PI: **Tran, Tuan Manh**	Title: Defining clinical and sterile immunity to Plasmodium falciparum infection using systems biology approaches	
Received: 07/12/2016	FOA: PA16-191	Council: 01/2017
Competition ID: FORMS-D	FOA Title: MENTORED CLINICAL SCIENTIST RESEARCH CAREER DEVELOPMENT AWARD (PARENT K08)	
1 K08 AI125682-01A1	Dual:	Accession Number: 3956054
IPF: 577806	Organization: INDIANA UNIV-PURDUE UNIV AT INDIANAPOLIS	
Former Number:	Department:	
IRG/SRG: MID-B	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: [redacted] Year 2: [redacted] Year 3: [redacted] Year 4: [redacted] Year 5:	Animals: N Humans: N Clinical Trial: N Current HS Code: E4 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Tuan Tran	INDIANA UNIVERSITY	PD/PI
Chandy John	Indiana University	Other (Specify)-Mentor
Wanzhu Tu Ph.D	Indiana University	Other (Specify)-Co-Mentor
Peter Crompton	National Institute of Allergy and Infectious Diseases	Other (Specify)-Co-mentor
Lang Li	Indiana University	Other (Specify)-Co-mentor
Sangeeta Bhatia	Massachusetts Institute of Technology	Other (Specify)-Collaborator
Philip Felgner	University of California	Other (Specify)-Collaborator
Scott Michaels	Indiana University	Other (Specify)-Collaborator
Kara Wools-Kaloustian	Indiana University	Other (Specify)-Collaborator

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

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE | **State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier [redacted]

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier** | **c. Previous Grants.gov Tracking Number**

5. APPLICANT INFORMATION **Organizational DUNS*:** [redacted]

Legal Name*: The Trustees of Indiana University
Department:
Division:
Street1*: [redacted]
Street2:
City*:
County:
State*:
Province:
Country*:
ZIP / Postal Code*:

Person to be contacted on matters involving this application
Prefix: Mr. First Name*: Jim Middle Name: Last Name*: Becker Suffix:
Position/Title: Executive Director, Grant Administration
Street1*: Office of Research Administration
Street2:
City*:
County:
State*:
Province:
Country*:
ZIP / Postal Code*:
Phone Number*: [redacted] Fax Number: [redacted] Email: [redacted]

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

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

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

8. TYPE OF APPLICATION*
❍ New ● Resubmission
❍ Renewal ❍ Continuation ❍ Revision

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

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

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Defining clinical and sterile immunity to Plasmodium falciparum infection using systems biology approaches

12. PROPOSED PROJECT
Start Date* Ending Date*
04/01/2017 03/31/2022

13. CONGRESSIONAL DISTRICTS OF APPLICANT
IN-007

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: Tuan Middle Name: Manh Last Name*: Tran Suffix:

Position/Title: Assistant Professor of Medicine

Organization Name*: INDIANA UNIVERSITY

Department:

Division:

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Street2: [redacted]

City*: [redacted]

County:

State*: [redacted]

Province:

Country*: [redacted]

ZIP / Postal Code*: [redacted]

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

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$[redacted]
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$[redacted]
d. Estimated Program Income*	$0.00

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

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

DATE:

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

❍ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

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

● I agree*

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

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Mr. First Name*: John Middle Name: W. Last Name*: Talbott Suffix:

Position/Title*: Assistant Vice President for Research Admin.

Organization Name*: The Trustees of Indiana University

Department: Office of Research Aministrati

Division:

Street1*: [redacted]

Street2:

City*: [redacted]

County: [redacted]

State*: [redacted]

Province:

Country*: [redacted]

ZIP / Postal Code*: [redacted]

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

Signature of Authorized Representative*	Date Signed*
John Talbott	07/12/2016

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:K08_Cover_Letter_07012016.pdf

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

	Page Numbers
SF 424 R&R Cover Page	1
Table of Contents	3
Performance Sites	4
Research & Related Other Project Information	5
Project Summary/Abstract(Description)	6
Project Narrative	7
Bibliography & References Cited	8
Facilities & Other Resources	14
Equipment	17
Research & Related Senior/Key Person	18
Research & Related Budget Year - 1	64
Research & Related Budget Year - 2	67
Research & Related Budget Year - 3	70
Research & Related Budget Year - 4	73
Research & Related Budget Year - 5	76
Budget Justification	79
Research & Related Cumulative Budget	80
PHS398 Cover Page Supplement	81
PHS 398 Career Development Award	83
Introduction to Application	85
Candidate Information and Goals for Career Development	86
Specific Aims	89
Research Strategy	90
Training in the Responsible Conduct of Research	99
Plans and Statements of Mentor and Co-Mentor(s)	100
Letters of Support from Collaborators,Contributors, and Consultants	106
Description of Institutional Environment	110
Institutional Commitment to Candidate's Research Career Development	111
Protection of Human Subjects	112
Inclusion of Women and Minorities	114
PHS Inclusion Enrollment Report	115
Inclusion of Children	116
Resource Sharing	117
Authentication of Key Biological and/or Chemical Resources	118

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

Project/Performance Site Location(s)

Project/Performance Site Primary Location

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

Organization Name:	The Trustees of Indiana University
Duns Number:	[redacted]
Street1*:	[redacted]
Street2:	[redacted]
City*:	[redacted]
County:	[redacted]
State*:	[redacted]
Province:	
Country*:	[redacted]
Zip / Postal Code*:	[redacted]
Project/Performance Site Congressional District*:	IN-007

File Name

Additional Location(s)

Tracking Number: GRANT12212200
Funding Opportunity Number: PA-16-191. Received Date: 2016-07-12T10:54:42.000-04:00

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

RESEARCH & RELATED Other Project Information

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

1.a. If YES to Human Subjects

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

If YES, check appropriate exemption number: — 1 — 2 — 3 ✔ 4 — 5 — 6

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

IRB Approval Date:

Human Subject Assurance Number 00003544

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*	K08_Project_Summary-Abstract_07082016.pdf
8. Project Narrative*	K08_A1__07012016__Project_Narrative.pdf
9. Bibliography & References Cited	K08_References_Cited_07062016.pdf
10.Facilities & Other Resources	K08__Facilities_and_Resources__07082016.pdf
11.Equipment	K08_Equipment_07082016.pdf

Tracking Number: GRANT12212200
Funding Opportunity Number: PA-16-191. Received Date: 2016-07-12T10:54:42.000-04:00

Project Summary/Abstract

Malaria afflicts ~198 million people yearly, with 438,000 malaria deaths due to *Plasmodium falciparum*, underscoring the need for a highly effective malaria vaccine. The first licensed malaria vaccine, RTS,S, may provide much-needed reductions in morbidity and mortality, but its modest efficacy in reducing clinical malaria in the target population of African infants leaves ample margin for improvement. A better understanding of immunity to *P. falciparum* in naturally exposed populations can inform efforts to improve malaria vaccine design. To date, there are no reliable correlates of protection from either symptomatic *P. falciparum* infection (clinical immunity) or parasitemia (sterile immunity). Systems biology utilizes computational modeling of large-scale data sets to elucidate complex biological networks and has the potential to reveal novel predictors and mechanisms of malaria protection when applied to well-designed clinical cohort studies.

In this project, the candidate proposes to assess immune predictors of natural protection from *P. falciparum* infection using systems biology approaches. By analyzing clinical data and blood specimens collected from a well-characterized, prospective cohort of Malian children who differ in their degree of immunity to *P. falciparum* infection, the candidate will address two main research aims: 1) determine immune parameters predictive of protection from symptomatic infection (clinical immunity) and protection from *P. falciparum* infection (sterile immunity) and 2) relate these immune parameters and outcomes to the ability of plasma obtained from these children to inhibit parasite invasion into liver and red blood cells *in vitro*. The practical implications of this work include identifying novel immune predictors and mechanisms of protection from *P. falciparum* infection and disease within the vaccine target population that could provide rational benchmarks for candidate malaria vaccines.

The candidate is firmly committed to a career in translational malaria research and systems biology and is strongly supported in his career and research goals by his mentors and his division at the Indiana University School of Medicine. He currently holds a position as an Assistant Professor of Medicine with 80% protected time for research, independent laboratory and office space, and funding for equipment. The current proposal includes a comprehensive mentorship and didactic plan to advance the candidate's skills and knowledge in biostatistics and computational biology required for developing expertise in systems biology. Under the guidance of his primary mentor, Dr. Chandy John, and his co-mentors, Dr. Wanzhu Tu, Dr. Lang Li, and Dr. Peter Crompton, he will advance his bioinformatics skills and learn predictive modeling methodologies that will be directly applied to this proposal. Completion of this comprehensive training plan will provide the candidate with the skills and experience necessary to become a successful independent investigator specializing in computational systems biology with a focus on host immunity to *Plasmodium* infection.

Project Narrative

Each year malaria afflicts ~200 million people and causes over 430,000 deaths, primarily among African infants. Although a first-generation malaria vaccine is now available, it is only modestly effective in the target population of African infants; thus, a better understanding of natural immunity to *Plasmodium falciparum*, the deadliest of malaria parasites, in young African children can inform efforts to develop the next generation of malaria vaccines. Using samples collected from young Malian children before and during natural *P. falciparum* infections, this study aims to identify predictors and mechanisms of malaria immunity that aid the development of future malaria vaccines.

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Facilities and Resources

Overview: IUSM was established in 1903, graduating its first class in 1907, and merged with other Indianapolis schools to form Indiana University – Purdue University Indianapolis (IUPUI) in 1969. The main medical center campus, located at IUPUI, occupies 93 acres in downtown Indianapolis and consists of 18 buildings and four hospitals: University, Eskenazi Health, Roudebush Veteran's Administration, and the James Whitcomb Riley Hospital for Children. A fifth downtown hospital, the not-for-profit Methodist Hospital, merged with Indiana University Hospital in 1997 to form Indiana University Health. Within IUSM facilities, research laboratory and support space encompasses approximately 619,000 net assignable square feet. An additional 41,000 net assignable square feet of IUSM-leased lab space is located off campus at partner health institutions. The academic faculty comprises of ~2,000 full-time faculty across more than 60 clinical departments and 29 research institutes. For FY2014-15, IUSM received approximately $302 million in sponsored programs funding, $111 million of which came from the NIH. As of June 2015, there were 564 externally funded primary investigators at IUSM. IUSM currently hosts 12 research centers or program projects supported by PHS/NIH or other federal funds, and at least 20 supported by non-federal funds. As of June 2015, there were 14 T32, one T35, three R25, one CTSI-based (TL1), eight HRSA, nine F-series, and 18 K-series federally-funded training programs and grants at IUSM.

Laboratory: The IU Cancer Research Institute (R4) at IUSM is a four-story, 112,000 square foot research and teaching facility that houses the Herman B. Wells Center for Pediatric Research and laboratories for researchers from seven multidisciplinary research groups. I have 500 square feet independent laboratory space (R4 Room 451B) adjacent to the laboratory of Dr. Chandy John, my primary mentor, which features shared biosafety cabinets and incubators for tissue culture work and a liquid nitrogen storage tower. My laboratory is equipped with: a -80°C freezer, a -20°C freezer/refrigerator, a refrigerated Eppendorf 5430R centrifuge, an Eppendorf 5424 centrifuge, a microwave, water baths, a heat block, Mettler-Toledo scale, vortexes, a Qubit 3.0 Fluorometer, a mini-incubator, a BioTek 96-well plate washer with magnetic adapter, a Bio-Rad C1000 Touch thermal cycler, and an upright and inverted phase microscope. A Bio-Rad MAGPIX multiplex reader for bead-based immunoassays, ELISA microplate reader, Bio-Rad plate washer, a LabChip GX Touch HT microfluidic device, a Bio-Rad Tetrad Thermal Cycler, and an Applied Biosystems QuantStudio 6 Flex Real-Time PCR System with 96- and 384-well blocks will be shared with Dr. John. The Wells Center also provides glass washing, common reagent preparation, and sterilization services for all investigators. As a member of the Wells Center, I will have unlimited access to a BD FACSCanto II capable of 6-color flow cytometer for routine FACS experiments. Importantly, R4 is adjacent to Walther Hall, which houses the flow-cytometry core facilities within the IU Simon Cancer Center, allowing access to the necessary equipment for conducting multi-parametric flow cytometry assays and cell sorting experiments (see below).

Clinical sites: IUSM and its affiliated hospitals are located on or near the IUPUI campus. The main campus complex is 93 acres in area. IU Health manages the administration of three of the teaching hospitals (University Hospital, Riley Hospital for Children, and Methodist Hospital). The School is nearly physically contiguous with the Richard L. Roudebush Veterans Administration Medical Center located less than ¼ mile from the western edge of campus. University Hospital is a tertiary care hospital and referral center for Indiana and nearby states. It has approximately 400 inpatient beds. Methodist Hospital has approximately 800 beds and was formerly an independent non-profit hospital. Methodist Hospital also has a full range of tertiary services including: Level 1 trauma Center, orthopedics, cardiovascular, neurosciences and outpatient surgery.
I will devote no more than 20% effort to clinical responsibilities. I will maintain a one half-day Infectious Diseases clinic every other week at the Indiana University Hospital Medical Diagnostic Center, focusing on patients with infectious diseases issues who need urgent appointments, timely hospital follow up, or travel medicine consultation. I will also provide 6 weeks of clinical service per year as the Infectious Diseases consult attending at Methodist, University, or Roudebush Hospitals.

Offices: I have a fully furnished office within the Wells Center in the Cancer Research Building (R4) on the same floor as my laboratory with a personal phone, computer, and printer, and internet access. Print, copy, scan, and fax capabilities are provided by a shared laboratory multi-function printer. My office is along the same hallway as the primary office of Dr. John. I also have an office in the Van Nuys Medical Science Building in the Division of Infectious Diseases within the Department of Medicine, with a separate phone, computer, and

shared printer, in close proximity to the office of my Division Director Dr. Kara Wools-Kaloustian (located in Emerson Hall).

Computers: I will be using a 15-inch MacBook Pro with a 2.8 GHZ Quad-core Intel Core i7 processor and a MacPro with a 3.5GHz 6-Core Intel Xeon E5 processor as primary research computers, equipped with external Dell 24" P2416D monitors. Appropriate software will be installed on the computer, including Microsoft Office, EndNote, R, GraphPad Prism, Adobe Illustrator, Adobe Acrobat, FlowJo, and Ingenuity Pathways Analysis. In addition, I have an iMac in my Medical Science Building office, which I use primarily for clinical work, and an iMac for use by a laboratory technician. A Dell Optiplex 9020 will be a common-use computer in my laboratory dedicated to running Windows-only applications such as ABI PeakScanner and SDS software packages. I maintain an NIH/NIAID MacBook Pro laptop through my Special Volunteer status at the NIH, in conjunction with my co-mentor Dr. Crompton, allowing access to an encrypted NIH server for collaborative work. The computers at IUSM are connected via Ethernet or secured Wi-Fi to the main server for email, Internet access, and secure storage of data files. Research data is backed up from the servers to on-site disk backup devices using 256-bit AES encryption.

The **IUSM Center for Computational Biology and Bioinformatics (CCBB)** can provide additional computational support. Statistical packages include R, SAS, S-PLUS, SigmaPlot, nQuery Advisor, DBMScopy, Solas, LogXact, StatXact, and SPSS. Databases are implemented using Microsoft SQL Server 2000. The center also maintains a network of UNIX systems, running Solaris or Linux for intensive data analysis. The staff accesses these UNIX machines over the network using Hummingbird Exceed or SSH.

For statistical analysis requiring high-performance parallel computing, I will have access to the IU supercomputers, which include IU's Big Red II supercomputer (one petaflops), Big Red supercomputer (40.96 teraflops), Quarry parallel computing cluster (26.22 teraflops), and Mason large-memory (8TB) computing cluster (3.81 teraflops).

Administrative support: The Division of Infectious Diseases provides administrative support for this project. The Department of Medicine provides support for website design and maintenance, grants management expertise, and appropriate laboratory and office space. In addition, the Department has a dedicated and engaged Vice Chair of Research Dr. Samir Gupta who provides career development support for junior faculty.

Other Resources – Research Environment

IU Bloomington Center for Genomics and Bioinformatics: The Center for Genomics and Bioinformatics (CGB), directed by Dr. Scott Michaels, offers in-house next generation sequencing services on Roche 454, Illumina NextSeq, and Illumina MiSeq platforms for IU faculty. Sequencing on Illumina HiSeq is also available via send out. CGB also provides RNA-seq mapping services, transcriptome annotation services, and sequence submission to the NCBI Sequence Read Archive and Gene Expression Omnibus.

IU Simon Cancer Center Flow Cytometry and Proteomics Services: As an investigator within the Wells Center, I have ready access to the necessary equipment for flow cytometry and cell sorting through the Flow Cytometry Core Facility at the IU Simon Cancer Center, located in Walther Hall, a building directly connected to R4. This facility contains two BD FACSCalibur (three and four colors) and two BD LSRII (nine and 10 colors) flow cytometers as well as BD FACSAria (10 colors), BD SORP Aria (15 colors), and iCyt Reflection (nine colors) cell sorters. Both the FACSAria and SORP Aria are BSL2+. In addition, IUSM recently acquired a CyTOF2 Mass cytometer, which allows simultaneous detection of more than 40 parameters using metal-conjugated markers at the single cell level.

University Libraries: As a member of IU faculty, I have access to nearly 700 databases, 60,000 electronic journal titles, and 815,000 electronic books.

The IUPUI University Library is a state-of-the-art library completed in 1993. It houses more than one million volumes, including current subscriptions to over 35,000 print and electronic journals.

The Ruth Lilly Medical Library (RLML) was established in 1908 and is the largest academic health sciences library in Indiana. It is the primary information resource for faculty, students, and staff of the Indiana University

School of Medicine and ancillary health sciences programs. The RLML has eight tenure-track library faculty, three non-tenure track librarians, and eight support staff members. The Library staff is available for consultation on a wide range of knowledge management issues and manages a collection of 8,000 electronic journals, 15,000 electronic books and nearly 200,000 print volumes.

Resources for Early Stage Investigators: IUSM is committed to promoting the development and advancement of early stage investigators and offers the following resources:

A. The **Indiana Clinical and Translational Sciences Institutes** (CTSI) offers **Young Investigator Awards in Clinical-Translational Research**, of which I am a recipient. These awards designed to provide junior investigator faculty with the opportunity to be mentored in research-intensive multi-disciplinary settings toward the goal of developing clinical-translational research careers. Clinical research can include epidemiological studies, clinical trials, or other investigations involving human subjects.

B. The **Independent Investigator Incubator (I^3)**, supported by the IUSM Transforming Research Initiative (TRI) in collaboration with the CTSI, develops junior faculty into successful researchers by providing access to one-on-one time and professional coaching from a senior faculty "super-mentor" and support services such as a professional grant writer, administrative support, and a biostatistician.

C. The **Leadership in Academic Medicine Program (LAMP**), organized through the Office of Faculty of Affairs and Professional Development at IUSM, is a year-long, cohort-based faculty development and orientation program designed for faculty in the second and third years of their appointment.

D. The **Indiana-CTSI Project Development Teams** are committees of multi-disciplinary researchers who help investigators develop concepts and hypotheses into translational research projects necessary for successful extramural grant submissions, providing access to protocol development, pilot funding, biostatistics support, IRB/regulatory support, and access to collaborators at IUPUI and other Indiana-CTSI institutions (IU, Purdue, and Notre Dame).

Collaborating sites: Human samples will be obtained from the NIH in collaboration with Dr. Peter Crompton at NIAID and with the support of Dr. Boubacar Traore in the MRTC in Mali. The laboratory of Dr. Philip L. Felgner within the Institute for Immunology, University of California, Irvine will support protein array studies. The Laboratory of Multiscale Regenerative Technologies, directed by Dr. Sangeeta Bhatia, will support studies involving the microscale human liver platform.

Major Equipment

Dr. Tran's laboratory is equipped with four BSL2 biosafety cabinets shared with two other investigators at the Wells Center. His laboratory will also have the following: a -80°C freezer, a -20°C freezer/refrigerator, a refrigerated Eppendorf 5430R centrifuge, an Eppendorf 5424 centrifuge, a microwave, water baths, a heat block, Mettler-Toledo scale, vortexes, a Qubit 3.0 Fluorometer, a mini-incubator, a BioTek 96-well plate washer with magnetic adapter, a Bio-Rad C1000 Touch thermal cycler, and an upright and inverted phase microscope. Dr. Tran will share the following equipment with Dr. John: Bio-Rad MAGPIX multiplex reader for bead-based immunoassays, ELISA microplate reader, Bio-Rad plate washer, a LabChip GX Touch HT microfluidic device, a Bio-Rad Tetrad Thermal Cycler, and an Applied Biosystems QuantStudio 6 Flex Real-Time PCR System with 96- and 384-well blocks.

Laboratory Core within the Wells Center for Pediatric Research: Dr. Tran will have routine access to a BD FACSCanto II 6-color flow cytometer and an additional Applied Biosystems real-time PCR machine. Other available equipment includes autoMACs cell separators, two NanoDrop 2000 Spectrophotometers, two Beckman ultracentrifuges, Leica conventional and GFP microscopes equipped with digital cameras, Z1 coulter counter, a Perkin-Elmer cell harvester and Top Count system, and double-deionizing water purification systems. There will be a walk-in 4°C cold room along the same hallway as Dr. Tran's lab.

Flow Cytometry Core Facility at IU Simon Cancer Center: Walther Hall, adjacent to the Wells Center, houses the flow cytometry core facility, which is equipped with two BD FACSCalibur (3 and 4 colors) and two BD LSRII (9 and 10 colors) flow cytometers as well as BD FACSAria (10 colors), BD SORP Aria (15 colors), and iCyt Reflection (9 colors) cell sorters. Both the FACSAria and SORP Aria are BSL2+. In addition, IUSM recently acquired a CyTOF2 Mass cytometer, which allows simultaneous detection of more than 40 parameters using metal-conjugated markers at the single cell level. Core services, available to all investigators at the Wells Center, include multi-parameter immunofluorescence analysis and cell sorting, single cell sorting, cell cycle analysis, and kinetic analysis.

IU Bloomington Center of Genomics and Bioinformatics: Dr. Tran will work with the genomics and bioinformatics team at CGB for his next generation sequencing studies. Available NGS equipment includes: **a)** Illumina NextSeq 500 **b)** Illumina MiSeq **c)** Roche 454. Bioinformatics specialists will also provide mapping and visualization of sequences for the projects outlined in the proposal.

IUSM Center for Computational Biology and Bioinformatics (CCBB): Dr. Tran will have access to additional computational support through the CCBB. The center maintains a network of UNIX systems, running Solaris or Linux for intensive data analysis. For statistical analysis requiring high-performance parallel computing, Dr. Tran can access the IU supercomputers, which include IU's Big Red II supercomputer (one petaflops), Big Red supercomputer (40.96 teraflops), Quarry parallel computing cluster (26.22 teraflops), and Mason large-memory (8TB) computing cluster (3.81 teraflops).

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

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Tuan Middle Name Manh Last Name*: Tran Suffix:

Position/Title*: Assistant Professor of Medicine
Organization Name*: Indiana Unviversity
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Credential, e.g., agency login: [redacted]

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

Degree Type: MD, PHD, BS Degree Year: 2007/2005/1999

	File Name
Attach Biographical Sketch*:	biosketch-Tran.Tuan_070716.pdf
Attach Current & Pending Support:	

Tracking Number: GRANT12212200
Funding Opportunity Number: PA-16-191 . Received Date: 2016-07-12T10:54:42.000-04:00

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Chandy Middle Name C. Last Name*: John Suffix:

Position/Title*: Professor, Pediatrics, Micro & Immunology
Organization Name*: Indiana University
Department: Medicine
Division: Pediatrics, Microbiology/Immun
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: E-Mail*: [redacted]

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

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

Degree Type: MD, MS Degree Year: 1988/2001

File Name
Attach Biographical Sketch*: John_Bios.pdf
Attach Current & Pending Support:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Wanzhu Middle Name Last Name*: Tu Suffix: Ph.D

Position/Title*: Professor
Organization Name*: Indiana University
Department: School of Medicine
Division: Biostatistics
Street1*: Wanzhu Tu, PHD
Street2: Dept. of Biostatistics
City*: Indianapolis
County: Marion
State*: IN: Indiana
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 462023012

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: PHD Degree Year: 1997

File Name
Attach Biographical Sketch*: Bios_TU_070716.pdf
Attach Current & Pending Support:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Peter Middle Name D. Last Name*: Crompton Suffix:

Position/Title*: Chief
Organization Name*: National Institute of Allergy and Infectious Diseases
Department: Intramural research
Division: Lymphocyte Activation Section
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number: E-Mail*: [redacted]

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

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

Degree Type: MD Degree Year: 2000

File Name
Attach Biographical Sketch*: CromptonBio_070116.pdf
Attach Current & Pending Support:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Lang Middle Name Last Name*: Li Suffix:

Position/Title*: Professor
Organization Name*: Indiana University
Department: Medicine
Division: Bioinformatics
Street1*: Indiana University
Street2: DEPT of MEDICINE, DIV of BIOSTAT
City*: Indianapolis
County: Marion
State*: IN: Indiana
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 462023012

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: PHD, MA, BA Degree Year: 2001/1996/1992

File Name
Attach Biographical Sketch*: LangLiBiosketch.pdf
Attach Current & Pending Support:

Tracking Number: GRANT12212200
Funding Opportunity Number: PA-16-191 . Received Date: 2016-07-12T10:54:42.000-04:00

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Sangeeta Middle Name N. Last Name*: Bhatia Suffix:

Position/Title*: Professor
Organization Name*: Massachusetts Institute of Technology
Department: IMES
Division: Molecular Biology
Street1*: MIT-Institute for Medical Engineering & Science
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
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, PHD Degree Year: 1999/1997

File Name
Attach Biographical Sketch*: SangeetaBhatiaBiosketch.pdf
Attach Current & Pending Support:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Philip Middle Name Louis Last Name*: Felgner Suffix:

Position/Title*: Adjunct Professor
Organization Name*: University of California
Department: Medicine
Division: Infectious Disease
Street1*: Division of Infectious Disease
Street2: Department of Medicine
City*: Irvine
County:
State*: CA: California
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 92869-0000

Phone Number*: [redacted] Fax Number: E-Mail*: [redacted]

Credential, e.g., agency login: pfelgner

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

Degree Type: PHD,MS,BS Degree Year: 1978/4975/1972

File Name
Attach Biographical Sketch*: PhillipFelgnerBiosketch.pdf
Attach Current & Pending Support:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Scott Middle Name D. Last Name*: Michaels Suffix:

Position/Title*: Professor
Organization Name*: Indiana University
Department: Medicine
Division: Biology
Street1*: [redacted]
Street2:
City*:
County:
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*:

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: PHD,BS Degree Year:

File Name
Attach Biographical Sketch*: MichaelsBiosketch.pdf
Attach Current & Pending Support:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Kara Middle Name Kay Last Name*: Wools-Kaloustian Suffix:

Position/Title*: Associate Professor
Organization Name*: Indiana University
Department: Medicine
Division: Infectious Diseases
Street1*: [redacted]
Street2:
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State*:
Province:
Country*: [redacted]
Zip / Postal Code*:

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, BA Degree Year: 1988/1984

File Name
Attach Biographical Sketch*: Kara_Wools-Kaloustian_Biosketch_Tuan_K08.pdf
Attach Current & Pending Support:

Tracking Number: GRANT12212200
Funding Opportunity Number: PA-16-191 . Received Date: 2016-07-12T10:54:42.000-04:00

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

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Trustees of Indiana University

Start Date*: 04-01-2017 **End Date*:** 06-30-2018 **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 .	Dr.	Tuan	Manh	Tran		PD/PI	[redacted]	9.0			[redacted]	[redacted]	[redacted]
2 .	Dr.	Tuan	Manh	Tran		PD/PI (IUHP)	[redacted]	9.0			[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)

Tracking Number: GRANT12212200
Funding Opportunity Number: PA-16-191 . Received Date: 2016-07-12T10:54:42.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Trustees of Indiana University

Start Date*: 04-01-2017 **End Date*:** 06-30-2018 **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)	0.00
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: GRANT12212200

Funding Opportunity Number: PA-16-191 . Received Date: 2016-07-12T10:54:42.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Trustees of Indiana University

Start Date*: 04-01-2017 **End Date*:** 06-30-2018 **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)	Department of Health and Human Services (D.H.H.S.) Arif Karim, (214) 767-3261

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

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: K08_Budget-Justification_070516revised.pdf (Only attach one file.)

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

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

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Trustees of Indiana University

Start Date*: 04-01-2018 **End Date*:** 03-31-2019 **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 .	Dr.	Tuan	Manh	Tran		PD/PI	[redacted]	9.0			[redacted]	[redacted]	[redacted]
2 .	Dr.	Tuan	Manh	Tran		PD/PI (IUHP)	[redacted]	9.0			[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)

Tracking Number: GRANT12212200
Funding Opportunity Number: PA-16-191 . Received Date: 2016-07-12T10:54:42.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Trustees of Indiana University

Start Date*: 04-01-2018 **End Date*:** 03-31-2019 **Budget Period:** 2

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)	0.00
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: GRANT12212200

Funding Opportunity Number: PA-16-191 . Received Date: 2016-07-12T10:54:42.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Trustees of Indiana University

Start Date*: 04-01-2018 **End Date*:** 03-31-2019 **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)	Department of Health and Human Services (D.H.H.S.) Arif Karim, (214) 767-3261

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

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: K08_Budget-Justification_070516revised.pdf (Only attach one file.)

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

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

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Trustees of Indiana University

Start Date*: 04-01-2019 **End Date*:** 03-31-2020 **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 .	Dr.	Tuan	Manh	Tran		PD/PI	[redacted]	9.0			[redacted]	[redacted]	[redacted]
2 .	Dr.	Tuan	Manh	Tran		PD/PI (IUHP)	[redacted]	9.0			[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)

Tracking Number: GRANT12212200
Funding Opportunity Number: PA-16-191 . Received Date: 2016-07-12T10:54:42.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Trustees of Indiana University

Start Date*: 04-01-2019 **End Date*:** 03-31-2020 **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)	0.00
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: GRANT12212200

Funding Opportunity Number: PA-16-191 . Received Date: 2016-07-12T10:54:42.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Trustees of Indiana University

Start Date*: 04-01-2019 **End Date*:** 03-31-2020 **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)	Department of Health and Human Services (D.H.H.S.) Arif Karim, (214) 767-3261

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

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: K08_Budget-Justification_070516revised.pdf (Only attach one file.)

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

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

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Trustees of Indiana University

Start Date*: 04-01-2020 **End Date*:** 03-31-2021 **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 .	Dr.	Tuan	Manh	Tran		PD/PI	[redacted]	9.0			[redacted]	[redacted]	[redacted]
2 .	Dr.	Tuan	Manh	Tran		PD/PI (IUHP)	[redacted]	9.0			[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)

Tracking Number: GRANT12212200
Funding Opportunity Number: PA-16-191 . Received Date: 2016-07-12T10:54:42.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Trustees of Indiana University

Start Date*: 04-01-2020 **End Date*:** 03-31-2021 **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)	0.00
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: GRANT12212200

Funding Opportunity Number: PA-16-191 . Received Date: 2016-07-12T10:54:42.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Trustees of Indiana University

Start Date*: 04-01-2020 **End Date*:** 03-31-2021 **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)	Department of Health and Human Services (D.H.H.S) Arif Karim (214) 767-3261

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

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: K08_Budget-Justification_070516revised.pdf (Only attach one file.)

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

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

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: The Trustees of Indiana University

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **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 .	Dr.	Tuan	Manh	Tran		PD/PI	[redacted]	9.0			[redacted]	[redacted]	[redacted]
2 .	Dr.	Tuan	Manh	Tran		PD/PI (IUHP)	[redacted]	9.0			[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)

Tracking Number: GRANT12212200
Funding Opportunity Number: PA-16-191 . Received Date: 2016-07-12T10:54:42.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Trustees of Indiana University

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **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)	0.00
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: GRANT12212200

Funding Opportunity Number: PA-16-191 . Received Date: 2016-07-12T10:54:42.000-04:00

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

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: The Trustees of Indiana University

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **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)	Department of Health and Human Services (D.H.H.S.) Arif Karim, (214) 767-3261

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

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: K08_Budget-Justification_070516revised.pdf (Only attach one file.)

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

Budget Justification:

Tuan Tran, M.D., PhD, Principal Investigator (9.00 calendar months): Dr. Tran is an Assistant Professor of Medicine in the Division of Infectious Diseases at Indiana University School of Medicine. His appointment began on Sept 1, 2015. Dr. Tran will conduct all work associated with this study as proposed and will interpret data.

Indiana University Health Physicians

Dr. Tran receives compensation from two sources as a faculty member with the Department of Medicine. The first source is from Indiana University (IU). The second source is from a third-party, non-profit corporation, Indiana University Health Physicians (IUHP), whose mission is to support the instruction, research and public service missions of the IU School of Medicine. It is certified the two sources of income comprise a single compensation package for Dr. Tran, which complies with the NIH guidelines on institutional-based salary (NIH Notice NOT-OD-05-061). Dr. Tran will devote 9.00 calendar months to the project; accordingly, the two sets of calendar month figures shown in the budget should not be added together to establish effort – the second figure is repeated to reflect the correct split between two sources for one institutional-based salary.

Materials and Supplies: $[redacted] yr.1; $[redacted] yr.2; $[redacted] yr.3; $[redacted] yr.4; and $[redacted] yr.5

RNA Extraction	[redacted]
RNA-Seq Library Preparation and Sequencing	[redacted]
Consumable plasticware	[redacted]
DNA extraction	[redacted]
PCR reagents	[redacted]
One meeting to present data	[redacted]
Shipping	[redacted]
RNA-seq Mapping Services	[redacted]
4 TB External Hard Drives	[redacted]
Protein Array Materials and Service	[redacted]

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

Tracking Number: GRANT12212200

Funding Opportunity Number: PA-16-191 . Received Date: 2016-07-12T10:54:42.000-04:00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ❍ Yes ● No

*Agency-Defined Phase III Clinical Trial? ❍ Yes ❍ No

2. 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 proved scientific justification

3. *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: GRANT12212200

Funding Opportunity Number: PA-16-191 . Received Date: 2016-07-12T10:54:42.000-04:00

PHS 398 Cover Page Supplement

4. 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, please 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):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ❍ Yes ❍ No

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

*Previously Reported: ❍ Yes ❍ No

6. 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: GRANT12212200

Funding Opportunity Number: PA-16-191 . Received Date: 2016-07-12T10:54:42.000-04:00

PHS 398 Career Development Award Supplemental Form

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction	
1. Introduction to Application (RESUBMISSION)	K08_Introduction_07072016.pdf
Candidate Section	
2. Candidate Information and Goals for Career Development	K08_Candidate_Information_07082016.pdf
Research Plan Section	
3. Specific Aims	K08_Specific_Aims_07062016.pdf
4. Research Strategy*	K08_Research_Strategy_07072016.pdf
5. Progress Report Publication List (for RENEWAL applications only)	
6. Training in the Responsible Conduct of Research	K08_Training_In_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)	Mentor_Statements_Chandy_Crompton_Li_Wantzu.pdf
9. Letters of Support from Collaborators, Contributors, and Consultants	Letters_ofS_support_Bhatia_Boubacar_Wools-Kaloustian_Felgner.pdf
Environment and Institutional Commitment to Candidate Section	
10. Description of Institutional Environment	K08_Description_of_the_Institutional_Environment__07072016.pdf
11. Institutional Commitment to Candidate's Research Career Development	TRAN_Institutional_Commitment_Ltr_K08_Geraci_Resubmission.pdf
Human Subject Section	
12. Protection of Human Subjects	K08_Protection_of_Human_Subjects_01.pdf
13. Data Safety Monitoring Plan	
14. Inclusion of Women and Minorities	K08__Inclusion_of_Women_and_Minorities_07012016.pdf
15. Inclusion of Children	K08_Inclusion_of_Children.pdf
Other Research Plan Section	
16. Vertebrate Animals	
17. Select Agent Research	
19. Consortium/Contractual Arrangements	
19. Resource Sharing	K08_Data_Resource_Sharing_Plan_07052016.pdf
20. Authentication of Key Biological and/or Chemical Resources	K08_Authentication_of_Key_Biological_Resources_07122016.pdf
Appendix	
21. Appendix	

Tracking Number: GRANT12212200
Funding Opportunity Number: PA-16-191. Received Date: 2016-07-12T10:54:42.000-04:00

PHS 398 Career Development Award Supplemental Form

Citizenship*:

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 with a temporary U.S. visa who has applied for permanent resident status and expect to hold a permanent resident visa by the earliest possible start date of the award, also check here: ❑

Introduction to Resubmission

This is a resubmission of the application 1 K08 AI125682-01, which was reviewed on February 29, 2016 (MID-B Study Section). As a junior investigator, I am grateful for the reviewers' comments and have made every effort to address each of their critiques. I believe the current application better delineates how systems biology approaches will be implemented to identify immune parameters predictive of immunity to *P. falciparum (Pf*) and broadens the scope of potential findings by incorporating host genetic and epidemiological factors relevant to malaria. The reviewers were enthusiastic of my potential as a K08 candidate, citing that my unique skill set, consisting of both field-research experience and knowledge of bioinformatics and systems biology, could allow me to excel in cutting-edge biomedical -omics fields. The panel highlighted my eminent team of qualified mentors and my institution's commitment to my success. They noted that the application addresses important questions in the understudied field of malaria systems immunology and has the potential for success given the track record of my mentors and unique access to a valuable collection of patient samples. However, several weaknesses related to the research and career development plans were outlined. To address these criticisms, the current proposal provides more robust classifications of clinical and sterile immunity to *P. falciparum* (*Pf*) and details the computational methods for integrating epidemiological, clinical, genomic, and immunological data in order to predict immune classes, while reinforcing statistical training in the didactic regime to enhance my competence in modeling approaches.

The primary concerns expressed by the panel include the lack of detailed methodologies on how the data can be integrated and modeled in order to predict immunity to *Pf* infection. To address this, we describe in the current application how we will test the accuracy of regularized regression models and machine learning algorithms in predicting the immune classes, focusing on approaches with robust feature selection that can accommodate mixed-type, high-dimensional data (**3.2.2h**). We outline how these approaches allow us to integrate clinical and epidemiological data with high-dimensional biological data from multiple assay platforms, and can select for groups of highly correlated variables to derive biological insight. The panel also suggested combining Aims 1 and 2 given the overlap in methods. We have indeed **combined the original Aims into a single Aim 1** and have **introduced a revised Aim 2** that adds a comparative, multi-platform analysis between clinical and sterile immunity (as suggested by the panel) and better integrates the proposed functional assays with the gene expression, protein array, and flow cytometry data.

Reviewer 1 expressed concern that we had overlooked malaria protection afforded by host genetics, most notably erythrocyte variants. The original application restricted the study to only HbAA individuals to avoid confounding due to HbS and HbC. The current proposal also accounts for ABO blood groups, α+-thalassemia, and G6PD deficiency and will incorporate these erythrocyte variants in the above models (**3.2.2b**).

Reviewers 2 and 3 suggested additional statistical training, with reviewer 3 emphasizing epidemiology. Based on reviewers' suggestions, we reconstructed my proposed educational program to better cover all the statistical and epidemiological training that I need, which includes formal courses integrated with directed reading and analyses that I perform under the direct supervision of my statistical mentors (**Didactic Training**).

Reviewer 3 was concerned with arbitrary cut-offs and statistical inference made from our power analysis. We originally chose 2500 parasites/µl as the threshold for defining malaria episodes to strike a balance between sensitivity and specificity for diagnosing malaria in a semi-immune population, where children with low-level *Pf* infections exhibiting fevers due to other pathogens may be misclassified. However, we recognize the need for increased sensitivity and **lowered the threshold to 500 parasites/µl**. By doing so, we expand the number of episodes for all children, allowing for an indeterminate group (children with 3 episodes) that demarcates the clinically immune and susceptible groups. To address concerns about power and sample size adequacy, we expanded the original analysis by **adding simulation-based estimates of effect sizes** using our proposed statistical methods and sample sizes based on the actual number of existing biological samples.

Reviewer 4 expressed concern for the lack of an existing mentoring relationship between IU mentors and myself and for a potential reduction in productivity during first years of the K08. I am currently involved in 7 projects (5 using data previously collected by members of our team and 2 involving new data collection). Three of these include collaborations with new mentors in my current position, and 4 involve collaboration only with my mentor from my prior position. Based on our prior experience, we expect these activities to produce 4-6 publications over the next 2 years. These activities will help maintain my name recognition in the field.

In summary, we thank the reviewers for their helpful suggestions. Their critiques have provided us with better focus and guided our revisions to significantly improve the resubmission. We believe that the better characterized immune and susceptible class definitions, more thorough description of computational methods, and more rigorous statistical didactic regime in the revised proposal have resulted in more powerful studies that should advance malaria systems immunology and help develop my expertise in this understudied field.

Candidate Information

I have been fascinated with host responses to microbes ever since grade school, when I was introduced to Louis Pasteur by way of a children's biography. This early fascination, nurtured by the support of my physician-father, led me towards formal training as a medical scientist. In high school, I was fortunate to work with Dr. Jill Verlander at the University of Florida (UF) College of Medicine. I investigated the effects of estrogen on the thiazide-sensitive NaCl co-transporter in the distal convoluted tubule. My continued research in Dr. Verlander's laboratory during my undergraduate studies at UF led to second authorship on a publication (19). I graduated from UF as the valedictorian and enrolled in the MSTP at Emory University. As a medical student, I happened upon a lecture by the late Dr. Robert Desowitz. Malaria, I learned that evening, was a disease that affected ~500 million people yearly, yet there was no effective vaccine. I switched my focus to malaria and began my studies in Dr. Mary Galinski's laboratory.

For my PhD thesis, I evaluated the *Plasmodium vivax* reticulocyte binding protein-1 (PvRBP1) as a vaccine candidate. I was awarded the Ben H. Kean Fellowship to work in the Brazilian Amazon, where Dr. Galinski and FIOCRUZ collaborator Dr. Joseli Oliveira-Ferreira were investigating naturally acquired immune responses to *P. vivax* vaccine candidate antigens. In Brazil, I performed field research and field-based immunological assays (20-22). I expressed recombinant fragments spanning the PvRBP1 ectodomain to map its binding domain (23) and to determine dominant B-cell epitopes (24). I also evaluated the genetic diversity of reticulocyte-binding homologues (25). My thesis work resulted in 3 first-author publications (23-25) and showed evidence that the N-terminal region of PvRBP1 would be a rationale *P. vivax* vaccine target (22). After graduating from Emory, I completed an Internal Medicine residency at the Johns Hopkins Hospital. As a resident, I was the PI on a Johns Hopkins Global Health Grant, which allowed me to independently study antigen-specific basophil responses during *P. falciparum* (*Pf*) infection in malaria-exposed Malians.

I completed the first clinical year in the Infectious Diseases Fellowship at NIAID before joining Dr. Susan Pierce's Laboratory of Immunogenetics (LIG), where I began the research phase of my fellowship under the supervision of Dr. Peter Crompton. Dr. Crompton's laboratory collaborates with Dr. Boubacar Traore at the Malaria Research and Training Center (MRTC) within the Mali International Center for Excellence in Research to study malaria immunity in individuals living in malaria-endemic areas. I helped establish a longitudinal cohort study in Kalifabougou, Mali to investigate the molecular and cellular differences between prospectively defined malaria-susceptible and immune individuals using systems biology approaches (8). I characterized the cohort to define clinically immune and susceptible individuals, which involved retrospectively identifying when individuals became infected with *Pf* and whether or not they progressed to malarial fever. The first year of this cohort study yielded much insight into naturally acquired malaria immunity. First, I provided evidence that, despite the natural acquisition of *clinical* immunity to malaria, most individuals in hyperendemic areas did not acquire *sterile* immunity despite years of malaria exposure (7). Second, I showed that naturally acquired IgG to *Pf* reticulocyte binding homologue 5 correlated with reduced malaria risk and inhibited parasite growth in vitro (17). Thorough parasitological characterization of the cohort allowed me to elucidate the relationship between *Schistosoma haematobium* infection and malaria risk (18). My analysis of the clinical cohort data and computational abilities allowed me to make contributions to other projects, leading to co-authorship on several publications (26-32). Since characterizing the initial clinical data, I have focused on determining the differences in whole-blood transcriptomes between malaria-immune and susceptible children using RNA-seq (33).

In September 2015, I joined the Division of Infectious Diseases within the Department of Medicine at the Indiana University School of Medicine (IUSM) as a tenure-track Assistant Professor. I chose IUSM due to the collaborative atmosphere and the commitment to global health research in the Division led by Dr. Kara Wools-Kaloustian. I was drawn to the opportunity for growth in malaria research due to the arrival of Dr. Chandy John, who has expertise in malaria immuno-epidemiology and severe human malaria pathogenesis. The IUSM Department of Medicine granted me a generous three-year start-up package that includes funding for equipment and a technician, independent lab and office space, and 80% protected research time to facilitate my development as an independent scientist and establish an extramurally funded translational research program focused on malaria immunity. Recently I have been awarded internally funded Indiana-CTSI Young Investigator and IU Global Health-Indiana CTSI grants, the latter to start field studies in Western Kenya with my mentor Chandy John. My clinical duties include an infectious diseases clinic twice per month and 6 weeks per year of service as the infectious diseases consult attending.

Candidate's Goals For Career Development

I am interested in unraveling the complexities of host immune responses to *Plasmodium* infection in the endemic setting, where children are exposed to a myriad of commensal and pathogenic organisms, since I

believe this is essential to understanding acquired immunity to the parasite and why vaccines that perform well in naïve donors have performed poorly in the field (34-36). In field settings, variables related to the host, parasite, vector, and environment can influence host responses to infection. Such complexity lends itself well to "systems-based" analysis, which requires advanced mathematical and computational approaches to determine if and how these parameters interact to influence the host response to the parasite. However, there is a dearth of biologists and clinicians who can understand the analysis at the mathematical and computational level, necessitating collaboration with computational specialists to help translate the biomedical data into lines of code and vice versa (37). As an investigator with training in infectious diseases, immunology, field research, and applied bioinformatics, I wish to take the next logical step in my career development and learn the advanced statistical and computational approaches required to integrate complex datasets from multiple platforms with the purpose of identifying correlates of protection from malaria disease and *Pf* infection. As such, my long-term goal is to acquire expertise in the field of systems biology of malaria, with a particular emphasis on modulation of immune responses to *Pf* by previous exposures/infections, the environment, and host factors, and how immunomodulation might influence susceptibility to infection and disease as well as ineffective vaccine responses. Building upon our previous observations (17, 18, 26, 32), I hope to further elucidate the mechanisms by which co-infections and diet influence malaria risk by applying systems biological methods to well-designed prospective cohort studies.

My short-term goals, and the focus of this K award proposal, are to determine the immune parameters that predict whether a child will be protected from malaria symptoms once parasitemic or protected from *Pf* parasitemia outright using systems biology approaches. Differences in immune parameters between children who become parasitemic but remain asymptomatic versus those who develop symptoms during parasitemia could provide insights for "anti-disease" vaccines that mitigate malarial disease but do not prevent *Pf* infection. Identifying differences in immune parameters between children who remain free of *P. falciparum* parasitemia, as documented by bi-weekly, negative PCR's throughout a 6-month high-transmission season and the complete absence of any *Pf*-positive clinic visits, and children who become parasitemic could provide novel immunological benchmarks for vaccines targeting sterile immunity. Career objectives for this K award are:

***Objective 1**:* To advance my knowledge and skills in biostatistics and computational biology and develop mathematical models that integrate high-dimensional clinical, epidemiological, parasitological, and immunological data in order to predict protection from *Pf* infection and/or malarial symptoms.

Objective 2: To elucidate mechanisms of immunity to *Pf* liver- and blood-stage infection by employing experimental approaches to complement computational findings, which may require training in new laboratory techniques to test specific hypotheses.

Both Dr. Crompton and Dr. Traore are committed to my success as an independent investigator and have kindly agreed to allow me access to biospecimens and clinical data from their clinical study for this grant proposal. Importantly, I have began to explore ways in which I can study naturally acquired immunity to malaria independent of the Malian cohort, including initiating an independent malaria studies in Kenya with guidance from Dr. John and institutional support from IUSM.

Candidate's Plan for Career Development/Training Activities During Award Period

I assembled an interdisciplinary team of mentors who have helped me develop a comprehensive training program to ensure that I acquire the skills to become a successful independent investigator specializing in computational systems biology with a focus on immunity to *Pf* infection. This plan includes both a didactic and research component, as outlined below, with expected milestones to be completed during the K08 award. My effort for the K08 will be 20% clinical and 80% dedicated to research and career development.

Mentors and Advisors:

Dr. Chandy C. John, Ryan White Professor of Pediatrics and Professor of Microbiology and Immunology at IUSM, will act as my **primary mentor**. He has mentored 44 graduate students, 39 post-doctoral fellows, and 7 junior faculty members. Dr. John has received continuous NIH funding since 1999 for studies on malaria immuno-epidemiology and severe malaria pathogenesis in Kenya and Uganda. Dr. John has already been a key mentor at IU, helping me establish field studies in Western Kenya, where I will lead a project to determine the prevalence and parasite densities of asymptomatic *P. falciparum* infections in communities with different malaria transmission intensities. For the proposed K08 project, we will meet individually on a biweekly basis to review data and plan research goals in addition to our current joint malaria research group meetings.

Dr. Wanzhu Tu, Professor of Biostatistics in the Department of Biostatistics at IUSM, will be a **co-mentor** on this application. Dr. Tu specializes in longitudinal data analyses and semiparametric regression, with a focus on modeling of biological processes and developing new analytic tools to better understand

common diseases. Thus, his novel modeling techniques on regularized regression methods, including lasso and elastic net, are directly applicable to my research plan. He helped formulate my K08 didactic curriculum and will serve as a co-mentor. We will meet monthly for biostatistics and modeling guidance to ensure that I become well-versed in advanced generalized linear models and machine learning techniques.

Dr. Lang Li, Professor and T.K. Li Endowed Chair in the Department of Medical and Molecular Genetics at IUSM and the Director of the Center for Computational Biology and Bioinformatics, will serve as a **co-mentor** for this proposal. Dr. Li has expertise in pharmacogenomics and uses systems pharmacology approaches to elucidate drug interactions and investigate the effects of genetics on drug efficacy and drug interactions. His independent research is actively funded by three R01s. Dr. Li has mentored 6 post-doctoral fellows and 8 Ph.D. students. He will meet with me monthly during the computational phase of my K08 to provide the guidance and resources to properly execute my predictive modeling algorithms.

Dr. Peter D. Crompton, Chief of the Malaria Infection Biology and Immunity Unit in the Laboratory of Immunogenetics at NIAID will serve as a **co-mentor**. I have known Dr. Crompton since 2008, and he served as my primary research mentor during my fellowship at NIAID. He provides expertise in human immunity to *Plasmodium* infection. We continue to collaborate on ongoing projects, and four collaborative projects will be finalized this year with the manuscripts submitted for publication prior to the start of the current proposal. We will have monthly teleconferences and in-person research discussions at 2 conferences per year.

Didactic Training:

Immunology: To remain updated on recent findings and techniques in immunology, I will take the AAI **Advanced Course in Immunology**, a one-week intensive course on new advances in immunology.

Statistics and Computational Biology: To learn how to develop and apply the appropriate statistical and computational models for predicting immune outcomes via integration of high-dimensional biological, epidemiological, and clinical data from prospective studies, I will undergo the following didactic regime at IUPUI or online: **Epidemiology**: 1) PBHL E517 Fundamentals of Epidemiology 2) PBHL E601 Advanced Epidemiology. **Advanced Biostatistics:** To advance my knowledge of advanced statistics, I will take: 1) B670 Linear Regression 2) B670 Categorical Data Analysis 3) B642 Survival Data Analysis 4) B670 Biostatistics Computing. **Bioinformatics/Computational Biology:** To learn how to apply statistical methods to a broad range of areas in bioinformatics research, I will take: 1) INFO B646 Computational Systems Biology 2) INFO B636 Next Generation Genomic Data Analytics 3) INFO B556 Biological Database Management 4) INFO B529 Machine Learning for Bioinformatics. All IUPUI courses are 3 credit hours. In addition, I will complete the following on-line "**Systems Biology Specialization**" courses through Coursera which includes: 1) Network Analysis in Systems Biology 2) Dynamic Modeling Methods for Systems Biology 3) Integrated Analysis in Systems Biology. Each Coursera course requires 6 hours/week of commitment for 6 weeks. To enhance my competence in statistical methods for analyzing -omics datasets, I will apply for the intensive one-week Cold Spring Harbor Laboratory course "**Statistical Methods for Functional Genomics**."

Writing: Prior to submitting my first R01 application in year 4 and 5, I will improve my scientific writing and grantsmanship by enrolling in a scientific writing course and a Grant Writer's Workshop.

Timeline of Career Development Activities (percent effort/year where applicable)										
Training Activity	**Year 1**		**Year 2**		**Year 3**		**Year 4**		**Year 5**	
Workshops										
AAI Immunology Seminar/ CSHL Stats Methods Genomics	(2)			(2)						
Bioinformatics & Computational Biology Courses										
Fundamentals of Epidemiology/Advanced Epidemiology	(10)	(10)								
Linear Regression/Categorical Data Analysis			(10)	(10)						
Computational Systems Biology/Biostatistics Computing					(10)	(10)				
Next Gen Genomic Data Analytics/Biological Database Mgmt							(10)	(10)		
Machine Learning for Bioinformatics									(10)	
Coursera Systems Biology Specialization (on-line)	(2)	(2)	(2)	(2)	(2)					
Conduct of research and ethics										
Introduction to Research Ethics course (GRAD G504)	(10)									
Responsible Conduct of Research and CITI renewals										
Grantsmanship and career development										
Grant Writer's Workshop & Scientific Writing Course										
Conference Attendance										
American Society of Tropical Medicine & Hygiene										
Gordon Research Conference/Keystone Symposium										

Specific Aims: Malaria afflicts ~200 million people yearly, with ~430,000 malaria deaths due to *Plasmodium falciparum* (*Pf*) (1), underscoring the need for a highly effective malaria vaccine. The first licensed malaria vaccine, RTS,S, will provide much-needed reductions in morbidity and mortality. However, its 25.9% efficacy in reducing clinical malaria in the target population of African infants (2) leaves ample margin for improvement. Other malaria vaccine candidates that performed well in experimental studies also proved less effective in pediatric field studies in Africa (3), suggesting that immune factors specific to children in endemic areas may influence vaccine-induced responses. Better understanding of immunity to *Pf* in naturally exposed populations will provide insights for improving malaria vaccine design. Despite the established role for *Pf*-specific IgG in conferring immunity to malarial symptoms (**clinical immunity**) (4, 5), unambiguous immune correlates of clinical protection remain elusive (6). Even less is known about immunity to *Pf* infection (**sterile immunity**) given that years of repeated exposure to the parasite does not reliably confer sterilizing protection from parasitemia (7). Systems biology, which relies on computational modeling of large-scale data sets to elucidate complex biological networks (8), has been used to predict vaccination outcomes (9-12) and classify diseases (13-15) but can also reveal novel insights into host immunity to infection in an unbiased manner (16).

The goal of this proposal is to use systems biology approaches to elucidate correlates and mechanisms of both clinical and sterile immunity to *Pf* infection in a well-characterized, prospective cohort of Malian children living in an area of intense, seasonal malaria transmission (7, 17, 18). We have defined children as clinically immune or susceptible to *Pf* infection based on 3 years of malaria surveillance data. Our initial data has shown that clinically immune children at their uninfected baseline have increased B cells and *Pf*-specific IgG levels—both features typical of asymptomatic carriers of *Pf*. Identification of uninfected and asymptomatically infected children who have been *prospectively* defined as clinically immune or susceptible provides an opportunity to investigate whether asymptomatic *Pf* blood-stage infection (**BSI**) confers clinical immunity or is a consequence of clinical immunity. We have also identified a subset of young children who, remarkably, remained free of **BSI** as determined by bi-weekly PCR surveillance during 6 months of intense malaria transmission and who demonstrated *Pf* exposure by boosting of *Pf*-specific IgG, suggesting that these children controlled BSI to below PCR-detectable levels—*i.e.* ***they developed the closest functional equivalent to sterile immunity seen in a naturally exposed population***. The present study will take advantage of samples collected from this unique cohort to identify immune parameters that characterize and best predict **clinical immunity** (no symptoms with BSI) or apparently **sterile immunity** (no evidence of active BSI by PCR despite evidence of *Pf* exposure). Our overall hypothesis is that children who have acquired either clinical or sterile immunity to *Pf* have distinct pre-infection immune profiles than predict *Pf* infection or malaria outcomes.

Specific Aim 1: Identify immune parameters predictive of clinical immunity to *Pf* infection (protection from symptomatic BSI) and sterile immunity to *Pf* infection (protection from BSI).

We will perform transcriptomics, multiplex cytokine analysis, *Pf*-specific antibody profiling, and flow cytometry on baseline blood samples and apply these parameters along with parasitological, demographic, and genetic data to both machine learning algorithms and statistical models to determine which parameters best predict clinical or sterile immunity. Although the goal is to identify novel predictors and signatures of both clinical and sterile immunity in an unbiased manner, based on previous data, we hypothesize that *Pf*-specific IgG antibodies, a B-cell signature, and *Pf* BSI status will be significant predictors of clinical immunity, whereas either a Type I or Type II interferon signature will predict sterile immunity.

Specific Aim 2: Compare cellular, molecular, and *Pf*-specific IgG reactivity profiles in the blood of children with clinical immunity vs. children with sterile immunity and relate these profiles and immune outcomes to the *in vitro* parasite-inhibitory activity of their plasma.

To determine biological signatures that might define clinical and sterile immunity, we will directly compare transcriptomic, cytokine, *Pf*-specific antibody reactivity, and flow cytometry data between the two immune classes. We hypothesize that the clinically immune signature will be biased towards B-cells and have a predominantly blood-stage specific antibody profile, whereas a signature of sterile immunity will be driven by interferon responses and an antibody profile targeting both pre-erythrocytic and blood-stage antigens. We will quantify the ability of plasma from children in our study to inhibit parasite invasion of human hepatocytes and erythrocytes *in vitro* and determine whether invasion-inhibitory activity in plasma correlates with protection from BSI or symptomatic BSI after accounting for confounding variables. We hypothesize that plasma from sterilely immune children will inhibit parasite invasion of both hepatocytes and erythrocytes *in vitro* and that this activity will correlate with increased IgG reactivity intensity against both pre-erythrocytic and blood-stage antigens.

Taken together, this work will enhance our understanding of natural immunity to both malarial disease and *Pf* infection and may identify novel predictors and mechanisms of immunity to *Pf* within the vaccine target population, thus informing the rational design of malaria vaccines for children in Africa.

Research Strategy

1. Significance

Malaria is a global health burden that afflicts ~200 million people annually (1). *Plasmodium falciparum* (*Pf*), the deadliest of human malaria parasites, is responsible for the overwhelming majority of ~438,000 malaria deaths, primarily in children under 5 years of age (1). A highly effective malaria vaccine is widely viewed as a much-needed tool for reducing malaria in endemic areas where healthcare delivery and vector control strategies are often disrupted by political conflict, natural disasters, and the ever-present potential for epidemics of rapidly transmissible viruses such as Ebola that can divert scarce resources from malaria control efforts (38). If licensed, the first malaria vaccine, RTS,S, will partially reduce the burden of malaria in certain endemic areas, but its 25.9% efficacy in the target population of African infants (2) leaves ample margin for improvement. Other malaria vaccine candidates that initially performed well in clinical trials with malaria-naïve adults have subsequently performed poorly in African children (3), suggesting that parasite and immunological factors specific to children in endemic areas may influence vaccine efficacy. Thus, a better understanding of immunity to malaria in naturally exposed populations may provide insights for improving malaria vaccine design.

Immunity to *Plasmodium* is inextricably linked to the parasite life cycle. After a human has been inoculated with *Pf* sporozoites by the bite of an infected female *Anopheles* mosquito, the parasite can, within minutes, migrate via the bloodstream to invade liver hepatocytes, where they develop and replicate for ~7 days, after which they rupture from hepatocytes as merozoites and begin the blood stage of infection. *Pf* blood-stage infection (BSI) begins as low-level, asymptomatic parasitemia which can rapidly progress to a spectrum of clinical illness ranging from uncomplicated malaria (defined as confirmed BSI with symptoms such as fever but in the absence of severe criteria) to severe malaria (criteria includes but not limited to hyperparasitemia, impaired consciousness, convulsions, or severe anemia) to death (39). In areas of intense malaria transmission, immunity to *Pf* infection can also be viewed as a sequential spectrum (40). Natural immunity is acquired to severe, life-threatening disease after a young child survives 1-2 severe malaria episodes early in life, but immunity to uncomplicated malaria requires years of repeated *Pf* infections (40). Thus, reliable <u>clinical immunity</u>, or immunity that prevents malarial symptoms, is generally not acquired until adolescence in individuals living in highly endemic areas. Importantly, such intense exposure to *Pf* in endemic settings does not readily confer <u>sterile immunity</u> (immunity that prevents establishment of BSI) even as children approach adulthood (7).

It is well established that *Pf*-specific IgG confers clinical immunity to malaria (4, 5); however, unambiguous targets of IgG-mediated protection still remain elusive (6). Similarly, *Pf*-elicited cellular interferon-gamma (IFNγ) responses have been associated with clinical immunity to malaria in humans (41-43), but specific cellular correlates of either naturally acquired or vaccine-induced immunity have yet to be validated across multiple settings. Furthermore, as IFNγ responses can also trigger pathological inflammation, the mechanisms required for maintaining a balance between immunity and immunopathology are not fully understood. Sterile immunity has been well studied in experimental models of *Plasmodium* infection. In mice and non-human primates, IFNγ plays a critical role in sterile protection afforded by immunization with irradiated sporozoites (44-46). More recently, type I IFN responses generated during liver-stage infections were shown to reduce *Plasmodium* liver infection load and delay the appearance of BSI (47, 48). Another study showed that antibodies generated against α-gal present on gut commensal bacteria can target sporozoites intradermally to prevent *Plasmodium* infection (26). In contrast to animal studies, much less is known about the mechanisms of sterile immunity in humans given the lack of accessible biomarkers specific for liver-stage infection that would help distinguish the relative contributions of effective liver-stage immunity from early blood-stage immunity in controlling initial parasitemia. *Because neither predictors of clinical immunity nor sterile immunity to Pf infection in humans are known, understanding the mechanisms responsible for differential immune outcomes after Pf exposure and infection in children residing in endemic settings will guide efforts to develop malaria vaccines that achieve better response in the primary target population.*

We are conducting an ongoing cohort study in Kalifabougou, Mali where *Pf* transmission is intense and seasonal. In this study, we prospectively monitor children for both *Pf* infection and febrile malaria episodes, allowing us to categorize children by the number of febrile malaria episodes over three malaria transmission seasons. During the first season, we collected dried blood spots (DBS), plasma, and whole-blood RNA at bi-weekly active surveillance visits and sick visits, allowing us to 1) retrospectively detect each child's first *Pf* BSI of the season by PCR of archived DBS and 2) perform whole-blood transcriptomics on uninfected and infected samples. Our initial results using clinical data over the first malaria season demonstrated that a [redacted]

[redacted] molecular signature of whole blood prior to infection associates with clinical immunity to *Pf* infection. Furthermore, a subset of children never demonstrated detectable BSI despite bi-weekly PCR of fingerprick blood samples. Importantly, these children showed evidence of having been exposed to infective mosquito bites as demonstrated by boosting of antibodies to pre-erythrocytic *Pf* antigens, which, taken together, suggests that they approached sterile immunity, i.e., BSI was never established or never achieved PCR-detectable levels. **These initial data provided proof-of-concept that baseline host-immune molecular signatures could differentiate prospectively defined immune phenotypes, serving as a basis for further studies to not only validate said signatures predictive of clinical immunity but also to determine signatures predictive of sterile immunity.** Thus, **we hypothesize** that differential host immune profiles at the pre-infection baseline can influence the immune response during subsequent *Pf* infection and predict whether the host will be protected from BSI or protected from disease.

2. Innovation

The innovation within this proposal lies in: 1) the availability of longitudinally collected biospecimens (plasma/whole-blood RNA/DBS/PBMCs) that are linked to >3 years of prospective clinical, parasitological, and epidemiological data in a cohort of children living in a village with intense malarial transmission 2) the use of unbiased systems biology approaches to assess differences in gene expression, antibody, and cellular profiles between clinical phenotypes followed by complementary experiments aimed at evaluating the functional basis for differences in host immune outcome and 3) the identification of a unique subset of children who were protected from BSI despite evidence for exposure to infective mosquito bites, facilitating the rational evaluation of host factors that may confer apparently sterile immunity. The proposed work will determine whether clinical or apparently sterile immunity to natural *Pf* infection can be predicted by host differences prior to exposure/infection and may provide valuable targets for assessing immune correlates of protection in malaria vaccine studies. The trove of biospecimens and clinical data provides a unique opportunity to rationally identify baseline biomarkers predictive of immunity to *Pf* using an unbiased, systems approach.

3. Approach

3.1. General Strategy: Children living in malaria-endemic areas gradually acquire immune mechanisms that protect against *Pf*. In this well-characterized cohort, we defined children as clinically immune, sterilely immune, or susceptible to *Pf*. **Our general hypothesis** proposes that these three groups can be distinguished by global analyses of host immune factors at baseline, prior to the first *Pf* exposure of the malaria season. Our overall strategy is to analyze biospecimens collected from children differing in levels of *Pf* immunity at baseline or during/after incident *Pf* exposure to assess differential host responses to the *Pf* parasite.

3.2. Specific Aim 1: Identify immune parameters predictive of clinical immunity to *Pf* infection (protection from symptomatic BSI) and apparently sterile immunity to *Pf* infection (protection from BSI).

3.2.1. Rationale and Preliminary Data: Our preliminary data has shown that gene expression profiles of whole blood obtained at the *Pf* PCR-negative baseline differed between children who remained asymptomatic during their first *Pf* infections of the ensuing malaria season (clinically immune) versus children who presented with febrile malaria during their first *Pf* infection of the season (susceptible, **Fig. 1A**). Pathways and regulator network analyses of differentially expressed genes prior to infection suggested that a [redacted] signature associated with clinical immunity to incident BSI during the 6 month follow-up period (**Fig. 1B**). Clinically immune children had higher *Pf*-specific antibody levels and [redacted] compared to susceptible subjects *at pre-infection baseline* (**Fig. 1C**), which are features typically found in another group of individuals who are protected from febrile malaria attacks presumably due to enhanced immunity: asymptomatic, long-term carriers of *Pf* (**Fig. 1D**) (18, 49). Two intriguing hypotheses may explain the relationship between clinical immunity to BSI and asymptomatic carriage of BSI: 1) elevated *Pf*-specific IgG levels, [redacted] signature observed in clinically immune children at their uninfected baseline are actually driven by recent or occult BSI below the detection limit of our PCR assay, which would explain why their immunological profile mirrors that of asymptomatic, chronic *Pf* carriers or 2) a distinct, malaria-protective immune profile is established first, which then predisposes children to clinically silent infections that go undetected and thus untreated. To test the first hypothesis, we will re-interrogate for occult BSI using a recently developed ultrasensitive quantitative real-time PCR (qPCR) assay that improves sensitivity by as much as 30-fold relative to our original PCR assay (50). For the second hypothesis, we must

Figure 1. Differences in immune and susceptible children at healthy, uninfected baseline. (A) MA smearplot showing the log-fold change (FC) between the 2 groups against the log counts per million (CPM) as determined by RNA-seq. Red points indicate differentially expressed genes (DEGs) with a false discovery rate (FDR)<5% determined using a generalized linear model in edgeR. (B) DEGs in A with FDR<5% were applied to pathways and upstream regulator analysis. Shown are top significant immune pathways. BH: Benjamini-Hochberg. A |z-score| >2 with *P*<0.01 is considered significant. Differences between % B cells and *Pf*-specific IgG responses are shown between (C) clinically immune and susceptible children at uninfected baseline and between (D) asymptomatic, *Pf* PCR (+) and PfPCR (-) children. **P*<0.05; ***P*<0.01; ****P*<0.001. Significance determined by t test or Wilcoxon test as appropriate.

[redacted]

first determine whether the malaria-protective, [redacted] signature that we observed at baseline is a unique feature of clinical immunity in uninfected children or if all clinically immune children, irrespective of asymptomatic BSI status, share pertubations in immunological pathways that might predict host control of malarial symptoms. Understanding the relationship between asymptomatic BSI and clinical immunity to malaria will clarify whether asymptomatic BSI confers/maintains clinical immunity or is a consequence of prior acquisition of immunity, which has important implications for malaria vaccine studies (51) and eradication efforts (52).

In our first Kalifabougou study, we observed no age-related differences in time-to-first *Pf* infection (irrespective of symptoms) of the season among individuals aged 4-25 years of age and concluded that there was *no evidence for acquisition of sterile immunity with increased duration of malaria exposure*. Children <4 years of age were excluded from this initial study, because we observed that they had *decreased* prospective risk of *Pf* infection, with ~20% never becoming positive for *Pf* by bi-weekly blood PCR despite 6 months of intense malaria transmission. This effect persisted even after separating children <6 months of age to account for maternal IgG (**Fig. 2A**). We hypothesized that the decreased infection risk was due to decreased exposure

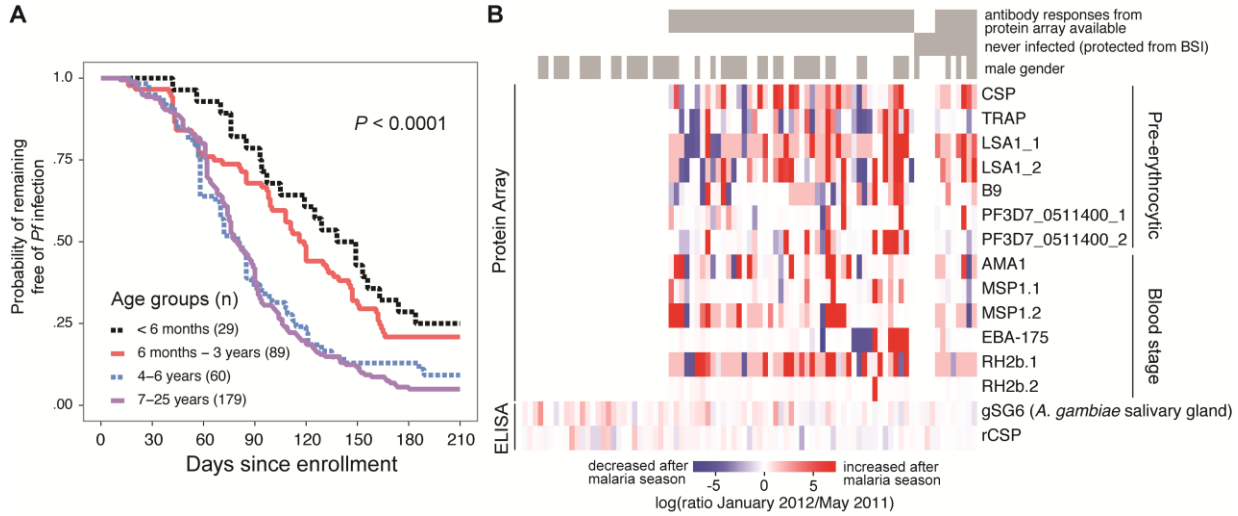

Figure 2: Evidence supporting apparently sterile immunity in a subset of children in the Kalifabougou cohort. (A) A proportion of children, predominantly <4 years of age, remained free of *Pf* infection after 6 months of intense malaria transmission and bi-weekly, retrospective determination of their *Pf* infection status by PCR. Significance testing was by log-rank analysis for all groups. (B) Heatmap showing the log ratio of post-malaria season (January 2012) and pre-malaria season (May 2011) antibody responses by ELISA or a protein array containing 1,087 *Pf* antigens for 12 sterilely immune (never infected, rightmost columns) and 75 non-protected children. Red indicates that antibody responses increased after the malaria season.

to infective mosquito bites, perhaps due to maternal behavioral factors (swaddling, etc.). To better address the question of *Pf* exposure, we asked whether these children had boosting of plasma antibodies after the 6-month malaria season specific for 1) a salivary gland antigen of the primary *Pf* vector *Anopheles gambiae,* gSG6*;* 2) pre-erythrocytic *Pf* antigens; or 3) blood-stage *Pf* antigens either by traditional ELISAs or *Pf* protein microarray, as increased antibodies would suggest vector, pre-erythrocytic, and blood-stage exposure, respectively. Surprisingly, 12 children who had complete 7-month follow-up and tested negative for *Pf* infection by PCR every 2-weeks and at sick visits also showed antibody boosting to ≥1 pre-erythrocytic antigen or gSG6 (53),

providing evidence of *Pf* exposure and *suggesting apparently sterile immunity to Pf infection* (**Fig. 2B**). This protected phenotype, rarely seen in studies conducted in areas of intense *Pf* transmission, offers a unique opportunity to investigate the mechanisms of sterile immunity in humans naturally exposed to *Pf* malaria.

3.2.2 Methodology

a) *Study site, subjects, and available biospecimens:* We will use cryopreserved biospecimens collected from subjects who are enrolled in an ongoing observational cohort study in the village of Kalifabougou, Mali. Here, malaria transmission is intense and seasonal from June through December. Biospecimens used in this proposal are from the first malaria season (May 2011-January 2012), but prospective clinical data has been ongoing. Subjects with the hemoglobin genotypes HbS/C and subjects co-infected with *Schistosoma haematobium* have been excluded from the current proposal as these factors can affect malaria risk (18, 54). The case definition for a malaria episode is an axillary temperature >37.5°C and a parasite density by blood smear of >500 asexual parasites/µl, a threshold chosen to provide sensitivity in diagnosing malaria while maintaining specificity in a semi-immune population who may develop fevers from other pathogens. Our preliminary study evaluated 20 children meeting a strict definition for clinical immunity: incident BSI with ***no*** malaria episodes and no receipt of anti-malarials during the first malaria season (**Fig. 1A**). Given that subjects meeting this strict definition of no episodes have already been evaluated, the current proposal defines clinically immune as ≤2 malaria episodes over 3 years and susceptible as ≥4 episodes over 3 years. Children with 3 malaria episodes over 3 years are interdeterminate and excluded. *The current definition treats clinical immunity more as a continuum and allows for partial immunity and the development of the occasional fever.* Class definitions and sample availability are shown for the clinical (**Table 1**) and sterile immunity (**Table 2**) studies.

Table 1: Clinical Immunity Class Definitions and Available Samples (n)

Class	Description	Definition	n	Time Point	Samples Available
A	Clinically Immune, Uninfected	≤2 malaria episodes over 3 years; *Pf* PCR (-) at baseline	10	May 2011 (pre-malaria season)	PBMCs, DBS,RNA, plasma, pellet
B	Clinically Immune, Infected	≤2 malaria episodes over 3 years; *Pf* PCR (+) at baseline	47		
C	Susceptible, Uninfected	≥4 malaria episodes over 3 years; *Pf* PCR (-) at baseline	18		
D	Susceptible, Infected	≥4 malaria episodes over 3 years; *Pf* PCR (+) at baseline	21		

Table 2: Sterile Immunity Class Definitions and Available Samples

Class	Description	Longitudinal *Pf* PCR status during the 2011 malaria season	*Pf*, vector exposure status	Number of available subjects	Samples available by time point
E	**apparently sterile immunity** (**protected** from BSI)	Negative at every 2 week surveillance visit and passive (sick) visit	Boosting of Abs to ≥1 *Pf* pre-erythrocytic antigen or gSG6 during first malaria season	12 (11 with boosting to ≥1 *Pf* pre-erythrocytic antigen ± gSG6	**Baseline** (May 2011): PBMC, whole blood RNA, plasma (2ml), DBS **Bi-weekly** time points: whole-blood RNA, plasma (50 µl), DBS
F	**non-protected**	Converted from negative to positive at least once		75	

b) *Evaluation of additional erythrocyte variants that may affect malaria risk*: Although HbS/C individuals have been excluded in this proposal, we will control for the presence of other erythrocyte variants that might affect malaria risk. ABO blood group typing has been performed for our cohort, and we will genotype for the most common α thalassemia and glucose-6-phosphate dehydrogenase deficiency variants present in Mali (54, 55).

c) *Re-interrogation of baseline samples by ultra-sensitive quantitative real-time PCR*: To verify that clinically immune groups from the our preliminary study and the current proposed study were not misclassified as uninfected at baseline, we will re-interrogate all samples using an ultrasensitive qPCR assay (50) with genomic DNA (gDNA) extracted from cryopreserved cell pellets obtained from ~200 µl of whole blood. This assay targets the TARE-2 repeat region in the *Pf* genome and has a sensitivity of 0.03-0.12 parasites/µl, which is 10-30x more sensitive than the method we originally used to determine *Pf* infection status (1 parasite/µl) (7, 56).

d) *Determining kinetics of P. falciparum specific IgG Abs by protein microarray*: We will measure *Pf*-specific IgG responses using protein array technology developed by **Dr. Philip Felgner (University of California Irvine)** as previously described (57). This technique involves probing plasma samples to glass slides spotted with 1,087 recombinant *Pf* antigens, allowing either single-antigen, stage-specific, or general assessments of *Pf*-specific IgG reactivity.

For the clinical immunity study, *Pf*-specific IgG responses will be measured at the uninfected baseline, and differential reactivity between classes will be assessed using the empirical Bayes method in the limma package (58). Sterilely immune children, by definition, do not have a *Pf* PCR positive timepoint, and biomarkers of

human liver-stage infection do not exist. Thus, for the sterile immunity study, we will rely on the kinetics of host antibody response to *Pf* to estimate the time of the first *Pf* exposure of the season (referred to as "incident exposure"). We will take advantage of the fingerprick blood specimens (whole-blood RNA/plasma) collected bi-weekly during the malaria season and determine longitudinal antibody responses. The 2-week timepoint showing the first significant increase in the sum intensity of antibody responses to pre-erythrocytic antigens (e.g. CSP, TRAP, LSA-1) from **baseline (T0)** will approximate the **incident exposure (T1)**. Samples from subjects who had documented *Pf* infection during the season (Class F) will be used as a control, as it is expected that T1 will coincide with the time point before or during incident ***infection***.

e) *Whole-blood transcriptomics*: We will use RNA-seq of fingerprick whole-blood samples collected directly in Tempus (ThermoFisher) to assess differential gene expression. Total RNA will be extracted, and rRNA and globin mRNA will be removed with the Globin-Zero rRNA Gold kit (Illumina). RNA-seq libraries will be prepared (25 million paired-end 2x75 bp reads per sample) and sequenced on a NextSeq 500 (Illumina). Samples will be randomized and processed at the same time to avoid batch effects. Reads will be mapped to the reference human genome (hg38), and count data will used for differential gene expression analysis using edgeR (59), DESeq2 (60), and limma-voom (58). Both edgeR and limma-voom allow for adjustment of any significant baseline differences between groups (e.g. possible age differences) if needed. Contrasts will be set up as 2-way comparisons between the classes (**Tables 1-2**) or as nested factorial designs (see below).

For the clinical immunity study, transcriptomics will be performed on whole blood collected in May 2011, prior to the first malaria season. The Class A vs. C contrast will address baseline transcriptional differences that may define clinical immunity prior to *Pf* infection, while the Class A vs. B contrast will be important to determine the effects of baseline asymptomatic infection on clinical immunity. For the sterile immunity study, transcriptomics will be performed on whole-blood samples collected from **protected (E)** and **non-protected (F)** children at T0 and T1. The contrast of Class E.T0 vs. F.T0 will determine if there are any baseline transcriptional differences between protected and non-protected children. The contrast of (E.T1 - E.T0) - (F.T1 - F.T0) with paired samples will determine transcriptional differences in host responses to incident *Pf* exposure between classes. Genes with an absolute fold-change of >1.5 and a false discovery rate (FDR) of <5% will be considered differentially expressed. Geneset testing will be performed within edgeR using the roast and camera functions (both in the limma package). Genes with a FDR <5% will be used in Ingenuity pathways analysis and modular analysis. We will use qPCR to confirm gene expression for selected genes.

Table 3 shows conservative power estimates for identifying 80% of DEGs whose absolute fold change is >2 at samples sizes for each 2-group comparison, assuming a nominal FDR of 5% and gene expression levels and dispersions estimated from our previous RNA-seq data. The statistical power of differential gene analysis (DGE) analysis is limited by the smaller sample sizes of Classes A and E. Thus, comparison of smaller groups will be considered exploratory.

Table 3: Power table for RNA-seq DGE using edgeR and the PROPER package (61) (100 simulations)

Group 1 (n_1)	Group 2 (n_2)	Actual FDR	Marginal Power	Avg # of True Discoveries	Avg # of False Discoveries
A (10)	B (47)	0.048	0.53	270	13
A (10)	C (18)	0.110	0.34	170	21
B (47)	D (21)	0.055	0.64	320	18
C (18)	D (21)	0.078	0.52	260	22
E (12)	F (75)	0.045	0.63	310	15
A+B (57)	C+D (39)	0.054	0.78	390	22
A (10)	E (12)	0.16	0.20	100	19

f) *Multi-parameter flow cytometry and multiplex cytokine analysis***:** Flow cytometric analysis (FACS) will be performed on cryopreserved PBMCs collected in May 2011 (contemporaneous with the RNA samples at T0) to determine expression of [redacted] and cytokine production among specific cell subsets. Specifically, we will determine the relative proportion of B cells (CD19+), CD3+CD4+ T cells, CD3+CD8+ T cells, NK cells (CD56+), monocytes (CD14+) and γδ T cells (TCR γδ+) and perform intracellular staining for IFNγ, IL-6, TNF, [redacted] Additional markers will be assessed based on the results of the transcriptomic analysis. Samples will be acquired on a BD LSRII flow cytometer and analyzed using FlowJo. Cytokine profiling of baseline plasma will also be performed for all classes using a multiplex cytokine profiling platform (BioRad) to test a broad panel of cytokines involved in inflammation that includes Type I/II interferons (IFNs), TNF, IL-1β, IL-6, and [redacted]. Significance group differences will be determined by t tests or Wilcoxon signed-rank tests as appropriate with Benjamini-Hochberg adjustments for multiple testing.

g) *Genetic analysis of P. falciparum isolates from clinically immune and susceptible children***:** To address the possibility that differences in clinical outcomes between immune and susceptible children are due to parasite rather than host factors, we will evaluate genetic differences between *Pf* isolates. We will extract gDNA from

cryopreserved blood pellets at all *Pf* PCR-positive visits and perform *Pf* genotyping using standard protocols for neutral loci *Pf* microsatellite analysis.

h) *Multi-parameter statistical modeling and machine learning to predict clinical or sterile immunity*: Given the lack of immune correlates of clinical or sterile protection from *Pf*, the overall goal of these analyses is to determine which variables examined at baseline best predicts clinical or sterile immunity. With the guidance of Drs. Tu and Li, I will use machine learning approaches to determine which variables (features) from demographic (i.e. age, gender), epidemiological, erythrocyte variant, parasitological (including baseline BSI), transcriptomic, FACS, multiplex cytokine, and *Pf*-specific IgG data best predicts clinical or sterile immunity in two independent models. In the clinical immunity model (Classes A+B, C+D), we will include all constant features and features collected at baseline. The outcome will be clinical immunity or malaria susceptibility. The sterile immunity model (Classes E, F) will include transcriptomic and *Pf*-specific Ab features collected at T1 in addition to all constant and T0 baseline features. The outcome will be sterile immunity or non-protected. We will first focus on regularized regression methods that consider all features simultaneously while also providing automatic feature selection (e.g. lasso, elastic net). Of these, elastic net is ideal for our study: it provides both automatic feature and grouped selection and performs well when the number of features >> number of samples and when the features are highly correlated (62). We will also test machine learning algorithms (e.g. k nearest neighbors, random forest, linear discriminant analysis). For algorithms without automatic feature selection, we will pre-select features using recursive feature elimination. We will perform model-specific parameter training and use repeated *k*-fold cross-validation to estimate the prediction error for each model. This approach ensures that we select models with the most accurate and stable predictions for our data set.

The sample sizes for this proposed study are pre-determined by the number of subjects meeting the selection criteria for this study with available samples (**Table 1**). Elastic net analysis of data simulated from our previous experimental data sets and group sample sizes of 57 and 39 (representing A+B and C+D) consistently identified ≥20 parameters from ~8300 features that can predict a binomial outcome with a cross-validated mean square error of 0.055-0.060 after 50 simulations. To further increase sample sizes and improve prediction accuracy, we will include data from the previous study (**Fig. 1A**). The definition of "clinically immune" in our current proposal (≤2 malaria episodes over 3 seasons) differs from the definition for the previous study (no malaria episodes over one season). Thus, we have reclassified the samples from the first study to match the definitions in the current proposal, adding 28 Class A and 33 Class C samples and allowing us to use samples from one study as a training set and the other as a test set or include all in a cross-validation scheme.

3.2.3. Expected outcomes – Aim 1

This work will attempt to validate findings in our previous study, which found increased immune activation in clinically immune children compared to susceptible children prior to their first *Pf* BSI of the malaria season, and determine if asymptomatic *Pf* BSI, which also is associated with decreased prospective risk of febrile malaria attacks, influences baseline immune activation. We previously showed that clinically immune children had [redacted] and enhanced *Pf*-specific IgG responses compared to susceptible children at their uninfected baseline, similar to asymptomatic, chronic carriers of *Pf*, but did not perform direct comparative transcriptomics between these groups. Of note, we do not expect to detect many, if any, occult infections by ultrasensitive qPCR as most of the children who were PCR negative at baseline had multiple, consecutive negative PCRs prior to their first positive PCR.

We will address our general aim of determining genome-wide, whole-blood predictors of clinical immunity to *Pf* infection with an emphasis on investigating whether the presence of asymptomatic *Pf* BSI significantly influences the immunological profile of clinical immunity at baseline. Overall, we expect to validate our previous findings and find [redacted] signature in both immune classes (A and B) compared to the susceptible classes (C and D) by transcriptomics and FACS, irrespective of infection status. Although we anticipate that the relative [redacted] will be similar between Class A and B, the [redacted] should differ qualitatively and quantitatively given evidence that asymptomatic infections have been associated with changes in [redacted].

We expect that regularized regression methods such as elastic net give us the best chance at determining a correlate of clinical protection from high-dimensional, multi-parameter data. We anticipate that baseline parameters of *Pf*-specific IgG response, B-cell percentage, and *Pf* BSI status to be positive predictors of clinical immunity given their correlation to previous malaria exposure, but we hope to gain novel insight as to which of the experimentally measured variables have the strongest independent effects on clinical immunity. Based on our experience with the previous dataset, we anticipate that certain features related to immune activation would be specifically enhanced in a majority of clinically immune children.

For the sterile immunity analyses, we anticipate that IgG responses against pre-erythrocytic antigens will show variable increases during the months of intense malaria transmission, allowing us to identify an appropriate T1 time point for our paired RNA-seq studies. Given the evidence that systems-based approaches can reveal unanticipated differences in host immune profile predictive of subsequent responses to immunological perturbation [**Fig. 1A**, (11)], we expect to see moderate whole-blood gene expression variability at T0 that will roughly align with the dichotomous classes E and F. We hypothesize that baseline Type I/II IFN activation will be seen in sterilely immune children relative to non-protected children based on the evidence that these pathways are involved in host control of the parasite during the pre-erythrocytic stages (47, 48). Furthermore, IFN pathways activation will likely be enhanced during incident *Pf* exposure (T1). Because the E vs. F comparison has a marginal power of 62% for observing ~300 differentially expressed genes (DEGs) at ≥2–fold change **(Table 3**), our genome-wide analysis will likely reveal novel insights into the genes associated with sterile immunity. We expect multiplex cytokine and FACS data to corroborate the transcriptomic findings provided that these assays are sufficiently sensitive to detect group differences. Should we identify a cytokine signature, FACS analysis will be critical for identifying the cell subset responsible for cytokine production.

3.2.4. Anticipated Problems and Limitations – Aim 1

An important limitation of this work is the lack of power to detect subtle gene expression differences between groups for comparisons involving Class A (n= 10). If DEGs were not detected between Class A vs. B and Class C vs. D, we would conclude that asymptomatic *Pf* infection at baseline does not have a *large* effect on baseline gene expression profiles for either clinically immune or susceptible children. We would then perform a secondary analysis in which the two immune classes, Class A and B, are combined, giving us larger sample sizes (clinically immune = 57, susceptible = 39) with a marginal power of 78% to detect a ≥2-fold difference between groups (**Table 3**). Small group sizes will also preclude more robust class prediction using all parameters. Inclusion of the previous study's independent dataset adds 28 Class A and 39 Class C subjects and will further improve our ability to accurately classify subjects.

Classes A and B would also be combined into a single group if validation of prior PCR results with ultra-sensitive qPCR reveals occult infection in clinically immune children previously labeled as uninfected. Such a scenario would provide a plausible, biological explanation for inflammatory activation and enhanced antibody responses previously seen in the clinically immune group (strict definition) and support the argument that asymptomatic parasitemia confers or maintains clinical immunity rather than being a consequence of immunity. Here, we would take advantage of having qPCR data for all immune children (Classes A+B, n=57) to address whether baseline parasite density correlates with changes of immunological markers and malaria outcomes.

In vivo biomarkers of *Pf* liver-stage infection have yet to be identified because 1) *Plasmodium* liver-stage infection is clinically silent and 2) host responses are likely confined locally to infected hepatocytes (47). Any changes in peripheral blood cells due to pre-erythrocytic *Pf* exposure are likely transient and difficult to detect, especially in an endemic pediatric population where blood can only be sampled at a certain resolution (here, every two weeks) for ethical and practical reasons. The main emphasis here is the prediction of sterile immunity by global analysis, with the primary biological parameters of interest being the ones determined using baseline (T0) samples, a strategy that has a moderate likelihood of success (**Table 3**). Attempting to detect biological changes at T1 carries higher inherent risk given that T1 is based on antibody response kinetics with only 2-week resolution and only represents the most proximal point to liver-stage infection among available time-points. This strategy might miss transient or subtle biological changes in blood and exhibit high intra-subject variability due to the unknown duration between actual *Pf* infection and T1. Despite these limitations, detecting a signal in *any* T1 sample will be informative as it would be the first time any surrogate marker suggestive of natural *Pf* liver-stage or initial blood-stage infection has been detected in a child living in an endemic area. Such a marker will have to be further evaluated for confounding by co-incident infections and validated in controlled human malaria infection studies.

3.3. Specific Aim 2: Compare cellular, molecular, and *Pf*-specific IgG reactivity profiles in the blood of children who are clinically immune vs. children with sterile immunity and relate these profiles and immune outcomes to the *in vitro* parasite-inhibitory activity of plasma

3.3.1. Rationale and Preliminary Data: Results from Aim 1 provide an opportunity to directly compare the immunological profiles of clinically and sterilely immune children prior to infection. Global analysis of all measured parameters (e.g. gene expression, FACS, *Pf*-specific IgG reactivity) from pre-perturbation samples may provide insights as to what type of immune profile could distinguish between sterile and clinical immunity or if both types of protection share common immune pathways. Whereas clinical immunity to *Pf* infection is

dependent on various facets of immunity elicited during BSI, sterilizing immunity to *Pf* infection could theoretically be achieved at four points along the parasite's intended path towards establishing BSI in the human host: 1) intradermally (immediately after inoculation of sporozoites by the mosquito) 2) in the blood/liver prior to hepatocyte invasion 3) during intrahepatocytic parasite development and 4) during the initial blood-stage inoculum when merozoites first exit the liver. Blood components could potentially inhibit the parasite at points 1, 2, or 4. Although *Pf* stage-specific IgG reactivity profiles from protein arrays might provide clues as to whether anti-parasite effects are mediated at the pre-erythrocytic stage (points 1 and 2) or during the blood-stage (point 4), inhibition of parasite invasion assays can provide functional evidence of pre-erythrocytic or blood-stage mediated sterile protection. We recently performed a pilot study in collaboration with **Dr. Sangeeta Bhatia** and Sanaria to assess the ability of plasma from individuals from Kalifabougou (n=24; distinct from current study) to inhibit invasion of sporozoites into human hepatocytes using a microscale human liver platform developed by her lab that recapitulates complete *Pf* liver-stage development *in vitro* (65, 66). [redacted] Similarly, we previously determined that IgG specific for the blood-stage antigen PfRH5 inhibited parasite growth *in vitro* using standardized growth inhibition assays (GIAs)(17), but at concentrations far greater than those occurring in malaria-immune Malians, implying that PfRH5-specific IgG levels only partially explain protection from clinical malaria. Parasite inhibitory activity exhibited by plasma components in these functional assays will be related back to *Pf* antigen-specific IgG reactivity profiles to identify new correlates and also incorporated into the multi-parameter models to assess the contribution of *in vitro* inhibitory activity to immune outcomes.

3.3.2. Methodology – Aim 2

a) *Qualititiave and statistical comparison of clinical and sterile immunity:* To determine biological signatures that might define clinical and sterile immunity, we will directly compare transcriptomic, *Pf*-specific antibody profiling, cytokine, and FACS data between clinically and sterilely immune children using statistical methods considered standard for each dataset (e.g. edgeR for RNA-seq, empirical Bayes for protein arrays) and adjust for multiple testing. Geneset enrichment, pathways, and modular analysis will be performed with transcriptomic data to gain insight into cellular processes and mechanisms that may differentiate clinical vs sterile immunity.

b) *Functional assessment of plasma from children with different immune outcomes:* To better understand the mechanisms underlying the immune phenotypes, we will assess the ability of baseline plasma collected from clinically immune, sterilely immune, and susceptible children to inhibit parasite invasion into human hepatocytes and erythrocytes using established methods. To assess differential inhibition of hepatocyte invasion and/or intrahepatocytic development by *Pf* NF54 sporozoites, plasma from protected or non-protected children will be added to the co-culture before or after establishment of the intrahepatocytic stage. Detection of sporozoite entry will be performed by fluorescence microscopy to assess intracellular sporozoites using anti-*Pf*CSP antibody, and the progression rate of liver-stage development will be measured by determining the percentage of *Pf* exoerythrocytic forms within hepatocytes over time as described (65, 66). Sterile or parasitemia-reducing immunity conferred by inhibition of the merozoites during the initial blood-stage inoculum is a distinct possibility given that boosting to prototypical *Pf* blood-stage antigens was also observed in Class E.T0 plasma (**Fig. 2B**). Therefore, to assess the ability of plasma to inhibit blood stage growth, we will use a well-established, standardized protocol for GIAs, in which we compare growth-inhibitory activity of IgG purified from the plasma of sterilely immune, clinically immune and susceptible individuals.

c) *Correlating parasite-inhibitory activity to Pf antigen-specific IgG reactivity*: Plasma parasite-inhibitory activity obtained from the microscale liver or GIA assays will be correlated to reactivity intensities to each antigen on the 1,087 *Pf* protein microarray and in combinations using a stage-specific combinatorial approach to calculate correlation coefficients and significance values with Benjamini-Hochberg adjustment for multiple testing.

d) *Statistical modeling to integrate functional data to multi-parameter data and immune outcomes***:** To integrate the parasite-inhibitory activity scores with parameters measured in Aim 1 and immune outcomes, we will

include inhibitory activity scores as two additional parameters (one each for microscale liver and for GIA) in the models for which the multinomial outcome is clinically immune (Class A+B), sterilely immune (Class E) or fully susceptible (Class C+D). We will use modeling and validation approaches described in Aim 1 to gain unbiased insight into the biology of parasite-inhibitory activity. Models will use parameters measured in Aim 1, including gender and age, as predictors and parasite-inhibitory activity scores as the outcomes. We will formally compare the accuracy of machine learning algorithms and regression models in selecting variables predictive of parasite inhibitory activity. We will also include functional scores as co-variates in Cox regression models to determine the effect of *in vitro* parasite-inhibitory activity on time-to-first *Pf* infection or malaria episode.

3.3.3. Expected outcomes – Aim 2

Direct comparisons of high-dimensional biological datasets between clinically and sterilely immune groups are expected to provide novel insights into naturally acquired immunity. We hypothesize that the sterilely immune signature will be driven by IFN responses and a balanced antibody profile targeting both pre-erythrocytic and blood-stage antigens, whereas the clinically immune signature will be biased towards [redacted] and have a predominantly blood-stage specific antibody profile. Our functional studies will formally test whether plasma from immune children will inhibit hepatocyte invasion, intrahepatocyte development, or erythrocyte invasion. We observed variability in the IgG responses against liver- and blood-stage antigens among protected children (**Fig. 2B**) as well as variability in hepatocyte-invasion activity of plasma from a separate group of Malian individuals in a pilot study (**Fig. 3**). Thus, in the current proposal, we also expect marked variation in the ability to inhibit *Pf* invasion of host cells, with certain samples conferring inhibition of invasion preferentially to hepatocytes or to erythrocytes. We hypothesize that plasma from sterilely immune children will inhibit parasite invasion of both hepatocytes and erythrocytes *in vitro*. We expect that parasite-inhibitory activity will correlate with increased IgG reactivity intensity against both pre-erythrocytic and merozoite antigens and independently associate with reduced risk of *Pf* infection in Cox regression models. This approach would allow us link functional responses to distinct antigen-specific IgG reactivity profiles and clinical outcomes.

3.3.4. Anticipated Problems and Limitations – Aim 2

Direct comparison of clinical and sterile immunity in the single platform comparison will be considered exploratory due to small sample sizes. However, pathways analysis might increase explanatory power in the case that we encounter only modest differences in gene expression between groups (67). Variability in the inhibitory activity of plasma samples in the functional assays could decrease our ability to detect group differences but may enhance our ability to correlate parasite-inhibitory activity to other parameters such as antigen-specific IgG or cell immuno-phenotypes. Nevertheless, identification of just a few individuals with an enhanced ability to inhibit hepatocyte or erythrocyte invasion will provide a basis for further studies into the mechanism of sterile protection, especially since these individuals remain enrolled in this cohort study.

4. Rigor of Experimental Design and Quality Control

For all experiments, sample processing will be randomized to ensure equal distribution of classes, gender, and age among batches as to minimize batch effects. Time points from the same child will be processed together, allowing each child to serve as his/her own healthy baseline control. Sample processing procedures will be thoroughly documented. The raw data and final scripts for the processing and analysis of the data will be made publicly available at the conclusion of the project to allow others to reproduce our analysis.

5. Research and Publication Timeline/Future Directions

Timeline (program year)	1	2	3	4	5
Aim 1					
Laboratory/data generation					
Computational analysis					
Presentations/Manuscripts					
Aim 2					
Laboratory/data generation					
Computational analysis					
Presentations/manuscripts					
R01 grant preparation					

Methodical analysis of high-throughput data generated from samples that are linked to a well-characterized observational malaria cohort study will allow us to determine predictors and elucidate mechanisms of immunity to *Pf* infection. Studies proposed in this application will provide the PI with valuable experience in generating, modeling, validating, and interpreting complex data and further his development as a systems biologist of human immune perturbations. The practical implications of this work include identifying novel molecular or cellular predictors of natural immunity to *Pf* within the vaccine target population that could provide rational benchmarks for candidate malaria vaccines in clinical trials. Results from these studies will help elucidate the molecular patterns and mechanisms underlying the natural immune response to *Pf* infection and generate hypotheses for further mechanistic studies of malaria immunity. Through successful completion of this proposal, the PI will gain technical expertise in mathematical modeling and additional experience analyzing large datasets while generating the results necessary for manuscripts that will form the basis for subsequent independent, R01-level funding in the fields of malaria immunity and systems biology.

Training in the Responsible Conduct of Research

I arrived at IUPUI in September 2015 and completed the Collaborative Institutional Training Initiative (CITI) Miami on-line courses on Good Clinical Practice, Responsible Conduct of Research and Human Subjects Research for Biomedical Researchers with inclusion of the International Research, Research with Children, and Genetic Research in Human Populations supplemental modules to provide me with formal training in these areas. I will maintain CITI certification at least every three years with refresher courses.

I will also enroll in the Introduction to Research Ethics course (GRAD G504) through the Indiana Clinical and Translational Sciences Institute to participate in face-to-face, small group discussions (meetings are for 1.5 hours, 15 times during a semester, 22.5 contact hours) on these ethical issues in research:

1. Science, Industry and Conflicts of Interest
2. Scientific Misconduct: Current Definitions, Policies, and Procedures
3. Responsible Authorship and Publications
4. Policies Regarding Human Subjects, Animal Subjects in Research, and Safe Laboratory Practices
5. International Research Ethics
6. Peer Review Practices
7. Scientists as Responsible Members of Society

IUSM's Center for Bioethics offers two venues for further in-depth group discussion in responsible conduct in research. I will participate in the Teaching Skills in International Research Ethics Workshop, which is an annual workshop aimed at building research ethics capacity between Indiana University and Moi University in Eldoret, Kenya, and the Global Bioethics Seminar Series offered every spring. These two workshops will address:

1. Mentor/mentee responsibilities and relationships
2. Collaborative research
3. Data acquisition, management, sharing, and ownership

My primary mentor Dr. John and my Division Director Dr. Wools-Kaloustian will ensure that I maintain current knowledge in the responsible conduct of research and that all my activities abroad have appropriate Institutional Review Board approval both in the United States and the site of my future collaborative malaria field research in Kenya. In addition, both Dr. John and Dr. Wools-Kaloustian will be available to discuss specific ethical issues that I may encounter while I develop my clinical research protocols for the collection of human biospecimens, which will be submitted for approval by the IUPUI and Kenyan IRB prior to future research proposals (anticipated to be in Years 4 and 5 of the current Research Plan).

[redacted]

[redacted]

[redacted]

PHS Inclusion Enrollment Report

This report format should NOT be used for collecting data from study participants.

OMB Number:0925-0001 and 0925-0002
Expiration Date: 10/31/2018

***Study Title:** Defining clinical and sterile immunity to Plasmodium falciparum infection using systems biology approaches

***Delayed Onset Study?** ❑ Yes ☑ No

If study is not delayed onset, the following selections are required:

Enrollment Type ❑ Planned ☑ Cumulative (Actual)

Using an Existing Dataset or Resource ☑ Yes ❑ No

Enrollment Location ❑ Domestic ☑ Foreign

Clinical Trial ❑ Yes ☑ No

NIH-Defined Phase III Clinical Trial ❑ Yes ☑ No

Comments: The proposal qualifies as Exempt Human Subject Research. The current proposal will only utilize existing, "in-the-freezer" human biological specimens and existing clinical data from an ongoing cohort study on naturally acquired malaria immunity in Kalifabougou, (Mali ClinicalTrials.gov Identifier NCT01322581). Both human speciments and clinical data have been coded so that the investigator cannot identify the subjects directly or through identifiers linked to the subjects.

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	0	0	0	0	0	0	0	0	0	**0**
Asian	0	0	0	0	0	0	0	0	0	**0**
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	**0**
Black or African American	344	351	0	0	0	0	0	0	0	**695**
White	0	0	0	0	0	0	0	0	0	**0**
More than One Race	0	0	0	0	0	0	0	0	0	**0**
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	**0**
Total	**344**	**351**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**695**

Inclusion of Children

The current proposal will use biospecimens and clinical data from ongoing clinical studies on naturally acquired malaria immunity in Kalifabougou, Mali (ClinicalTrials.gov Identifier NCT01322581). Enrollment for this study was based on a randomized, age-stratified census. **This proposal specifically will utilize blood samples obtained from children aged 6 months-12 years**. This age range captures the period during which immunity to *P. falciparum* infection is acquired in areas of intense malaria transmission like Mali and, thus, **the target population for a malaria vaccine**. Age will be included as a covariate in statistical models.

Assent forms were used for all study subjects between 7 and 18 years of age who are capable of giving their assent to participate. Parental consent was obtained for all study subjects since they were under 18 years of age. For minor's parents who were both under 18 years of age, consent was obtained from a grandparent, in accordance with Malian custom.

All experiments outlined in this proposal will use existing, cryopreserved biospecimens.

No additional children will be recruited for this proposal.

There is no additional risk to the subjects, since this proposal makes use of biospecimens that have been previously collected and stored.

Data and resource sharing plan

Materials generated under the project will be disseminated in accordance with Indiana University School of Medicine and NIH policies. Depending on such policies, materials may be transferred to others under the terms of a material transfer agreement. Raw sequence data files will be submitted to the NCBI Sequence Read Archive (SRA), and processed sequence data files will be submitted to the NCBI Gene Expression Omnibus (GEO). Processed data files containing *Plasmodium falciparum* sequences will be submitted to EuPathDB (PlasmoDB). Transcriptomic and antibody profiling data will be presented at scientific meetings. Custom scripts for processing and analyzing high-throughput data sets will be made publicly available at the conclusion of the project either prior to or at the time of publication. Presentations from the proposed projects will be presented at the following national and international meetings: Annual Meeting of the American Society of Tropical Medicine and Hygiene, Infectious Diseases Society of America Annual Meeting, and the Gordon Research Conference on Malaria. Publication of data shall occur during the project, if appropriate, or at the end of the project, consistent with normal scientific practices.

Authentication of Key Biological and/or Chemical Resources

Growth Inhibition Assays

Type O+ human erythrocytes used in the growth inhibition assays (GIAs) will be obtained from the Indiana Blood Center. *Plasmodium falciparum* 3D7 blood stage parasites used in GIAs will be cultured from cryopreserved stocks (courtesy of Jianbing Mu, National Institute of Allergy and Infectious Diseases) that have been authenticated as 3D7 by 6-loci microsatellite genotyping.

Hepatocyte Invasion Assays

For the microscale human liver platform (also called micropatterned co-culture assay), Dr. Sangeeta Bhatia's laboratory uses commercially available cryopreserved primary human hepatocytes (BioreclamationIVT; Invitrogen) and 3T3-J2 murine embryonic fibroblasts (courtesy of Howard Green, Harvard Medical School). Sanaria (Rockville, Maryland) will supply the *P. falciparum* NF54 for this study.

Flow cytometry

Antibodies used for flow cytometry will be purchased from three commercial vendors: BioLegend, BD Biosciences and R&D systems. The following are anticipated antibodies based on our prior experience:

Panel	Marker	Fluorochrome	Clone	Catalog
Surface	CD19	Brilliant Violet 785	HIB19	302240
Surface	CD21	APC	HB5	17-0219-42
Surface	CD27	Brilliant Violet 421	M-T271	356418
Surface	CD10	Brilliant Violet 510	HI10a	312219
Surface	CD23	PE-Cy7	B3B4	101613
Surface	IgG	PE-CF594	G18-145	562538
Surface	CD95	Brilliant Violet 605	DX2	305627
Surface	CD14	Brillian Violet 785	M5E2	301840
Surface	CD16	Brilliant Violet 605	3G8	302040
Surface	anti-FcεRI, common γ subunit	FITC	2472959	FCABS400F
Surface	CD3	Horizon V500	UCHT1	561416
Surface	CD4	PerCP	RPA-T4	300527
Surface	CD8	Brilliant Violet 570	RPA-T8	301038
Surface	CD56	Brilliant Violet 711	HCD56	318335
Surface	TCR γ/δ	APC	B1	331211
Surface	FCRL5	PE	509f6	340304
Intracellular	Phospho-STAT6 (Y641)	PE		IC3717P
Intracellular	T-bet	PE-CF594	O4-46	562467
Intracellular	STAT6	APC	253906	IC2167A
Intracellular	GATA3	PE-Cy7	L50-823	560405
Intracellular	IFN-γ	APC/Cy7	4S.B3	502529
Intracellular	IL-6	PE	MQ2-13A5	501106