topics.

During my next phase of training, I will continue to update my RCR training. Specifically, I will attend 1-2 in-person Research Integrity Colloquia per year and 1 in-person Department of Medicine meeting where an RCR topic is discussed per year. In years 4-5 of the award period, I will engage with the National Research Mentoring Network to take their online training as well as certify to be a NRMN Mentor. [redacted] I will take a formal RCR course in year 3 of the award as well. This plan will ensure that I am in compliance with the 5 instructional components outlined in the NIH Policy on Instruction in the Responsible Conduct of Research.

1) **Format:** My plan incorporates substantial face-to-face discussions among trainees, junior faculty, and senior faculty both via the Research Integrity Colloquia and the Department meeting on an RCR topic and via informal conversations with my mentor and co-mentors.

2) **Subject Matter:** My plan covers a variety of topics relevant to the responsible conduct of research, including conflict of interest, policies regarding human subjects, mentor/mentee responsibilities, collaborative research, peer review, data acquisition and laboratory tools, management, sharing and ownership, research misconduct and policies for handling misconduct, responsible authorship and publication, the scientist as a responsible member of society, contemporary ethical issues in biomedical research, and environmental and societal impacts of scientific research.

3) **Faculty Participation:** I will receive informal training from my primary mentor and my two co-mentors who are experienced basic, translational, and clinical investigators. Faculty will also lead the Research Integrity Colloquia and the Departmental meeting on an RCR topic that I will attend.

4) **Duration of Instruction:** My plan will involve well over the 8 contact hours of suggested instruction and will be spread over the 5 year award period.

5) **Frequency of Instruction:** My plan includes 2-3 formal contact hours per year and many informal contact hours per year of the award period, and complete recertification in the School of Medicine RCR program is required every four years. This is in accordance with NIH guidelines.

Description of the Institutional Environment
Johns Hopkins has strong, well-established, and collaborative research programs in basic, translational, and clinical HIV research pertinent to Dr. Antar's K08 application. Specifically, key mentors and advisory committee members at Johns Hopkins (Drs. Siliciano, Moore, Blankson, Siliciano, and Nonyane) will advise and guide Dr. Antar's scientific endeavors and career development as described in her career development plan.

The Robert Siliciano Laboratory includes 2200 square feet of laboratory space and a 670 square foot BSL-3 laboratory in which Dr. Antar has personal office and laboratory benchtop space. Dr. Siliciano is funded by the Howard Hughes Medical Institute and also by the NIH, foundations, and industry. All equipment necessary to carry out the research proposal is available in the Siliciano laboratory, most notably the droplet digital PCR system and dedicated ddPCR set-up room. All pertinent equipment and resources are described in the Facilities and Other Resources Section. Weekly laboratory meetings provide opportunities for formal research feedback and journal clubs encourage discussion of relevant research.

The Johns Hopkins Center for AIDS Research (CFAR) coordinates HIV research at Johns Hopkins and is comprised of 5 cores: 1) Administrative, 2) Developmental, 3) Clinical, 4) Prevention, and 5) Laboratory. The JH CFAR promotes interdisciplinary innovation and an extraordinarily collaborative environment in research; provides mentoring, support, and pilot funding for junior faculty members; and assists researchers with design and analysis of research studies and training in research methodologies. CFAR funding directly relevant to Dr. Antar's application include the $[redacted] CFAR Scholar Grants for Faculty Development open only to junior investigators and opportunities to apply on behalf of the CFAR for NIH administrative supplements. JH CFAR programming relevant to Dr. Antar's application include support for Institutional Review Board submissions, internal scientific review of grant applications, and the K2R Club, which provides programming for junior investigators related to grant writing and facilitating career independence.

The Johns Hopkins Division of Infectious Diseases has over 70 full-time faculty members, including global leaders in HIV cure, HIV immunity, HIV clinical and outcomes research, HIV implementation science, antiretroviral pharmacology, tuberculosis, viral hepatitis, transplant-associated infections, infectious diarrhea, antimicrobial stewardship, and epidemiology, many of whom the PI has formed close relationships with during residency and fellowship training. The ID Division prioritizes the research success of its faculty and promotes a collaborative research environment. The PI has frequent opportunities to interact with ID faculty, including grand rounds, HIV case conference, research conference, and clinical management conference, each held weekly. The division also has a formal program to guide and promote the interests of junior faculty that includes regular meetings with the division chief Dr. Thomas and other senior faculty. Additional networking opportunities arise from the regular Women in ID meetings, attended by senior and junior female faculty.

The Johns Hopkins School of Medicine has a long history of outstanding research and clinical care. It devotes many resources to the success of junior faculty. It sponsors the Junior Faculty Leadership Program, a 14-hour leadership training program in which I will participate during this award. The Johns Hopkins Institute for Clinical and Translational Research (ICTR) aids junior investigators via their "K-to-R" transition program which holds bi-monthly programmatic activities aimed at enhancing success in R01 applications. The ICTR also provides courses for translational researchers, funding for pilot studies, biostatistical consults, regulatory guidance, and information on enhancing community involvement. The Department of Medicine within the School also hosts an active Task Force on Women's Academic Careers in Medicine that focuses on providing programming to support the career advancement of women faculty and fellows and increasing leadership development and opportunities for women within the department. There are 14 graduate programs within the School of Medicine. The Immunology program hosts the *Graduate Immunology* course and the weekly immunology forum in which local and outside immunologists present their research.

The Johns Hopkins Bloomberg School of Public Health is the largest public health school in the world, is a research-oriented institution with more than 600 full-time faculty, and is home to the Feinstone Department of Molecular Microbiology and Immunology. It has a wide selection of excellent formal coursework in biostatistics and translational research, several of which Dr. Antar has proposed to take.

The Johns Hopkins Krieger School of Arts & Sciences' Advanced Academic Programs host a range of high-quality coursework including those in systems biology and bioinformatics proposed in this application.

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	○ Yes ● No
Is the Project Exempt from Federal regulations?	○ Yes ○ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Does the proposed research involve human specimens and/or data	● Yes ○ No
If Yes, provide an explanation of why the application does not involve human subjects research	Human_Subjects_Explanation.pdf
Other Requested information	

The research proposed in this K08 application involves the study of blood and tissue samples that have already been collected for another purpose and stored by one of four cohorts – SCOPE run out of UCSF, Johns Hopkins HIV Clinical Cohort (JHHCC), the Cleveland Immune Failure (CLIF) Cohort, and the AIDS Clinical Trials Group Longitudinal Linked Randomized Trials (ALLRT) for Aims 1 and 2 – or one of five clinical trials – ESPRIT, ICARUS, ILIADE, ACTG 5214, and NCT02191098 for Aim 3.

The people who provide us with the data and biological specimens will be study team members from each of one of the four cohorts (SCOPE, JHHCC, CLIF, and ALLRT), or study team members for each of one of the four trials (ESPRIT, ICARUS, ILIADE, NCT02191098), or an employee of the AIDS Clinical Trials Group (ACTG) for ACTG 5214. These people have no role in the research proposed in this application. All of the data from these cohorts and trials have been de-identified prior to their sample and minimal clinical info (CD4, viral load) and a trial or cohort number being sent to us for the proposed research. In the case of the cohorts, the study team member (who has no role in this application) will typically have access to the participants' identities. In the case of the clinical trials, the person sending us the sample may or may not have access to the participants' identities. The PI and her mentor do not have access to the participants' identities nor can the participants' identities be determined from the minimal clinical information we are given with the sample. The privacy of the participants is protected according to each research cohort and clinical trials' protocol.

Select Agent Research

Not applicable

Resource Sharing Plan

Data Sharing Plan: HIV *env* sequences obtained as part of these studies will be deposited in annotated form in Genbank. These sequences then become part of the Los Alamos HIV Sequence Database (https://www.hiv.lanl.gov/content/sequence/HIV/mainpage.html). Results of these assays will be published.

Sharing Model Organisms: Not applicable

Genome-Wide Association Studies: Not applicable

Authentication of Key Biological and/or Chemical Resources

Key biological resources in this K08 application include:

1. PBMCs and tissue from individuals with HIV

These were collected by the four cohorts and the clinical trials referenced in the K08 application. These are cryopreserved and shipped on dry ice to the Siliciano lab, where samples are stored in liquid nitrogen in a manner to minimize freeze-thaw cycles. All work performed after thawing until the completion of DNA or RNA extraction is done [redacted] Cells are counted after thawing to corroborate the cell number obtained by the cohort or trial. All cell handling is done in such a way as to minimize contamination.

2. Resting CD4 isolation kits

Resting $CD4^+$ T cells are isolated from PBMCs and lymphoid tissue from shipped samples in two steps. In the first, the Miltenyi $CD4^+$ T Cell Isolation kit (Miltenyi 130-096-533) is used to isolate untouched CD4s. In the second step, resting CD4s are isolated via negative selection after binding of sample to anti CD25 microbeads, anti HLA-DR microbeads or anti-CD69 biotin Ab and anti-biotin microbeads, all of which are obtained directly from Miltenyi. Miltenyi performs regular quality control checks on these products. CD4s are isolated within 24 hours of thaw and used on the day of isolation and the purity of their isolation is confirmed by flow cytometry using the PE Mouse Anti-Human CD4 antibody, obtained from BD biosciences (cat 555347).

3. MOLT-4 cell line

This cell line is used to amplify HIV in the quantitative viral outgrowth assay (QVOA). They are obtained from Sigma-Aldrich which provides a certificate of analysis. These are examined by microscopy for contamination twice weekly and maintained for three - four weeks prior to discarding the culture and thawing a new vial.

4. Primers/probes for QVIA and IPDA

All primers and probes are obtained from Integrated DNA Technologies (www.idtdna.com) and are aliquoted after receipt to minimize freeze/thaw cycles. Negative controls are included in every plate tested by IPDA and QVIA.