PI: **Friedman-Klabanoff, DeAnna**	Title: Serological markers of natural immunity to Plasmodium falciparum infection	
Received: 11/11/2020	Opportunity: PA-20-205	Council: 05/2021
Competition ID: FORMS-F	FOA Title: Mentored Patient-Oriented Research Career Development Award (Parent K23 Independent Clinical Trial Not Allowed)	
1K23AI155838-01A1	Dual:	Accession Number: 4517955
IPF: 820104	Organization: UNIVERSITY OF MARYLAND BALTIMORE	
Former Number: 1K23AI155838-01	Department: CVD PEDS ID	
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: [redacted]	Animals: Y Humans: Y Clinical Trial: N Current HS Code: 30 HESC: N HFT: N	New Investigator: Early Stage Investigator:

Senior/Key Personnel:	*Organization:*	*Role Category:*
DeAnna Friedman-Klabanoff	University of Maryland, Baltimore	PD/PI
Miriam Laufer	University of Maryland, Baltimore	Other (Specify)-Primary Mentor
Shannon Harrison	University of Maryland, Baltimore	Other (Specify)-Co-Mentor
Michael Cummings	University of MD Inst for Adv Comput Studys	Other (Specify)-Co-Mentor
Kathy Neuzil	University of Maryland, Baltimore	Other (Specify)-Advisor
Andrea Berry	University of Maryland, Baltimore	Other (Specify)-Advisor
John Adams	University of South Florida	Other (Specify)-Advisor

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

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

1. TYPE OF SUBMISSION*

❍ Pre-application ❍ Application ● Changed/Corrected Application

4.a. Federal Identifier AI155838

b. Agency Routing Number

2. DATE SUBMITTED 2020-11-11

Application Identifier 57352

c. Previous Grants.gov Tracking Number GRANT13241916

5. APPLICANT INFORMATION

Organizational DUNS*: [redacted]

Legal Name*: University of Maryland, Baltimore
Department: CVD PEDS ID
Division: CVD and Global Health
Street1*: Office of Research and Development
Street2: [redacted]
City*: Baltimore
County: Baltimore City
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]

Person to be contacted on matters involving this application

Prefix: First Name*: Christine Middle Name: R. Last Name*: Toalepai Suffix:

Position/Title: Senior Administrator SPA
Street1*: [redacted]
Street2:
City*: Baltimore
County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email: [redacted]

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*
NIH-National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Serological markers of natural immunity to Plasmodium falciparum infection

12. PROPOSED PROJECT
Start Date* 07/01/2021
Ending Date* 06/30/2026

13. CONGRESSIONAL DISTRICTS OF APPLICANT
MD-007

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: DeAnna Middle Name: J. Last Name*: Friedman-Klabanoff Suffix:
Position/Title: Instructor
Organization Name*: University of Maryland, Baltimore
Department: CVD PEDS ID
Division: CVD and Global Health
Street1*: Health Sciences Facility 480
Street2: [redacted]
City*: Baltimore
County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
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* [redacted]
c. Total Federal & Non-Federal Funds* [redacted]
d. Estimated Program Income* [redacted]

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

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

DATE:

b. NO ○ PROGRAM IS NOT COVERED BY E.O. 12372; OR
● PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

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

● I agree*

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

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Towanda Middle Name: M. Last Name*: Gilliam Suffix:
Position/Title*: Sponsored Prog Administrator
Organization Name*: University of Maryland, Baltimore
Department:
Division:
Street1*: [redacted]
Street2:
City*: Baltimore
County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: [redacted]
Phone Number*: 410-706-0013 Fax Number: Email*: [redacted]

Signature of Authorized Representative*

[redacted]

Date Signed*

11/11/2020

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:RRSF424_Cover_Letter.pdf

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

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

Project/Performance Site Location(s)

Project/Performance Site Primary Location

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

Organization Name: University of Maryland, Baltimore
Duns Number: [redacted]
Street1*: Office of Research and Development
Street2: [redacted]
City*: Baltimore
County: Baltimore City
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: MD-007

Project/Performance Site Location 1

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

Organization Name: Center for Vaccine Development
DUNS Number:
Street1*: [redacted]
Street2: [redacted]
City*: Baltimore
County: Baltimore
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: MD-007

Additional Location(s) File Name:

2020-11-11T16:27:19.000-05:00

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

RESEARCH & RELATED Other Project Information

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

1.a. If YES to Human Subjects

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

If YES, check appropriate exemption number: __ 1 __ 2 __ 3 __ 4 __ 5 __ 6 __ 7 ____8

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

IRB Approval Date:

Human Subject Assurance Number 00007145

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 A3200-01

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

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

4.b. If yes, please explain:

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

4.d. If yes, please explain:

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

5.a. If yes, please explain:

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

6.a. If yes, identify countries: Malawi

6.b. Optional Explanation: Collaborators in Malawi

	Filename
7. Project Summary/Abstract*	ProjectSummary.pdf
8. Project Narrative*	Narrative.pdf
9. Bibliography & References Cited	Bibliography.pdf
10.Facilities & Other Resources	Facilities.pdf
11.Equipment	Equipment.pdf
12. Other Attachments	Foreign_Justification.pdf

Tracking Number: GRANT13242170

Funding Opportunity Number: PA-20-205. Received Date: 2020-11-11T16:27:19.000-05:00

PROJECT SUMMARY

Plasmodium falciparum is the most common and deadly cause of malaria. An effective malaria vaccine has the potential to make a pivotal change in malaria control and eradication. For a vaccine to contribute significantly to malaria eradication, it must target the early, pre-erythrocytic part of the lifecycle to block both symptomatic disease and asymptomatic infection, which perpetuates transmission. Naturally acquired immunity to pre- erythrocytic infection is acquired with exposure but remains poorly understood and continues to impede vaccine efforts. DeAnna Friedman-Klabanoff, M.D., a pediatric infectious disease specialist at the University of Maryland School of Medicine, developed this career development award proposal to use novel high-throughput tools to define naturally acquired humoral immunity to diverse pre-erythrocytic epitopes associated with protection, which could lead to novel vaccine candidates. Dr. Friedman-Klabanoff's long-term goal is to become an independent clinical and translational researcher dedicated to the development of a malaria vaccine, applying immunology and data science to inform and optimize vaccine development. To gain the skills necessary to achieve this goal, Dr. Friedman-Klabanoff proposes a career development plan that includes mentoring from Drs. Miriam Laufer, Shannon Takala Harrison, Michael Cummings, Andrea Berry, Kathleen Neuzil, and John Adams, leaders in the fields of international research design and leadership, molecular epidemiology, data science for analysis of large data sets, use of peptide microarrays to study malaria, vaccinology, and *in vitro* models of pre-erythrocytic immunity. This project will utilize samples and data from a cohort study of malaria in Malawi led by Dr. Laufer, the primary mentor for this proposal. Household members were followed monthly for detection of malaria infection and mosquitoes were collected from the houses to identify bloodmeal sources. Bloodmeal sources will be identified by matching the human DNA found in the mosquito bloodmeals to DNA from enrolled participants . Mosquito salivary glands will also be tested for *P. falciparum* infection to determine if the mosquitoes were infectious. Children will be defined as protected or infected based on whether they develop blood-stage infection during the month after an infectious bite. Aim 1 of this proposal will be to identify serologic responses associated with natural protection against *P. falciparum* infection after exposure to an infectious bite. Serum from the day of exposure will be probed on a custom-developed peptide microarray designed from diverse, field-deriv ed sequences to characterize pre-exposure immunity to pre-erythrocytic antigens. Aim 2 of this proposal is to assess the functional role of antibodies targeting *P. falciparum* pre-erythrocytic antigens of interest. B- and T- cell epitope prediction tools will be used to find predicted epitopes in pre-erythrocytic proteins and their variants, and *in vitro* liver models will be used to assess the functional role of antibodies to these epitopes to validate and down select the potential epitopes. The practical implications of this work will be to identify promising epitopes that are targets of protective immunity.

PROJECT NARRATIVE

Plasmodium falciparum, the most common species of malaria, kills more children than any other pathogen, and the development of a highly effective vaccine has been challenging. Children acquire immunity to malaria with repeated exposure, but the mechanism is poorly understood. We will collect samples from children who are exposed to *Plasmodium*-infected mosquito bites and compare the immune responses among those who develop infection to those who do not to identify possible targets for vaccines to prevent malaria infection.

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FACILITIES AND OTHER RESOURCES

Center for Vaccine Development and Global Health (CVD), University of Maryland School of Medicine

Founded by Dr. Myron Levine in 1974, the CVD has earned an international reputation as an academic researc h vaccine center and an enterprise for creating and testing vaccine candidates against a variety of diseases , including malaria, cholera, typhoid fever, paratyphoid fever, shigellosis, *Escherichia coli* diarrhea, influenza, measles and many other infectious diseases. The CVD is engaged in the full range of vaccinology from basic laboratory science research through vaccine development, early clinical evaluation, large-scale pre-licensure field studies and post-licensure assessments. To support these efforts, the CVD has been recipient of many grants and contracts from NIH and private foundations.

Laboratories: CVD occupies 32,000 sq. feet of modern laboratory, office and clinical space spanning 2 floors within the Health Science Facility (HSF) I and the Health Science Facility II (HSF II) buildings at the University of Maryland Baltimore Campus.

Clinical Facilities: No clinical activities are planned for our Baltimore facility in this proposal. The CVD has over 40 years of experience performing high quality clinical trials. State of the art facilities for recruitment, teaching, vaccination and physical assessment of volunteers are available for vaccine studies. An office staffed with experienced personnel is dedicated to regulatory affairs and compliance. For the past 2 decades, CVD has been one of the NIH/NIAID funded Vaccine and Treatment Evaluation Units sites. The CVD is also engaged in international clinical and field research and field CVD field units have been established in Mali, Chile and Malawi.

Animal Facilities: ABSL2 and ABSL3 animal facilities are available in HSF I, 6th floor and the Howard Hall (Suite 600) Bldg. Several rooms are available for animal housing, animal proc edures and tissue collection. The ABSL2 containment equipment includes high-vacuum double door pass-through steam sterilizer, class II type A/B3 and class III biological safety cabinets, and mobile VCL cage autoclave carts. The facilities are fully accredited by AAALAC and have a program of animal care directed by a full-time specially trained veterinarian (Program of Comparative Medicine). The Institution has an Animal Welfare Assurance on file with the NIH Office for Protection from Research Risks, Assurance No [redacted] Technical and veterinary service for animal care and support for procedures are available through Veterinary Resources, School of Medicine.

Antigen Purification Core Facility: The CVD antigen purification facility is fully equipped to perform biochemical protein and polysaccharide purification and analysis, production of microbial antigens for immunological studies, and construction of subunit and conjugate vaccines. Expertise is also available through this facility for antigen purification, including microbial fermentation, bioprocess development (TFF, chromatography), and molecular analyses (HPLC, colorimetric assays, SDS-PAGE). The laboratory maintains a full suite of protein and polysaccharide purification apparatus.

Other Facilities: The CVD has common facilities for specimen/supply storage, dishwashing, sterilization and production of MilliQ water. The School of Medicine provides maintenance service for lab facilities. The University of Maryland has several core facilities on campus, close to the CVD, that are available to the investigators. The Biopolymer-Genomics Core Facility performs oligonucleotides synthesis and DNA sequencing. A Freezer Program centralized vendor is available on campus for the purchase a variety of reagents & supplies.

Malaria Research Program (MRP), CVD, University of Maryland School of Medicine

Laboratory: The Malaria Research Group has a renovated and expanded malaria laboratory facility in Health Sciences Facility II, including a 3200 ft^2 molecular parasitology laboratory, an insectary, a *Plasmodia* culture facility, and laboratory bench space. The laboratory has three separated rooms with direct connection, and a total of 16 work bench areas that accommodate laboratory staff, graduate students and post-doctoral fellows. Common facilities include a dark room, cold room, warm room, isotope room, freezer facility, and liquid nitrogen storage room.

Animal: Not applicable

Biohazards: MRP is equipped with BSL-2 laboratory safety and protective equipment including laminar flow hoods, chemical fume hoods, eye wash stations, and personal protective equipment to protect personnel from blood borne pathogens, chemicals and other biohazardous materials/conditions. Biohazardous waste is collected in appropriately labeled and indicated bins and disposed of both by autoclave and incineration. Laboratory staff, students and researchers are trained annually in the proper disposal of laboratory waste, prevention of blood borne pathogens, and laboratory chemical safety.

Offices: ~142 ft^2 offices assigned to faculty and staff are located on the 3th and 4th floor of HSFII. A 288 ft^2 conference room includes file cabinets, scanner and fax machine, 3 printers, a desktop, 2 TV screens (43' and 65'), a large conference table, and video conference capability. Additional scanners, common fax, network printers, photocopy machine, and color printer are located in the main CVD office on the 4th floor of Health Sciences Facility I. Dr. Friedman-Klabanoff's office is located on the 4th floor of HSFII within a short walk of the malaria laboratory, remaining faculty offices, staff offices, and the conference room. The office has a large L- shaped desk, a Dell PWS T3400 desktop computer with two flat screens, file cabinets, and bookshelves. Her office is across the hall from the shared laser and color printers and meeting room space. Dr. Friedman-Klabanof f also has access to a Dell Latitude 3490 laptop computer for use when she is off campus and/or traveling for training and/or meetings.

Computers: The offices have Dell PWS T3400 desktop computers with network connection and two flat double screen monitors; Dell Latitude E6540 laptop computers; and laser printers. The laboratory benches contain computer carrels, each equipped with a Dell Pentium desktop with printer and network access, for lab staff, trainees and students. The laboratory has a Custom Dell Linux Data Analysis Server.

Clinical: Not applicable

Other: Not applicable

Center for Bioinformatics and Computational Biology, University of Maryland College Park

Laboratory: Not applicable

Animal: Not applicable

Computer: Current research computing equipment in the Center for Bioinformatics and Computational Biology , part of the University of Maryland Institute for Advanced Computer Studies (UMIACS), includes scores of workstations running a variety of operating systems, numerous clusters, multiple mass storage systems, and various other computing resources required for leading-edge computer-based research. These systems are maintained by a highly qualified team of permanent staff members in UMIACS, and backed up regularly using multiple tiered, and redundant, data storage systems, including off-site archiving. Included among these storage systems are those that serve for backup of desktop and notebook computers. Among the various researc h computers available for use by Dr. Friedman-Klabanoff while working with Dr. Cummings and his group, and which are most relevant to the proposed project include the following:

Intel Xeon Phi: A Ninja server with liquid-cooled Knights Landing (72 cores, 16 GB MCDRAM) and 96 GB RAM, 800 GB SSD, 1 AMD Fiji Nano card for GPGPU computing.

Dell PowerEdge R730: This server has dual Xeon E5-2680 v4 CPUs (28 cores total), 512 GB RAM, 800 GB SSD, 2 AMD FirePro S9170 cards for GPGPU computing.

Dell PowerEdge R730: This server has dual Xeon E5-2697v4 CPUs (36 cores total), 512 GB RAM, 800 GB SSD, 2 NVIDIA G100 cards for GPGPU computing.

Networking and Storage High speed data transfers and multicast applications are available through the Mid Atlantic Crossroads (MAX), the Next Generation Internet Exchange (NGIX), and the Internet2 with peers at several remote sites. There is application level support to ensure high speed wide-area network connectivity for the Access Grid, Conference XP, and the Storage Resource Broker. These facilities are supported by several

disk and tape storage systems, including enterprise-class systems, commodity systems, and enterprise DBMS systems.

The ensemble of computing resources available to the project greatly exceed the project need.

Office: The Center for Bioinformatics and Computational Biology has modern, well-equipped office space for all project personnel located in the Brendan Iribe Center for Computer Science and Engineering, the newest building at the University of Maryland, College Park (opened in April 2019). Offices are equipped with all necessary resources for personnel to perform project functions. The office for Dr. Cummings is approximately 158 ft^2, and the graduate student office is shared space with ample individual areas for each student that Dr. Friedman- Klabanoff can use while completing her independent study work. A rich diversity of meeting space, including open spaces, huddle rooms, and conference rooms are available for project meetings as needed.

Clinical: Not applicable

Other: Not applicable

College of Medicine, University of Malawi

The University of Malawi (UM) was founded just after Malawi gained independence in 1962, with the College of Medicine formed in 1991 as a constituent college. The College of Medicine provides clinical care and teaching at Queen Elizabeth Central Hospital, the largest public hospital in the nation. The UM Malaria Alert Center provides postgraduate training, provision of laboratories to support for malaria research, and access to field sites where observational and intervention studies can be conducted. Dr. Don Mathanga, Professor in the Faculty of Public Health and Co-Principal Investigator of the International Center for Excellence in Malaria Research (ICEMR) grant from which the samples will be obtained, is an infectious diseases epidemiologist with a special focus on malaria.

University of Malawi College of Medicine, Malaria Alert Center (MAC)

Laboratory: On site, MAC has laboratory facilities with microscopy and molecular capabilities. Five laboratory technicians perform standard work such as malaria microscopy, mosquito dissection and identification, PCR, gel electrophoresis, and ELISA. MAC also uses a molecular parasitology lab in the Department of Biochemistry within the College of Medicine located on an adjacent campus, which is also the Molecular and Genomics Core Laboratory of the ICEMR project. This lab occupies a 2000 ft^2 space divided into three rooms. All rooms are climate controlled with dedicated air conditioners and diesel generated back up power. The laboratory has the necessary equipment and infrastructure for standard PCR, real-time PCR, and parasite cryopreservation and culture. Three -80ºC freezers and a liquid nitrogen storage system are on site. There is also a 110-kVA diesel back-up generator.

Biohazards: The laboratory has been certified as a BSL-2 lab in accordance with the handling of *Plasmodia* sp. Access to the laboratory is limited and restricted by an electronic lock system. The two Class II biosafety cabinets are tested and certified for appropriate airflow and HEPA filter integrity on an annual basis. Laboratory staff, students and researchers are trained annually in the proper disposal of laboratory waste, prevention of blood borne pathogens, and laboratory chemical safety. Field staff and supervisors responsible for specimen collection and transport also receive training in blood borne pathogens.

Animal: Not applicable.

Computers: Laptop computers are used by Dr. Mathanga, research coordinators, data managers, laboratory and administrative staff. Skype and Zoom are used for regular communication including scheduled weekly teleconferences with international collaborators. Internet connectivity is accessed through the College of Medicine and linked to the MAC via a wireless/RF link. Service is provided by Globe Internet Limited and is a fiber optic connection. ICEMR supports a back-up connection that is a wireless broadband link provided by a different Internet service provider (Skyband).

Office: The offices have desks, chairs, lockable filing cabinets, wired Internet access and a local area network for use by visitors with computers. The Data Management unit in this office suite has a room with secure and locked cabinets to store patient confidential information. Temporary office space is available for visitors. There is a general printer and scanner available for use. There are two seminar rooms with a combined capacity of 90 participants.

Clinical: Not applicable

Other: Not applicable

Center for Global Health and Infectious Diseases Research, University of South Florida

The University of South Florida (USF) Center for Global Health and Infectious Diseases Research (GHIDR) is an interdisciplinary center of research and training brings together faculty, students and staff from across the USF with the goal of improving the health and lives of people afflicted by infectious diseases through the development of improved diagnostics, treatments, and preventive measures. GHIDR focuses on vector-borne diseases of public health importance, including malaria, leishmaniasis, dengue fever, viral encephalopathi es, onchocerciasis and filariasis.

Laboratory: The Adams laboratories are part of the GHIDR facilities on the third and fourth floors of the Interdisciplinary Research Building (IDRB) in USF Research Park and are equipped for biochemical, cell biological, and molecular biological experiments. The two BSL-2 lab suites are partially shared and total ~3000 sq. ft, plus associated shared supporting laboratories for microbiology applications, live cell fluorescent microscopy, nucleic acid detection, autoclaves, and glassware decontamination and washing. A separate 1750 sq. ft. ACL-2 insectary suite has walk-in environmental chambers, BSL1 and BSL 2 animal rooms (managed by USF Division of Comparative Medicine), an animal room prep lab, and an isolation lab to work with *P. falciparum/ P. vivax*-infected mosquitoes.

Animal: USF Comparative Medicine maintains an Animal Care and Use Program is fully AAALAC accredited, as accredited unit [redacted]. Veterinarians and staff provide oversight of animal health and well-being, guidance and assistance and equipment and room sanitation. Associated with GHIDR Vector Borne Pathogen lab are BSL-1 and BSL-2 animal facilities within IDRB, combined space with prep room and autoclave is approximately 800 sq. ft., that can support most of laboratory animal research needs of the ongoing projects.

Insectary: The insectary at USF is a 1700sq. ft. facility divided into three work areas: maintenance insectary , vivarium, and ACL-2 laboratory. The facility has permits for work with the major human malaria parasites, *P. falciparum* and *P. vivax,* including laboratory lines and clinical isolates, and rodent malaria parasites. Integrated into the facility are a BSL-1 vivarium for the rodent parasite infections and a BSL- 2 vivarium with a secure pass- through into the ACL-2 area to allow safe transfer of animals (e.g. humanized mouse) and parasite material between containment areas.

Biohazards: The Adams lab is approved to work with BSL-2 agents by the USF Biosafety Committee in accordance with PHS guidelines. This includes *P. falciparum, P. vivax, P. cynomolgi, P. knowlesi, P. ovale,* various rodent malaria parasites, and various *Anopheles spp.* Laboratory facilities, equipment, and protocols are in place to provide appropriate personal protection from exposure to infectious agents while working with infectious agents as well as decontamination of work areas and destruction of potentially infectious materials when the work is completed. This includes seven 6-foot class II biosafety cabinets (BSC) and five 4-foot BSC. In addition, USF Environmental Health and Safety has approved the laboratory for work with chemical hazards and chemical storage and has approved procedures for hazardous waste management, biomedical waste management, and accident management.

Offices: The IDRB office suite at GHIDR has networked computers, scanners and printers for access by researc h staff, postdoctoral fellows, graduate assistants and visiting researchers. The GHIDR third floor IDRB office suite has two conference rooms, including built-in projection and Cisco/Tandberg MOVI video conference systems, and a lecture room. Dr. Adams has a fourth floor 250 sq. ft office in IDRB with a network computer that has videoconferencing capabilities for meetings with Dr. Friedman-Klabanoff and her mentoring team.

Computers: Dr. Adams has multiple (Mac, Windows) computers in his office and lab for personnel. All have full internet access connected to a campus-wide network either by WiFi or ethernet. USF has modern, well- established intranet and internet systems with automatic back-up and security.

Clinical: Not applicable

Other: Not applicable

EQUIPMENT

Malaria Laboratory, Center for Vaccine Development and Global Health, University of Maryland School of Medicine (partial list)
Biotage PyroMark Q96 MD automated pyrosequencer, BioRad Molecular Imager Gel Doc XR system, four BioRad T100 thermal cyclers, 2 BioRad C1000 thermal cyclers, QIAGEN Qiaxcel automated nucleic acid fragment analysis system, BioRad Molecular Imager Gel Doc XR system, eight gel electrophoresis rigs, Applied Biosystems 7300 real time PCR system, Nimblegen MS200 microarray reader, CapitalBio12-array processing system, Molecular Devices SpectraMax M2 microplate fluorometer and absorbance spectrophotometer, 264 ghz (80 core) data analysis server, two 4-foot biosafety cabinets, one 6-foot biosafety cabinet, Napco Series 8000 CO_2/O_2 incubator with gas system, Sanyo cycling incubator, ThermoFisher environmental chamber, three fume hoods, analytical balance, pH meter, NanoDrop spectrophotometer, two tabletop refrigerated centrifuges, Jouan C4i centrifuge, four tabletop micro-centrifuges, microwave oven, dual chamber benchtop water bath, two upright adjustable temp incubators, four light microscopes, two dissecting microscopes, four -80°C Freezers, Thermo- Scientific 7404 CryoPlus 3 nitrogen vapor freezer, Eppendorf vacuum centrifuge, eight -20°C lab freezers, three lab refrigerators.

Access to a full range of laboratory equipment and shared facilities of the 14 units and sections of the CVD, including Molecular Diagnostics, Microbiology, Physiology, Bacterial Genetics, Applied Immunology, Cellular Immunology, Biochemistry, and Molecular Structure-Function. Access to equipment housed at the Institute of Genome Sciences within the University of Maryland School of Medicine.

University of Malawi Malaria Alert Centre
The equipment described here, combined with the capacity described in "Facilities and Other Resources," are enough to capture the requisite data and samples from the field sites.

Blantyre Offices:
We will have access to the following equipment for data collection, office use and backup of study -related information: 65 Lenovo Tab4: these are for data collection, 13 Dell Latitude 5580 laptops for study staff, 32 Seagate portable external hard drives, 2 routers for internet connectivity at the filed sites, 1 Dell PowerEdge R320, 3 Dell PowerEdge R210, 3 Dell R630 SFF, 1 Amazon Cloud Server.

Field Sites:
Vehicles: We will have access to the two vehicles for the ICEMR project: a Toyota Land Cruiser and a Nissan Hardbody double cabin 4x4.

Field Offices: We have 4-roomed office blocks at each of the study sites. Each of these offices has electricity and internet. Back-up power is provided by a diesel generator. The field offices have one desktop computer and color printer/scanner for the site administrator. Site coordinators and data officers have laptop computers as detailed above.

Center for Global Health and Infectious Diseases Research, University of South Florida
Six dual chamber 3-gas incubators, two dual CO2 incubators, programmable temperature cycling incubator, multiple 37°C shaker incubators, Qiaexcel capillary electrophoresis analyzer, automated DNA extractor, BioRad gel documentation system, Nanodrop, BioRad and Nucleofector electroporators, multiple thermal cyclers, numerous horizontal and vertical units with low and high power supplies for eletrophoresis of proteins and nucleic acids, blotting systems, hybridization oven, a convection vacuum oven, New Brunswick BioFlo 310, GE AKTA Pure FPLC, Hitachi LaChrom Elite HPLC, 6 chromatography refrigerators, automated microplate washer, ELISA plate reader, Accuri C6 Flow cytometer, Canto II flow cytometer, BD FACS Aria with UV, CASY Cell Analyzer, Leica laser capture microscope, Double Water distiller, Nikon inverted fluorescent microscope with digital camera, BioMek 3000 robot for aseptic media changing/liquid handling, Stratagene MX3000 qPCR, fluorescenc e plate reader, Biotek synergy HT capable of reading absorbance, fluorescence and luminescence with reagent automated injector, ultracentrifuges, high speed centrifuges, microcentrifuges, analytical electronic balance, waterbaths, hotplates, stirring plates, luminometer with automated injector for bioassays, DNA sequencer, MagnaPure Nucleic acid purification system, ELISA plate reader, spectrophotomer, Sonicator, Pyromark ID Pyrosequencer, Applied Biosystems 7500Fast, RT-PCR machine, G:BOX Chemi XRQ gel doc system, Illumina

MiSeq, Illumina NextSeq and 10X Chromium, multiple -80C freezers, controlled temperature & RH environmental chambers, LN2 storage, refrigerators, and - 20C freezer. Microscopes include a Zeiss upright and inverted DIC/phase fluorescence microscopes with CCV cameras and a DeltaVision DV CORE for live cell imaging.

FOREIGN JUSTIFICATION

I am collaborating with investigators at the Malaria Alert Center (MAC) at the University of Malawi College of Medicine (COM) who are conducting longitudinal cohort studies on mosquito biting patterns and malaria transmission in Malawi. Specifically, I have proposed a project to better understand the humoral responses that contribute to natural immunity to *Plasmodium falciparum* in Malawi. The results of this project will inform future malaria vaccine development and contribute to elimination and eradication efforts in the region and potentially around the world. The collaboration with MAC provides unique access to study populations in a moderate to high transmission area of Malawi. This type of research cannot be performed in the United States, where there is no malaria transmission. My primary mentor, Dr. Miriam Laufer, has a longstanding collaboration with MAC and the University of Malawi COM that has spanned from 2010 to the present and has resulted in 20 coauthored publications. We have an established Cooperative Research and Development Agreement to work with the MAC team, and Dr. Laufer and Dr. Don Mathanga from the University of Maryland and the MAC, respectively, are currently listed as investigators for five study protocols. Thus, the collaboration with MAC is justified based on our existing relationship and the topic of the proposed research.

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

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: DeAnna Middle Name J. Last Name*: Friedman-Klabanoff Suffix:

Position/Title*: Instructor
Organization Name*: University of Maryland, Baltimore
Department: CVD PEDS ID
Division: CVD and Global Health
Street1*: [redacted]
Street2:
City*: Baltimore
County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

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

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

Degree Type: Medical Doctor Degree Year: 2009

Attach Biographical Sketch*: File Name: DeAnna_J._Friedman-Klabanoff_Biosketch.pdf

Attach Current & Pending Support: File Name:

Funding Opportunity Number: PA-20-205 . Received Date: 2020-11-11T16:27:19.000-05:00

PROFILE - Senior/Key Person

Prefix: First Name*: Miriam Middle Name K Last Name*: Laufer Suffix:

Position/Title*: Associate Professor
Organization Name*: University of Maryland, Baltimore
Department: CVD PEDS ID
Division: CVD and Global Health
Street1*: [redacted]
Street2:
City*: Baltimore
County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

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

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

Degree Type: Medical Doctor Degree Year: 1997

Attach Biographical Sketch*: File Name: Miriam_K_Laufer_Biosketch.pdf

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

PROFILE - Senior/Key Person

Prefix: First Name*: Shannon Middle Name Takala Last Name*: Harrison Suffix:

Position/Title*: Associate Professor
Organization Name*: University of Maryland, Baltimore
Department: CVD Geographic Medicine
Division: CVD and Global Health
Street1*: [redacted]
Street2:
City*: Baltimore
County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

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

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

Degree Type: Doctor of Philosophy Degree Year: 2006

Attach Biographical Sketch*: File Name: Shannon_Takala_Harrison_Biosketch.pdf

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

PROFILE - Senior/Key Person

Prefix: First Name*: Michael Middle Name P Last Name*: Cummings Suffix:

Position/Title*: Associate Professor
Organization Name*: University of MD Inst for Adv Comput Studys
Department: CVD PEDS ID
Division:
Street1*: Cntr for Bioinfor and Computnl Bio
Street2: [redacted]
City*: College Park
County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

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

E-Mail*: [redacted]

Credential, e.g., agency login:

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

Degree Type: Doctor of Philosophy Degree Year: 1992

Attach Biographical Sketch*: File Name: Michael_P_Cummings_Biosketch.pdf

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

PROFILE - Senior/Key Person

Prefix: First Name*: Kathy Middle Name Maletic Last Name*: Neuzil Suffix:

Position/Title*: Professor
Organization Name*: University of Maryland, Baltimore
Department: CVD Geographic Medicine
Division: CVD and Global Health
Street1*: [redacted]
Street2:
City*: Baltimore
County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

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

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

Degree Type: Medical Doctor Degree Year: 1987

Attach Biographical Sketch*: File Name: Kathy_Maletic_Neuzil_Biosketch.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: Andrea Middle Name Ahn-Yee Last Name*: Berry Suffix:

Position/Title*: Assistant Professor
Organization Name*: University of Maryland, Baltimore
Department: CVD PEDS ID
Division: CVD and Global Health
Street1*: [redacted]
Street2:
City*: Baltimore
County:
State*: MD: Maryland
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

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

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

Degree Type: Doctor of Philosophy Degree Year: 2003

Attach Biographical Sketch*: File Name: Andrea_Ahn-Yee_Berry_Biosketch.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: First Name*: John Middle Name Last Name*: Adams Suffix:

Position/Title*: Professor
Organization Name*: University of South Florida
Department: CVD PEDS ID
Division:
Street1*: [redacted]
Street2:
City*: Tampa
County:
State*: FL: Florida
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

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

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

Degree Type: Doctor of Philosophy Degree Year: 1986

Attach Biographical Sketch*: File Name: John_Adams_Biosketch.pdf

Attach Current & Pending Support: File Name:

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

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

NAME: Friedman-Klabanoff, DeAnna

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

POSITION TITLE: Instructor

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
University of Wisconsin, Madison, WI	BS	05/2004	Medical Microbiology and Immunology
University of Wisconsin School of Medicine and Public Health, Madison, WI	MD	05/2009	Medicine
University of Minnesota School of Medicine, Minneapolis, MN	Resident	06/2012	Pediatrics Residency
University of Maryland Medical Center/University of Maryland School of Medicine, Baltimore, MD	Fellow	06/2019	Pediatric Infectious Diseases
University of Maryland School of Medicine, Baltimore, MD		07/2020	Postdoctoral fellowship

A. Personal Statement

My long-term goal is to become an independent investigator dedicated to the development of a highly efficacious malaria vaccine, utilizing domestic and international clinical and translational research in pursuit of this goal. This Mentored Patient-Oriented Research Career Development Award (K23) application entitled “Serological markers of natural immunity to *Plasmodium falciparum* infection” will provide me with the training and mentorship I need to transition to independence in pursuit of this goal.

My education and training so far have provided me with a solid foundation in both clinical and basic science research. Through basic science experiences in college, I learned how to do PCR, plasmid insertion, and cloning. In medical school, I worked on two epidemiological studies, which provided me with experience in cross-cultural research activities, study design and implementation, analysis and interpretation of epidemiological data, and scientific writing. Clinical rotations in Cambodia and Thailand during residency, and capacity building and medical education experience in Rwanda after residency gave me experience in tropical medicine and conducting small research projects in resource limited settings. Through these experiences, I found that a career in pediatric infectious diseases would be the perfect combination of interesting clinical work, global health, and clinical and translational research opportunities for me. I pursued a fellowship in Pediatric Infectious Diseases and chose the program at the University of Maryland because of its world-renowned Center for Vaccine Development. I currently work on a Phase I trial of a candidate malaria vaccine, gaining experience in clinical research implementation and management. I also have developed a sub-study to use peptide microarrays to assess vaccine-induced antibody responses, which has given me experience in peptide microarray design. As part of the preparation for this work and to better understand natural immunity to diverse circumsporozoite protein (CSP) variants, I completed an analysis of humoral responses to CSP in adults and children in Mali, and now have experience with peptide microarray analysis and manipulation of large data sets. With restrictions on research due to the COVID pandemic, I also currently help to lead our site activities for a large-scale, Centers for Disease Control and Prevention SARS CoV-2 syndromic and sero-surveillance project that is providing me with training on the use of serological assays in epidemiological research and skills in project leadership, committee leadership, and multisite collaboration.

My proposed project is intended to continue my training in trial implementation and leadership, expanding to large-scale international trials, to learn analytical approaches for large datasets including data science approaches for peptide array analyses, and to gain training in the use of *in vitro* liver models to examine pre- erythrocytic *P. falciparum* antibody function. By utilizing the peptide array platform to identify naturally acquired humoral immunity associated with protection from malaria blood stage infection and using *in vitro* models to understand the functionality of these antibodies, I will be able to ensure I gain proficiency in these short-term goals and could identify potential new malaria vaccine candidates. Through the training detailed in my Career Development Plan, mentoring from Drs. Miriam Laufer, Shannon Takala Harrison, Michael Cummings, Andrea

Berry, John Adams, and Kathy Neuzil, and the research proposed in my K23, I will gain skills necessary to develop my research niche. Once the project is complete, I will use the data to apply for an R01 to perform targeted *in vitro* and *in vivo* functional studies to select the most promising candidates for further development.

1. **Friedman-Klabanoff DJ**, Travassos MA, Ifeonu OO, Agrawal S, Ouattara A, Pike A, Bailey JA, Adams M, Coulibaly D, Lyke KE, Laurens MB, Takala-Harrison S, Kouriba B, Kone AK, Doumbo OK, Patel JJ, Thera MA, Felgner PL, Tan JC, Plowe CV, Berry AA. Epitope-specific antibody responses to a *Plasmodium falciparum* subunit vaccine target in a malaria-endemic population. *J Infect Dis.* Published ahead of print. https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa611/5913015
2. **Friedman-Klabanoff DJ**, Laurens MB, Berry AA, Travassos MA, Adams M, Strauss KA, Shrestha B, Levine MM, Edelman R, Lyke KE. The controlled human malaria infection experience at the University of Maryland. *Am J Trop Med Hyg*. 2019; 100(3): 556-565.

B. Positions and Honors

Positions and Employment

2011 - 2014	On-call Physician, University of Minnesota Amplatz Children's Hospital, Pediatric Bone Marrow Transplant Unit, Minneapolis, MN
2012 - 2014	Attending Physician, University of Minnesota Amplatz Children's Hospital, Pediatric Emergency Room, Minneapolis, MN
2012 - 2014	Assistant Professor, University of Minnesota, Department of Pediatrics, Minneapolis, MN
2014 - 2015	Clinical Instructor, Duke University/Human Resources for Health in Rwanda, Department of Pediatrics, Kigali
2015 - 2016	Assistant Professor, Medical College of Wisconsin, Department of Pediatrics, Milwaukee, WI
2016 - 2017	Clinical Fellow, University of Maryland Medical Center, Pediatric Infectious Diseases and Tropical Pediatrics, Baltimore, MD
2017-2020	Post-doctoral Fellow, University of Maryland School of Medicine, Department of Pediatrics, Division of Infectious Diseases and Tropical Pediatrics, Baltimore, MD
2019 - present	PRN Pediatric Hospitalist, Baltimore Washington Healthcare Services, Inc., Glen Burnie, MD
2020 - present	Instructor, University of Maryland School of Medicine, Department of Pediatrics, Division of Infectious Diseases and Tropical Pediatrics, Baltimore, MD

Other Experience and Professional Memberships

2007 - 2013	Member, Member, American Academy of Pediatrics
2012 - present	Member, American Society of Tropical Medicine and Hygiene
2013 - present	Fellow, American Academy of Pediatrics
2016 - present	Member, Pediatric Infectious Disease Society
2016 - present	Member, Infectious Disease Society of America

Honors

2006	Lora L. Marshall Scholarship for academic achievement, University of Wisconsin School of Medicine and Public Health (UWSMPH)
2008	Lewis E. and Edith Phillips Scholarship for academic achievement, UWSMPH
2008	Alpha Omega Alpha (AOA) Medical Honor Society, UWSMPH AOA
2009	Dr. Elizabeth M. Smithwick Scholarship for achievement in Pediatrics, UWSMPH
2018-2020	National Institutes of Health Loan Repayment Program
2019-2021	Burroughs Wellcome Fund/American Society of Tropical Medicine and Hygiene Postdoctoral Fellowship in Tropical Infectious Diseases
2019-2021	Pichichero Family Foundation Research Development, Vaccines for Children Initiative Award in Pediatric Infectious Diseases

Certifications

2012	Pediatrics, American Board of Pediatrics, Active
2012	Certificate of Knowledge in Clinical Tropical Medicine and Traveler's Health (CTropMed®), American Society of Tropical Medicine and Hygiene
2019	Pediatric Infectious Diseases, American Board of Pediatrics, Active

Medical Licensures

2011 - 2016 Minnesota Board of Medical Practice, License to Practice Medicine and Surgery
2015 - 2019 Wisconsin Medical Examining Board, License to Practice Medicine and Surgery
2019 - present Maryland Board of Physicians, License to Practice Medicine and Surgery

C. Contribution to Science

1. **Controlled Human Malaria Infection Experience at the University of Maryland:** With mentoring from Drs. Kirsten Lyke and Matthew Laurens, I completed a manuscript summarizing the 47-year experience of Controlled Human Malaria Infection (CHMI) at the University of Maryland Center for Vaccine Development. Our goal in this project was to summarize this 47-year experience, highlighting novel advances in the development of CHMI and the CVD's role in this progress. Through new analysis of compiled data, we found differences in the median time to patent *Plasmodium falciparum* (Pf) between the NF54 strain and the 7G8 clone and no difference in time to patent Pf infection between those infected with sporozoites through direct venous inoculation and those infected via mosquito bite challenge. We also found that participants diagnosed with malaria by ultrasensitive PCR had fewer symptoms associated with the malaria event than those diagnosed with malaria by smear or standard PCR. Interestingly, historical studies with NF54 showed a shorter median prepatency period compared with more recent studies despite significantly lower salivary gland scores in earlier studies, raising concerns about attenuation of NF54 over time. This research will provide important data for centers considering the use of CHMI in clinical trials for vaccine development. For this project, I collected and compiled data, analyzed and interpreted the data, reported findings at national meetings, and prepared and edited the manuscript.
   a. **Friedman-Klabanoff DJ**, Laurens MB, Berry AA, Travassos MA, Adams M, Strauss KA, Shrestha B, Levine MM, Edelman R, Lyke KE. "A Review of Controlled Human Malaria Infection Trials at the University of Maryland." 2018 Annual Meeting of the American Society of Tropical Medicine and Hygiene. Oral abstract presented in scientific session on October 30, 2018. New Orleans, Louisiana.
   b. **Friedman-Klabanoff DJ**, Laurens MB, Berry AA, Travassos MA, Adams M, Strauss KA, Shrestha B, Levine MM, Edelman R, Lyke KE. The controlled human malaria infection experience at the University of Maryland. *Am J Trop Med Hyg*. 2019; 100(3): 556-565.

2. **Antibodies to peptides representing *Plasmodium falciparum* circumsporozoite protein reflect acquisition of naturally acquired immunity in Malian adults and children:** Circumsporozoite protein (CSP) is a major *Plasmodium falciparum* vaccine target. Previously recognized CSP epitopes include the immunodominant NANP repeat region, the conserved junction between Region 1 (R1) and the NANP repeats, and the polymorphic Th2R and Th3R in the C-terminus. However, little is known about naturally acquired humoral immunity to precise and diverse epitopes along the CSP sequence. Our goal was to use novel high-throughput tools to examine immunity to diverse CSP epitopes, especially in the R1-NANP junctional region. To investigate naturally acquired CSP immunity, we probed sera from ten adults and ten children from Bandiagara, Mali, a region with intense, seasonal malaria transmission, on a diversity-reflecting peptide microarray. We identified precise CSP epitopes where serologic responses differed between adults and children and in children over a malaria season. Adults showed responses to more variants and higher antibody responses at the R1-NANP junctional region and the NANP repeat region, but not to the 3D7 variant sequence in the Th2R epitope, which is included in RTS,S. Children acquired some short-lived immunity to the R1-NANP junctional region and a Th2R epitope during the season but not the NANP repeat region. This work provides groundwork for the work described in this K23 differentiating immunodominant from protectiv e responses in a larger study with longitudinal infection surveillance data and contributes to the growing body of literature that the R1-NANP junctional region of CSP may contain an important epitope. For this project, I compiled data, analyzed and interpreted the data, reported findings at a national meeting, and prepared and edited the manuscript.
   a. **Friedman-Klabanoff DJ**, Travassos MA, Agrawal S, Ouattara A, Pike A, Bailey JA, Adams M, Coulibaly D, Lyke KE, Laurens MB, Takala-Harrison S, Kouriba B, Kone AK, Doumbo OK, Patel JJ, Thera MA, Felgner PL, Tan JC, Plowe CV, Berry AA. "Antibodies to peptides representing *Plasmodium falciparum* circumsporozoite protein reflect acquisition of naturally acquired immunity in Malian adults and children." 2019 Annual Meeting of the American Society of Tropical Medicine and Hygiene. Oral abstract presented in scientific session on November 21, 2019. National Harbor, Maryland.

b. **Friedman-Klabanoff DJ**, Travassos MA, Ifeonu OO, Agrawal S, Ouattara A, Pike A, Bailey JA, Adams M, Coulibaly D, Lyke KE, Laurens MB, Takala-Harrison S, Kouriba B, Kone AK, Doumbo OK, Patel JJ, Thera MA, Felgner PL, Tan JC, Plowe CV, Berry AA. Epitope-specific antibody responses to a *Plasmodium falciparum* subunit vaccine target in a malaria-endemic population. *J Infect Dis.* Published ahead of print. https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiaa611/5913015

3. **Low dose recombinant full-length circumsporozoite protein-based *Plasmodium falciparum* vaccine is highly immunogenic in Phase 1 first-in-human clinical testing:** RTS,S, the most advanced malaria vaccine to date, includes a truncated version of circumsporozoite protein (CSP), the major sporozoite surface protein. A full-length recombinant CSP (rCSP) based strategy may improve the modest efficacy provided by RTS,S, which lacks part of the amino-terminal region critical to sporozoite attachment and invasion of hepatocytes. In my current work, I help to lead a first-in-human Phase 1 clinical trial of a full-length, biologically active rCSP-based vaccine with and without adjuvant, Glucopyranosyl Lipid A-liposome *Quillaja saponaria* 21 formulation (GLA-LSQ). In interim safety and immunogenicity assessment of the first three groups, we found that rCSP/GLA-LSQ demonstrated a favorable safety, tolerability, and immunogenicity profile. The lowest adjuvanted vaccine dose achieved >50-fold rise in geometric mean anti-CSP IgG antibody titer. Recent studies suggest that low dose CSP-based vaccines may be more immunogenic compared to higher doses, and indeed we observed similar anti-rCSP titers in groups despite differing doses in groups that received rCSP with GLA-LSQ. The preliminary low adverse event rate supports GLA-LSQ safety and tolerability in humans. The combined safety and immunogenicity results support advancement in clinical product development. The next phase of the trial will measure preliminary efficacy against controlled human malaria infection (CHMI). We will also measure humoral immunity using high throughput peptide microarrays to help identify correlates of any protection against CHMI. For this project, I contribute to protocol writing, IRB submissions, participant screening and enrollment, participant follow-up, interactions with the study sponsor, data clarification, microarray analyses, the clinical study report, and other essential clinical trial activities. For the interim analysis, I helped analyze and interpret the data and prepared and edited the manuscript, which is under review for publication in *Vaccine*.
   a. **Friedman-Klabanoff DJ**, Berry AA, Travassos MA, Cox C, Zhou Y, Nomicos EYH, Deye GA, Pasetti MF, and Laurens MB. Low dose recombinant full-length circumsporozoite protein-based *Plasmodium falciparum* vaccine is well-tolerated and highly immunogenic in Phase 1 first-in-human clinical testing. *Vaccine.* Under review.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/deanna.friedman-klabanoff.1/bibliography/public/

D. Additional Information: Research Support
Ongoing Research Support

[redacted]
(PI: Friedman-Klabanoff) 11/01/2019 – 10/31/2021
[redacted]

[redacted]
(PI: [redacted]anoff) 07/01/2019 – 06/30/2021
[redacted]

[redacted]
(PI: Laurens) 11/30/2018 – 11/29/2020
[redacted]
Role: Co-Investigator

HHSN272201300022I / 27200019.FY.2018.A1B1C1D1.077 (PI: Kotloff) 08/25/2017 – 05/31/2021
NIH Vaccine and Treatment Evaluation Units contract
A sub-study to evaluate epitope mapping of antibodies elicited by controlled human malaria infection, vaccination, and natural infection with Plasmodium falciparum malaria in 14-0040, 15-0052, and 13-0088

Role: Co-Investigator

HHSN272201300022I / 27200003-13-0088.B1C1.003 (PI: Kotloff) 03/20/2015 – 12/31/2022
NIH Vaccine and Treatment Evaluation Units contract
A Phase 1 Challenge Study to Evaluate Safety, Immunogenicity and Efficacy of a Malaria Vaccine (rCSP adjuvanted with GLA-LSQ), in Healthy Adults, DMID 13-0088
Role: Co-Investigator

CDC 75D30120C08405 (Site PI: Chen) 07/01/2020 – 06/30/2021
Vysnova Partners, Inc. (subaward)
Applied Research to Address the COVID-19 Emerging Public Health Emergency
Role: Co-Investigator

Completed Research Support

[redacted]
(Site PI: Neuzil) 04/01/2020 – 08/31/2020
[redacted]
Role: Co-Investigator

5T32AI007524-20, NIH T32 (PI: Levine) 07/01/2017 – 06/30/2019
Fellowship Training Program in Vaccinology
Role: Post-doctoral fellow

[redacted]
(MPI: Graziano, Kekitiinwa) 05/31/2005 – 08/31/2005
[redacted]
Role: Co-Investigator

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.

Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Laufer, Miriam K.

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

POSITION TITLE: Professor of Pediatrics; Secondary appointments in Medicine, Epidemiology and Public Health; Assistant Dean for Student Research

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Brown University, Providence, RI	BA	06/1992	Public Policy
University of Pennsylvania, Philadelphia, PA	MD	06/1997	Medicine
Columbia University, New York, NY	Residency	06/2000	Pediatrics
Johns Hopkins University, Baltimore, MD	Clinical Fellowship	06/2004	Pediatric Infectious Diseases
Center for Vaccine Development, University of Maryland, Baltimore, MD	Research Fellowship	06/2004	Malaria Research
Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD	MPH	05/2007	Public Health

A. Personal Statement

I am Professor of Pediatrics and the Associate Director of the Center for Vaccine Development and Global Health at the University of Maryland School of Medicine. I was also recently appointed as the Assistant Dean for Student Research. I lead a multi-disciplinary and international team of investigators, trainees and students, in the US and in Africa, who are dedicated in developing strategies and tools to support the global effort towards malaria elimination. In my laboratory at the University of Maryland, my team uses molecular, genomic, and immunological approaches to address some of the most pressing challenges in controlling the burden of malaria. I have been conducting epidemiological and translational research focusing on infectious diseases for 20 years. I lead large NIH-sponsored clinical trials and epidemiological studies linked to molecular and genomic analyses that translate scientific discovery into clinically relevant strategies to improve the health of people living in malaria-endemic countries. I currently serve as the PI for an NIAID cooperative agreement for a clinical trial in Malawi that studies the interaction between HIV and malaria. In addition, I have ongoing studies that look at exposure to malaria and HIV during pregnancy and its impact on infant health and immune response. I am Project Leader for the malaria transmission project of the Malawi International Center for Excellence in Malaria Research. This project examining longitudinal cohorts and conducting entomological studies to identify the key reservoirs of malaria transmission will provide the data and samples for Dr. Friedman-Klabanoff's research. Dr. Friedman-Klabanoff will also assist with upcoming ICEMR cohort studies to gain experience with international clinical trials design, implementation, and leadership.

All of my recently completed and on-going studies are the basis for research training of medical students, pediatric residents, undergraduate and graduate students, and post-doctoral fellows in the United States and Malawi. I currently have a K24 mentoring award to support my efforts training and mentoring young investigators in the technical, scientific and professional skills required to succeed in patient-oriented research. I serve as the PI for a D43 Fogarty training grant that supports graduate training in biostatistics, vector biology and molecular epidemiology for Malawian researchers. Two of my trainees, Dr. Lauren Cohee and Dr. Robert McCann have been awarded NIH K-awards and are now faculty at the Center for Vaccine Development and Global Health. I have co-mentored Dr. Friedman-Klabanoff since 2016 and will serve as primary mentor in her K23 award.

Below are some examples of publications from my pre- and post-doctoral trainees.

1. Divala TH, Mungwira RG, Mawindo PM, Nyirenda OM, Kanjala M, Ndaferankhande M, Tsirizani LE, Masonga R, Muwalo F, Potter GE, Kennedy J, Goswami J, Wylie BJ, Ndovie L, Mvula P, Mbilizi Y, Tomoka T, **Laufer**

MK. A Randomized, Controlled Clinical Trial of Chloroquine as Chemoprophylaxis or Intermittent Preventiv e Therapy to Prevent Malaria in Pregnancy in Malawi. *Lancet Inf Dis*. 2018 Oct 18 (10):1097-1107.
2. Boudova S, Divala TH, Mungwira R, Mawindo P, Tomoka T, **Laufer MK**. Placental but not peripheral *Plasmodium falciparum* infection during pregnancy is associated with increased risk of malaria in infancy. *J Infect Dis*. 2017 Sep 15;216(6):732-735.
3. Buchwald AG, Sixpence A, Chimenya M, Damson M, Sorkin JD, Wilson ML, Seydel K, Hochman S, Mathanga DP, Taylor TE, **Laufer MK**. Clinical implications of asymptomatic Plasmodium falciparum infections in Malawi. *Clin Infect Dis*. 2019 Jan 1;68(1):106-112.
4. Walldorf JA, Cohee LM, Coalson JE, Bauleni A, Nkanaunena K, Kapito-Tembo A, Seydel KB, Ali D, Mathanga D, Taylor TE, Valim C, **Laufer MK**. School-Age Children Are a Reservoir of Malaria Infection in Malawi. PLoS One Jul 24;10(7):e0134061, 2014.

B. Positions and Honors

Positions and Employment

1997 – 2000 Pediatric Resident, Babies and Children's Hospital of New York, Columbia University
2000 – 2001 Field Representative, Health Frontiers, Vientiane, Lao PDR
2001 – 2004 Pediatric Infectious Diseases Clinical Fellow, Johns Hopkins Hospital
2002 – 2004 Visiting Research Fellow, Center for Vaccine Development, University of Maryland School of Medicine
2004 – 2011 Assistant Professor of Pediatrics, Tenure Track, Division of Infectious Diseases and Tropical Pediatrics, University of Maryland School of Medicine
2004 – pres. Secondary appointment, Department of Medicine, University of Maryland School of Medicine
2008 – pres. Secondary appointment, Department of Epidemiology and Public Health, University of Maryland School of Medicine
2010 – 2012 Director, Global Health Resource Center (Global Health Interprofessional Council), University of Maryland, Baltimore
2010 – 2017 Program Director, Pediatric Infectious Diseases Fellowship, University of Maryland School of Medicine
2011 – 2018. Associate Professor of Pediatrics with Tenure (2013), Division of Infectious Diseases and Tropical Pediatrics, University of Maryland School of Medicine with secondary appointments in Epidemiology and Public Health and Medicine
2012 – 2017 Advisory Committee Member, MSTP (MD/PhD Program), University of Maryland School of Medicine
2012 – pres. Faculty, Graduate Program in Molecular Microbiology & Immunology Department of Microbiology and Immunology, University of Maryland School of Medicine
2015 – 2018 Associate Director for Global Health, Institute for Global Health, University of Maryland School of Medicine
2017 – pres. Director, Division of Malaria Research, Institute for Global Health, University of Maryland School of Medicine. 2018 organizational name change to: Associate Director for Malaria Research, Center for Vaccine Development and Global Health, University of Maryland School of Medicine
2018 – pres. Professor of Pediatrics with Tenure, Division of Infectious Diseases and Tropical Pediatrics, University of Maryland School of Medicine with secondary appointments in Epidemiology and Public Health and Medicine
2020 – pres. Assistant Dean for Student Research and Education, University of Maryland School of Medicine

Honors

1996 Stolley Fellowship in Internal Epidemiology, University of Pennsylvania School of Medicine
2000 – 2001 American Academy of Pediatrics Travel Grant
2002 – 2004 Pediatric Infectious Diseases Society Fellowship Award
2003 Infectious Diseases Society of America Travel Grant
2004 Infectious Diseases Society of America Travel Grant
2007 Faculty Research Award, Global Health Resource Center
2011 – 2017 Award for outstanding teaching, Host Defenses and Infectious Diseases
2014 Alpha Omega Alpha, elected by University of Maryland Chapter
2014 Global Health Faculty Award: Malaria Prevention in Adolescents in Malawi
2017 J. Tyson Tildon Award for Pediatric Research, University of Maryland School of Medicine
2018 Joseph Augustin LePrince Medal, American Society of Tropical Medicine & Hygiene

Professional Societies and Public Advisory Committees

2005 – 2009 Member, Public Policy Committee, International Affairs Committee Pediatric Infectious Diseases Society
2006 – 2008 Member, working group of the World Antimalarial Resistance Network (WWARN)
2007 – pres. Program Committee, American Society for Tropical Medicine and Hygiene
2007 – 2016 Judge and Committee Member, Young Investigator Award American Society for Tropical Medicine and Hygiene
2008 Committee member, Institute of Medicine, Committee on Intermittent Preventive Treatment for Malaria in Infants
2008 Grant reviewer, ZRG1 AARR-C 40 Special Emphasis Panel, NICHD
2008 – 2009 Grant reviewer, ZHD1 DSR-A IM in response to RFA, "Considerations for the Safe and Effective Use of Iron Interventions in Areas of High Malaria Burden (U01)," NICHD.
2008 Grant reviewer, Physicians' Services Incorporated Foundation, Canada
2009 – 2011 Member, Young Investigators Group, Malaria Eradication Research Agenda (MalERA)
2010 – 2012 Scientific Advisory Board, Military Infectious Diseases Research Program (MIDRP) Antiparasitic Drug Program Area
2010 – 2014. Malaria Subcommittee member, International Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT)
2012 Grant reviewer, NIH New Innovator Award and ZRG1 AARR-D, NICHD
2012 – pres. Steering committee member, T32 Vaccinology Training Grant, University of Maryland School of Medicine
2013 – pres Grant reviewer for foundations: Wellcome-Trust Fund Enhancements program, Doris Duke Charitable Foundation, Innovations in Clinical Research Award
2013 – 2015 Grant reviewer, Clinical Trial Units for NIAID Networks, MID-B, NIAID: ZAI1-JRR, NIAID; ZHL1 CSR-G, NHLBI
2015 – 2016 Member, Pediatric Terminology Working Group, NIAID
2015 – Reviewer, WHO Evidence Review Group on Intermittent Screening and Treatment and ACT Treatment of Malaria in Pregnancy
2015 – pres. Section Editor, American Journal of Tropical Medicine
2016 – 2017 Grant reviewer, CRFS and IRAP NIH study section
2017 Grant reviewer, Thrasher Foundation
2016 – 2019 WHO Technical Expert Group on Malaria Chemotherapy
2018 – WHO Malaria Vaccine Advisory Committee
2019 – MID NIH study section

C. Contribution to Science

1. **Reservoirs of residual malaria transmission**. Despite the roll out of malaria control efforts, malaria infection and disease persist in many of the hardest hit malaria-endemic areas. Through epidemiological and molecular studies, we have demonstrated that school-age children serve as a reservoir of malaria infection and are likely a key source of persistent malaria transmission in Malawi and other countries in the region. Dr. McCann and I have been invited to write a review on the impact of disease-focused interventions on malaria transmission that we will submit to *Trends in Parasitology*.
   a. McCann RS, Cohee LM, Goupeyou-Youmsi J, **Laufer MK.** Maximizing Impact: Can Interventions to Prevent Clinical Malaria Reduce Parasite Transmission? Trends Parasitol. 2020 Nov;36(11):906-91 3. doi: 10.1016/j.pt.2020.07.013. Epub 2020 Sep 9.
   b. Buchwald AG, Sixpence A, Chimenya M, Damson M, Sorkin JD, Wilson ML, Seydel K, Hochman S, Mathanga DP, Taylor TE, Laufer MK. Clinical Implications of Asymptomatic Plasmodium falciparum Infections in Malawi. Clin Infect Dis. 2019 Jan 1;68(1):106-112
   c. Buchwald AG, Walldorf JA, Cohee LM, Coalson E, Chimbiya N, Bauleni A, Nkanaunena K, Ngwira A, Kapito-Tembo A, Mathanga DP, Taylor TE, **Laufer MK**. Bed net use among school-aged children after a universal bed net campaign in Malawi. Malaria J. Feb 29;15(1):127, 2016.
   d. Walldorf JA, Cohee LM, Coalson JE, Bauleni A, Nkanaunena K, Kapito-Tembo A, Seydel KB, Ali D, Mathanga D, Taylor TE, Valim C, **Laufer MK**. School-Age Children Are a Reservoir of Malaria Infection in Malawi. PLoS One Jul 24;10(7):e0134061, 2014.

2. **The molecular and epidemiological basis for the emergence and spread of drug resistance.** Motivated by our observation of the disappearance of chloroquine-resistant malaria in Malawi, I further investigated the

molecular and genetic basis underlying this phenomenon. Together with colleagues in molecular epidemiology and genomics, I have also explored the impact of the removal of sulfadoxine-pyrimethamine drug pressure and extrapolated the genetic basis of the emergence of artemisinin-resistant malaria in Southeast Asia to hypothesize about the possible emergence and spread of artemisinin-resistance in sub- Saharan Africa.

a. Artimovich E, Schneider K, Taylor TE, Kublin JG, Dzinjalamala FK, Escalante AA, Plowe CV, **Laufer MK**, Takala-Harrison S. Persistence of Sulfadoxine-Pyrimethamine Resistance Despite Reduction of Drug Pressure in Malawi. J Infect Dis. 2015 Sep 1;212(5):694-701
b. Sisya TJ, Kamn'gona RM, Vareta JA, Fulakeza JM, Mukaka MF, Seydel KB**, Laufer MK**, Taylor TE, Nkhoma SC. Subtle changes in Plasmodium falciparum infection complexity following enhanced intervention in Malawi. *Acta Trop.* 2015 Feb;142:108-14.
c. **Laufer MK**, Takala-Harrison S, Dzinjalamala FK, Stine OC, Taylor TE, Plowe CV. Return of chloroquine -susceptible falciparum malaria in Malawi was a reexpansion of diverse susceptible parasites. *J Infect Dis.* 2010 Sep 1;202(5):801-8.
d. **Laufer MK**, Djimdé AA, Plowe CV. Monitoring and deterring drug-resistant malaria in the era of combination therapy. *Am J Trop Med Hyg*. 2007 Dec;77(6 Suppl):160-9.

3. **The return of chloroquine-susceptible malaria in Africa**. I was the leading investigator in the first study to document the return of chloroquine efficacy for the treatment of malaria in sub-Saharan Africa. Malawi was the first country to stop using chloroquine due to widespread resistance. In 1993, Malawi switched its first line treatment for chloroquine to sulfadoxine-pyrimethamine and we found molecular evidence that the chloroquine-resistant malaria decreased significantly thereafter. Our studies have shown that chloroquine -susceptible malaria now predominates and is highly effective for the treatment of malaria.
   a. Divala TH, Mungwira RG, Mawindo PM, Nyirenda OM, Kanjala M, Ndaferankhande M, Tsirizani LE, Masonga R, Muwalo F, Potter GE, Kennedy J, Goswami J, Wylie BJ, Muehlenbachs A, Ndovie L, Mvula P, Mbilizi Y, Tomoka T, **Laufer MK**. Chloroquine as weekly chemoprophylaxis or intermittent treatment to prevent malaria in pregnancy in Malawi: a randomised controlled trial. *Lancet Infect Dis*. 2018 Oct;18(10):1097-1107
   b. Mwanza S, Joshi S, Nambozi M, Chileshe J, Malunga P, Kabuya JB, Hachizovu S, Manyando C, Mulenga M, **Laufer M**. The return of chloroquine-susceptible Plasmodium falciparum malaria in Zambia. *Malar J.* 2016 Dec 5;15(1):584.
   c. Frosch AE, Venkatesan M, **Laufer MK**. Patterns of chloroquine use and resistance in sub-Saharan Africa: a systematic review of household survey and molecular data. *Malar J.* 2011 May 9;10:116.
   d. **Laufer MK**, Thesing PC, Eddington ND, Masonga R, Dzinjalamala FK, Takala SL, Taylor TE, Plowe CV. Return of chloroquine antimalarial efficacy in Malawi. *N Engl J Med.* 2006 Nov 9;355(19):1959-66.

4. **Malaria in pregnancy and impact on infant health.** I have led several studies of malaria during pregnancy. We have shown that the burden of malaria is highest at the first antenatal visit, suggesting that interventions to prevent pregnancy-associated malaria must occur prior to initiation of antenatal care. Not only do women early in pregnancy have high rates of malaria infection, associated with adverse maternal and fetal outcomes, but they also have high rates of gametocyte, the transmissible form of malaria infection, suggesting that they are an important source of persistent malaria transmission in the community.
   a. Boudová S, Divala T, Mungwira R, Mawindo P, Tomoka T, **Laufer MK**. Placental but Not Peripheral *Plasmodium Falciparum* Infection During Pregnancy Is Associated With Increased Risk of Malaria in Infancy. J Infect Dis. 2017 Sep 15;216(6):732-735
   b. Cohee LM, Kalilani-Phiri L, Mawindo P, Joshi S, Adams M, Kenefic L, Jacob CG, Taylor TE, **Laufer MK**. Parasite dynamics in the peripheral blood and the placenta during pregnancy-associated malaria infection. *Malar J.* 2016 Sep 21;15(1):483
   c. Hsu H, Boudova S, Mvula G, Divala TH, Mungwira RG, Harman C, **Laufer MK**, Pauza CD, Cairo C. Prolonged PD1 Expression on Neonatal Vδ2 Lymphocytes Dampens Proinflammatory Responses: Role of Epigenetic Regulation. *J Immunol.* 2016 Sep 1;197(5):1884-92.
   d. Kalilani-Phiri L, Thesing PC, Nyirenda OM, Mawindo P, Madanitsa M, Membe G, Wylie B, Masonbrink A, Makwakwa K, Kamiza S, Muehlenbachs A, Taylor TE, **Laufer MK**. Timing of malaria infection during pregnancy has characteristic maternal, infant and placental outcomes. *PLoS One*. 2013 Sep 18;8(9).

5. **HIV-malaria interactions in sub-Saharan Africa.** Although HIV and malaria infections frequently co-exist in sub-Saharan Africa, their interaction has not been well described. We have demonstrated that HIV infection does not lead to increased severity of malaria although increasing immunosuppression is associated with

higher rates of febrile malaria.

a. **Laufer MK,** van Oosterhout JJ, Thesing PC, Dzinjalamala FK, Hsi T, Beraho L, Graham SM, Taylor TE, Plowe CV. Malaria treatment efficacy among people living with HIV: the role of host and parasite factors. *Am J Trop Med Hyg.* 2007 Oct;77(4):627-32.
b. **Laufer MK**, van Oosterhout JJ, Perez MA, Kanyanganlika J, Taylor TE, Plowe CV, Graham SM. Observational cohort study of HIV-infected African children. *Pediatr Infect Dis J.* 2006 Jul;25(7):623-7.
c. **Laufer MK,** van Oosterhout JJ, Thesing PC, Thumba F, Zijlstra EE, Graham SM, Taylor TE, Plowe CV. Impact of HIV-associated immunosuppression on malaria infection and disease in Malawi. *J Infect Dis.* 2006 Mar 15;193(6):872-8.
d. Laurens MB, Mungwira RG, Nyirenda OM, Divala TH, Kanjala M, Mkandawire FA, Tsirizani L, Nyangulu W, Mwinjiwa E, Taylor TE, Mallewa J, Blackwelder WC, Plowe CV, **Laufer MK**, van Oosterhout JJ. TSCQ Study: A randomized, controlled, open-label trial of daily trimethoprim-sulfamethoxazole or weekly chloroquine among adults on antiretroviral therapy in Malawi: study protocol for a randomized controlled trial. *Trials* 2016 July 18;17(1):322.

Complete List of Published Work in MyBibliography
https://www.ncbi.nlm.nih.gov/sites/myncbi/miriam.laufer.1/bibliography/44032816/public/?sort=date&direction=ascending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

NIH U01AI089342 (PI: Laufer) 08/01/2011 – 07/31/2021
Clinical trial of trimethoprim-sulfamethoxazole or chloroquine in adults on ART.
A clinical trial in Malawi that will provide evidence to determine whether to stop TS prophylaxis in persons on ART and the importance of preventing HIV-associated opportunistic infections and malaria in persons who are stable on ART.

NIH K24AI114996 (PI: Laufer) 01/01/2016 – 12/31/2020
Mentoring and patient-oriented research in malaria.
This grant is designed to support mentorship of the next generation of translational, patient-oriented malaria researchers in the United States and Malawi.

NIH D43TW010075 (MPI: Laufer, Mathanga) 03/01/2016 – 02/28/2021
Interdisciplinary malaria research training in Malawi.
A global infectious diseases research training program that supports training in molecular epidemiology, biostatistics and vector biology to support the next generation of infectious diseases researchers.

NIH U01HD092308 (MPI: Cairo, Laufer) 06/07/2017 – 05/31/2022
The impact of in utero HIV exposure on infant T and B cell responses in Malawi.
This study is a longitudinal analysis of T and B cell subsets in HUE infants from birth to 9 months of age, to assess the relationship between viremia below detectable levels before conception and immunologic alterations in infants.

NIH U19AI089683 (PI: Taylor) 07/01/2017 – 03/31/2024
The Intransigence of Malaria in Malawi: Understanding Hidden Reservoirs, Successful Vectors and Prevention Failures.
International Center of Excellence in Malaria Research (ICEMR). This award seeks to understand the persistence of malaria in Malawi. Pertinent to the research proposed are studies to systematically characterize human to mosquito transmission of P. falciparum.
Role: Project Leader

NIH R61HD103066 (MPI: Laufer/Gladstone) 08/10/2020 – 06/30/2022
Long-term neurocognitive outcomes of HIV-exposed uninfected children.
The goal of this study is to determine the impact of HIV and antiretroviral exposure on neurocognitive development in HIV-exposed uninfected children.

NIH R01HD100235 (MPI: Laufer/Gladstone) 07/01/2020 – 06/30/2025
Neurocognitive development of HIV-exposed and uninfected infants in Malawi.
This study will discern the role of HIV exposure compared to psycho-social factors that impact development of children who are HIV exposed and uninfected.

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

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

NAME: Harrison, Shannon Takala

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

POSITION TITLE: Associate Professor of Medicine and Epidemiology and Public Health

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Brigham Young University	B.S.	1999	Zoology/Chemistry
University of Maryland School of Medicine	Ph.D.	2006	Molecular Epidemiology
University of Maryland School of Medicine and Arizona State University	Postdoctoral Fellowship	2006-2008	Molecular Epidemiology and Evolution

A. Personal Statement

I am an Associate Professor of Medicine and of Epidemiology and Public Health at University of Maryland School of Medicine who has led pioneering molecular and genomic epidemiological studies of malaria. I am the head of the Genomic Epidemiology Unit within the Malaria Research Program in the Center for Vaccine Development and Global Health. I have 20 years of experience conducting rigorous multi-disciplinary translational researc h with publications focused primarily on understanding the evolution of the malaria parasite in response to the human immune system and interventions such as drugs and vaccines. I have training in epidemiological methods, biostatistics, and population genetics, as well as extensive laboratory experience in molecular biological methods. I have successfully applied these skills toward understanding the genetic diversity of malaria vaccine candidate antigens and the implications of parasite diversity for vaccine efficacy. My most recent work involves genomic epidemiological studies of the malaria parasite to understand the genetic basis of emerging anti-malarial drug resistance, as well as to estimate parasite gene flow/migration to inform malaria elimination strategies. I currently lead the Molecular Epidemiology track within the doctoral program in Epidemiology and Human Genetics at University of Maryland School of Medicine, serving as an advisor to doctoral students within the program, and currently serve on the Graduate Program Committee for that program . During my time at University of Maryland School of Medicine, I have mentored or co-mentored 20 graduate students (MS or PhD) and three junior faculty members and have trained or collaborated with investigators from Mali, Malawi, Cote d'Ivoire, Pakistan, China, Thailand, and Cambodia. As part of this career development award, I will mentor Dr. Friedman-Klabanoff on biostatistical and epidemiological methods needed to analyze the peptide microarray data, such as multivariable regression.

B. Positions and Honors

Positions and Employment

1996-1999 Research Assistant, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM
1999-2001 Emerging Infectious Diseases Training Fellow, Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, GA
2001-2006 Graduate Research Assistant, University of Maryland School of Medicine, Baltimore, MD
2006-2008 Postdoctoral Fellow, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD
2008-2015 Assistant Professor, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD
2010-present Director, Genomic Epidemiology Unit, Malaria Group, Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD
2015-present Associate Professor, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD

Other Experience and Professional Memberships

1999-present American Society of Tropical Medicine and Hygiene

2004	DIMACS Working Group: Methodologies for Comparing Vaccination Strategies, Rutgers University, New Brunswick, NJ
2008-2009	Member, Artemisinin Confirmation, Characterization, and Containment Collaboration
2008-2013	Member, MalariaGEN *P. falciparum* Community Project
2014	K13 Expert Review Group, World Health Organization, Geneva, Switzerland
2015, 2019	Proposal Reviewer: Medical Research Council, London, United Kingdom
2016	Proposal Reviewer: Wellcome Trust, London, United Kingdom
2016-2017	Study Section, Program Project Grant, National Institutes of Health, Rockville, MD
2016-2017	Peer Review Panel, Department of Defense CDMRP, Washington, DC
2017	Reviewer, Falk Trust Program, The Medical Foundation, Boston, MA
2018-present	Scientific Program Committee, American Society of Tropical Medicine and Hygiene
2018	Study Section, Clinical Research and Field Studies of Infectious Diseases, National Institutes of Health, Chicago, IL
2018	Proposal Reviewer: British Society for Antimicrobial Chemotherapy, United Kingdom
2018-present	Member, Women in Medicine and Science, University of Maryland School of Medicine
2019	Study Section, Pathogenic Eukaryotes, National Institutes of Health, San Francisco, CA
2019	Technical Consultation on Malaria Genomic Surveillance, World Health Organization, Geneva, Switzerland

Honors and Awards

1997	Barry M. Goldwater Scholar
2001	Graduate Merit Award, University of Maryland School of Medicine
2006	Trudy Bush Women's Health Research Award, University of Maryland School of Medicine
2007	Honorable Mention, Young Investigator Award, American Society of Tropical Medicine and Hygiene
2010, 2013	Clinical Publication of the Year, Department of Medicine, University of Maryland School of Medicine
2019	Bailey K. Ashford Medal, American Society of Tropical Medicine and Hygiene

C. Contributions to Science

1. **Parasite diversity and immune evasion.** My earlier studies done while a research fellow at the Centers for Disease Control and Prevention focused on understanding the role of highly diverse antigens, specifically the Block 2 region of Merozoite Surface Protein 1 (MSP1), in *P. falciparum* immune evasion. Our hypothesis was that multiple-genotype infections could result in delayed acquisition of immunity by presenting a "smoke screen" of antigens that result in immunomodulation or interference with responses to protective antigens. What we found was that larger within-host diversity of malaria infections, as assessed by genotyping MSP1 Block 2, was associated with increased susceptibility to subsequent malaria infection. We concluded that diversity in MSP-1 Block 2 may play a role in *Plasmodium falciparum* immune evasion. In addition, we identified a novel allele family within MSP1 Block 2 that appeared to result from a recombination event between alleles from two other allele families. The presence of a new allele family suggests that diversity in this region of the protein is greater than previously thought and should be taken into account when assessing MSP1 diversity.
   a. Branch OH, **Takala S**, Kariuki S, Nahlen BL, Kolczak M, Hawley W, and Lal AA. December 2001. *Plasmodium falciparum* genotypes, low complexity of infection, and resistance to subsequent malaria in participants in the Asembo Bay Cohort Project. *Infection & Immunity.* **69**(12):7783-92. PMC98874
   b. **Takala SL,** Branch OH**,** Escalante AA, Kariuki S, Wootton J, and Lal AA. November 2002. Evidence for intragenic recombination in *P. falciparum*: Identification of a novel allele family in Block 2 of Merozoite Surface Protein-1: Asembo Bay Area Cohort Project XIV. *Molecular and Biochemical Parasitology* . **125**(1-2):163-71. PMC1853304
   c. **Takala SL**, Escalante AA, Branch OH, Kariuki S, Biswas S, Chaiyaroj SC, and Lal AA. September 2006. Genetic diversity in the Block 2 region of the Merozoite Surface Protein 1 (MSP-1) of *Plasmodium falciparum*: additional complexity and selection and convergence in fragment size polymorphism . *Infection, Genetics, and Evolution.* **6**(5):417-24. PMC1853307

2. **Vaccine antigen diversity and strain-specific efficacy of malaria vaccines.** As a graduate student and post-doctoral fellow my research focused on understanding the natural diversity and dynamics of

polymorphic malaria vaccine antigens and the implications for vaccine design, efficacy, and testing. For two common blood-stage malaria vaccine antigens, we demonstrated that the variants of these antigens that were most common at a vaccine-testing site in Mali were not the variants on which early generation vaccines were based. These findings highlighted the need to take into account antigen diversity within vaccine target populations when designing and testing subunit vaccines. In addition, by examining the within-host dynamics of vaccine antigen variants, we were able to identify specific regions of these two antigens that were associated with clinical disease and inferred that these regions were important in immune escape. Subsequent efficacy analyses within a Phase 2 vaccine trial provided evidence of allele-specific efficacy based on these important regions of the vaccine antigen.

a. **Takala SL**, Smith DL, Stine OC, Coulibaly D, Thera MA, Doumbo OK, and Plowe CV. April 2006. A high-throughput method for quantifying alleles and haplotypes of the malaria vaccine candidate *Plasmodium falciparum* merozoite surface protein-1 19kDa (MSP-1_{19}). *Malaria Journal.* **5**:31. PMC1459863
b. **Takala SL**, Coulibaly D, Thera MA, Dicko A, Smith DL, Guindo AB, Kone AK, Traore K, Ouattara A, Djimde A, Sehdev P, Lyke K, Diallo DA, Doumbo OK, and Plowe CV. March 2007. Dynamics of polymorphism in a malaria vaccine antigen at a vaccine-testing site in Mali. *PLoS Medicine*. **4**(3):e93. PMC1820605
c. **Takala SL**, Coulibaly D, Thera MA, Batchelor AH, Cummings MP, Escalante AA, Ouattara A, Traore K, Niangaly A, Djimde AA, Doumbo OK, and Plowe CV. October 2009. Extreme polymorphism in a vaccine antigen and risk of clinical malaria: implications for vaccine development. *Science Translational Medicine.* **1**(2):2ra5. PMC2822345
d. Ouattara A, **Takala-Harrison** S, Thera MA, Coulibaly D, Niangaly A, Saye R, Tolo Y, Dutta S, Heppner DG, Soisson L, Diggs CL, Vekemans J, Cohen J, Blackwelder WC, Dube T, Laurens MB, Doumbo OK, Plowe CV. February 2013. Molecular basis of allele-specific efficacy of a blood-stage malaria vaccine: vaccine development implications. *Journal of Infectious Diseases*. **207**(3):511-9 PMC3537449

3. **Evolution of antimalarial drug resistance.** As a member of the faculty at University of Maryland School of Medicine, my focus shifted from investigation of vaccine resistance to understanding the evolution of antimalarial drug resistance. In response to failing chloroquine efficacy, Malawi was the first African nation to switch from chloroquine to an antifolate as the first-line treatment for uncomplicated malaria. Utilizing a molecular marker for chloroquine, we were able to show that within a decade of this policy change, chloroquine sensitivity returned to Malawi, suggesting a fitness cost of drug resistance mutations in the absence of drug pressure. By examining selective sweeps associated with the directional selection of chloroquine resistance alleles, we were able to show that this return in chloroquine sensitivity was due to the resurgence of diverse drug-sensitive parasites that had survived chloroquine drug pressure and were able to out-compete less fit drug-resistant parasites. In contrast, using similar methods, we were later able to show that resistance to sulfadoxine-pyrimethamine did not wane after the drug was removed as the first-line therapy, suggesting little to no fitness cost of sulfadoxine-pyrimethamine resistance mutations in the absence of drug pressure.
   a. Laufer MK, Thesing PC, Eddington ND, Masonga R, Dzinjalamala FK, **Takala SL**, Taylor TE, and Plowe CV. November 2006. Return of chloroquine anti-malarial efficacy in Malawi. *New England Journal of Medicine*. **355**(19):1959-66. PMID: 17093247
   b. Laufer MK, **Takala-Harrison S**, Dzinjalamala FK, Stine OC, Taylor TE, and Plowe CV. September 2010. Return of chloroquine-susceptible falciparum malaria in Malawi was a re-expansion of diverse susceptible parasites. *Journal of Infectious Diseases.* **202**(5):801-8. PMC3380613
   c. Artimovich E, Schneider K, Taylor TE, Kublin JG, Dzinjalamala FK, Escalante AA, Plowe CV, Laufer MK, and **Takala-Harrison S**. September 2015. Persistence of sulfadoxine-pyrimethamine resistance despite reduction of drug pressure in Malawi. *Journal of Infectious Diseases.* **212**(5):694-701. PMC4539899
   d. Artimovich E, Kapito-Tembo A, Pensulo P, Nyirenda O, Brown S, Joshi S, Taylor TE, Mathanga D, Escalante AA, Laufer MK, and **Takala-Harrison S**. The effect of local variation in malaria transmission on the prevalence of sulfadoxine-pyrimetham ine resistant haplotypes and selective sweep characteristic s in Malawi. *Malaria Journal*, **14**:387. PMC4595317

4. **Genome-wide studies of the genetic basis of antimalarial drug resistance.** In response to reports of progressively increased parasite clearance time following treatment with artesunate or ACTs and unusually high treatment failure rates on the Thailand-Cambodia border, a collaboration was formed, led by the World Health Organization, to confirm, characterize, and plan for containment of artemisinin resistance. As part of

this collaboration, our group initiated efforts to apply genomic approaches to identify loci within the malaria parasite genome associated with artemisinin resistance in order to identify a molecular marker that could be used in surveillance and to understand the molecular mechanisms underlying resistance. We conducted the first genome-wide association study of delayed parasite clearance following treatment with artemisinins and identified a region of chromosome 13 that contains a kelch protein, mutations in which were later shown to cause artemisinin resistance. In subsequent studies we were able to show that artemisinin resistanc e mutations within this kelch protein both spread between geographic locations and emerged independently within geographic locations, suggesting that efforts to prevent spread of resistance westward from Cambodia may not prevent emergence of resistance in other areas.

a. **Takala-Harrison S**, Clark TG, Jacob CG, Cummings MP, Miotto O, Dondorp AM, Fukuda MM, Nosten F, Noedl H, Imwong M, Bethell D, Se Y, Lon C, Tyner SD, Saunders DL, Socheat D, Ariey F, Phyo AP, Starzengruber P, Fuehrer HP, Swoboda P, Stepniewska K, Flegg J, Arze C, Cerqueira GC, Silva JC, Ricklefs SM, Porcella SF, Stephens RM, Adams M, Kenefic LJ, Campino S, Auburn S, MacInnis B, Kwiatkowski DP, Su XZ, White NJ, Ringwald P, Plowe CV. January 2013. Genetic loci associated with delayed clearance of Plasmodium falciparum following artemisinin treatment in Southeast Asia. *Proc Natl Acad Sci U S A.* **110**(1):240-5. PMC3538248
b. **Takala-Harrison S**, Jacob CG, Arze C, Cummings MP, Silva JC, Dondorp AM, Fukuda MM, Hien TT, Mayxay M, Noedl H, Nosten F, Kyaw MP, Nhien NTT, Imwong M, Bethell D, Se Y, Lon C, Tyner SD, Saunders DL, Ariey F, Mercereau-Puijalon O, Menard D, Newton PN, Khanthavong M, Hongvanthong B, Starzengruber P, Fuehrer HP, Swoboda P, Khan WA, Phyo AP, Nyunt MM, Nyunt MH, Brown TS, Adam s M, Pepin CS, Bailey J, Tan JC, Ferdig MT, Clark TG, Miotto O, MacInnis B, Kwiatkowski DP, White NJ, Ringwald P, and Plowe CV. March 2015. Independent emergence of artemisinin resistance mutations among *Plasmodium falciparum* in Southeast Asia. *Journal of Infectious Diseases.* **211**(5):670-9. PMC4334802
c. Miotto O, Amato R, Ashley EA, MacInnis B, Almagro-Garcia J, Amaratunga C, Lim P, Mead D, Oyola S, Dhorda M, Imwong M, Woodrow C, Manske M, Stalker J, Drury E, Campino S, Amenga-Etego L, Thanh TNN, Hien TT,Ringwald P, Bethell D, Nosten F, Phyo AP, Pukrittayakamee S, Chotivanich K, Chuor CM, Nguon C, Suon S, Sreng S,Newton PN, Mayxay M, Khanthavong M, Hongvanthong B, Htut Y, Han KT, Kyaw MP, Faiz MA, Fanello CI, Mokuolu OA, Jacob CG, **Takala-Harrison S**, Plowe CV, Day NP,Dondorp AM, Spencer CCA, McVean G, Fairhurst RM, White NJ, Kwiatkowski DP. March 2015. Genetic architecture of artemisinin resistant *Plasmodium falciparum. Nature Genetics.* **47**(3):226-34. PMC4545236
d. Agrawal S, Moser KA, Morton L, Cummings MP, Parihar A, Dwivedi A, Shetty AC, Drabek EF, Jacob CG, Henrich PP, Parobek CM, Jongsakul K, Huy R, Spring MD, Lanteri CA, Chaorattanakawee S, Lon C, Fukuda MM, Saunders DL, Fidock DA, Lin JT, Juliano JJ, Plowe CV, Silva JC, and **Takala-Harrison S**. August 2017. Association of a novel mutation within the *Plasmodium falciparum* chloroquine resistanc e transporter with decreased piperaquine sensitivity. *Journal of Infectious Diseases.* 216(**4**):468-47.

5. **Genomic and geospatial analyses to understand parasite population demography.** As efforts to eliminate malaria are underway in areas such as the Greater Mekong Subregion, local information about factors driving malaria risk will be important for prioritizing resources and optimizing strategies for malaria elimination, including estimates of parasite migration. We are currently applying approaches that explicitly model the spatial structure in genomic data to understand parasite migration patterns and are collaborating with geospatial scientists to understand the contribution of local human movement to spatial patterns of parasite migration. Our approaches provide a framework to identify specific geographic areas for targeted intervention that can inform malaria elimination strategies.
   a. Shetty AC, Jacob CG, Huang F, Li Y, Agrawal S, Saunders DL, Lon C, Fukuda MM, Ringwald P, Ashley EA, Han KT, Hlaing TM, Nyunt MM, Silva JC, Stewart KE, Plowe CV, O'Connor TD, **Takala-Harrison S**, Artemisinin Resistance Confirmation, Characterization, and Containment (ARC3), Artemisinin Resistance Containment and Elimination (ARCE), and Tracking Resistance to Artemisinin Collaboration (TRAC). June 2019. Genomic structure and diversity of *Plasmodium falciparum* in Southeast Asia reveal recent parasite migration patterns. *Nature Communications* 10(**1**):2665.
   b. Li Y, Shetty AC, Lon C, Spring M, Saunders DL, Fukuda MM, Hien TT, Pukrittayakamee S, Fairhurst RM, Dondorp AM, Plowe CV, O'Connor TD, **Takala-Harrison S,** and Stewart K. Detecting geospatial patterns of *Plasmodium falciparum* parasite migration in Cambodia using optimized estimated effective migration surfaces. *International Journal of Health Geographics.* **19**(1):13.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/shannon.harrison.1/bibliography/public/

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R01AI125579 (PI: Takala Harrison) 02/03/2017 – 01/31/2022
NIH
Identification and validation of molecular markers of piperaquine resistance
The goal of this grant is to use genome-wide approaches to identify loci associated with piperaquine resistance and to validate candidate loci, in vitro, using gene-editing approaches, and in drug efficacy studies.

R01AI141900 (PI: Silva) 01/11/2019 – 01/10/2024
NIH
Genome-wide sieve analysis and immunological validation to identify targets of protective efficacy in field trials of a whole-organism malaria vaccine
This project aims to generate and compare parasite whole genome sequence data from the vaccine and control arms of field efficacy trials of PfSPZ Vaccine to identify parasite loci that are the target of the vaccine-induced protective efficacy.
Role: Co-Investigator

[redacted] (MPI: Barry, Takala Harrison) 01/01/2019 – 12/31/2022
NHMRC
[redacted]
The goal of this project is to identify specific genes and polymorphisms that contribute to antigenic escape in *P.falciparum.*

2U19 AI110820-06 (MPI: Rasko, Fraser, White) 04/04/2019 – 03/31/2024
NIH
Genomics-based investigation of the determinants of polymicrobial infectious disease outcomes – Project 4: Genomic studies of the impact of external factors on parasite development and disease outcome
This project examines the impact of co-infection on parasite development and disease outcome for tropical parasitic diseases.
Role: Co-investigator

1R01AI145852 (MPI: Takala Harrison, O'Connor) 03/01/2020 – 02/28/2025
NIH
Genomic and geospatial analyses of malaria parasite migration to inform elimination
This grant aims to use genomic and geospatial approaches to understand malaria parasite population structure and migration to identify geographic units of intervention in support of malaria elimination.

Completed Research Support

5R01HL130750-03 (MPI: Plowe, Takala Harrison) 09/15/2015 – 05/31/2020
NIH
Variant surface antigens in cerebral malaria pathogenesis
This project aims to understand the mechanisms underlying cerebral malaria pathophysiology and acquired protective immunity, with a focus on parasite variant surface antigens.

R01-AI101713 (PI: Takala Harrison) 02/01/2013 – 01/31/2018
NIH
Genome-wide studies to identify markers of artemisinin-resistant malaria
The goal of this project is to identify genetic loci associated with artemisinin resistance that can be used in surveillance and containment efforts.

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

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

NAME: Cummings, Michael P.

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

POSITION TITLE: Professor

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of California, Davis	B.S.	06/1983	Botany
Harvard University	Ph.D.	04/1992	Biology
University of California, Berkeley	Postdoctoral	08/1995	Molecular Evolution
University of California, Riverside	Postdoctoral	12/1997	Plant Genetics

A. Personal Statement

I have been doing computer-based biological research since my undergraduate training at the University of California, Davis. I have a broad background in computational biology, bioinformatics, machine learning/statistics and molecular evolution. Most relevant to this proposal are my research accomplishments in the area of machine learning and statistical methods, which have been applied to identify genetic features associated with clinical characteristics in malaria, drug resistance in Plasmodium falciparum and Mycobacterium tuberculosis, limb loss in vertebrate evolution, RNA editing in plant mitochondrial genomes, peptide binding to MHC molecules, and estimation of software run times. I have served as PI, Co-PI, or collaborator on numerous grants from NIH, NSF, DOE, NASA and the Alfred P. Sloan Foundation.

1. Takala SL, Coulibaly D, Thera MA, Batchelor AH, **Cummings MP**, Escalante AA, Ouattara A, Traoré K, Niangaly A, Djimdé AA, Doumbo OK, Plowe CV. Extreme polymorphism in a vaccine antigen and risk of clinical malaria: implications for vaccine development. Sci Transl Med. 2009 Oct 14;1(2):2ra5. PubMed PMID:20165550; PMCID: PMC2822345.
2. Takala-Harrison S, Clark TG, Jacob CG, **Cummings MP**, Miotto O, Dondorp AM, Fukuda MM, Nosten F, Noedl H, Imwong M, Bethell D, Se Y, Lon C, Tyner SD, Saunders D, Socheat D, Ariey F, Phyo AP, Starzengruber P, Fuehrer H-P, Swoboda P, Stepniewska K, Flegg J, Arze C, Cerqueira GC, Silva JC, Ricklefs S, Porcella SF, Stephens RM, Adams M, Kenefic L, Campino S, Auburn S, MacInnis B, Kwiatkowsk i DP, Su X-z, White NJ, Ringwald P, Plowe CV. Genetic loci associated with delayed clearance of *Plasmodium falciparum* following artemisinin treatment in Southeast Asia. Proc Natl Acad Sci USA. 2013 Jan 2;110(1):240-5. PMID: 23248304; PMCID: PMC3538248.
3. Takala-Harrison S, Jacob CG, Arze C, **Cummings MP**, Silva JC, Dondorp AM, Fukuda MM, Tinh Hien T, Mayxay M, Noedl H, Nosten F, Kyaw MP, Thanh Thuy Nhien N, Imwong M, Bethell D, Se Y, Lon C, Tyner SD, Saunders DL, Ariey F, Mercereau-Puijalon O, Menard D, Newton PN, Khanthavong M, Hongvanthong B, Starzengruber P, Fuehrer H-P, Swoboda P, Khan WA, Pyae Phyo A, Nyunt MM, Nyunt MH, Brown TS, Adams M, Pepin CS, Bailey J, Tan JC, Ferdig MT, Clark TG, Miotto O, MacInnis B, Kwiatkowski DP, White NJ, Ringwald P, Plowe CV. Independent emergence of *Plasmodium falciparum* artemisinin resistanc e mutations in Southeast Asia. J Infect Dis. 2015 Mar 1;211(5):670-679. PMID: 25180241; PMCID: PMC4334802.
4. Agrawal S, Moser KA, Morton L, **Cummings MP**, Parihar A, Dwivedi A, Shetty AC, Drabek E, Jacob CG, Henrich PP, Parobek, CM, Jongsakul K, Huy R, Spring MD, Lanteri CA, Chaorattanakawee S, Lon C, Fukuda MM, Saunders DL, Fidock DA, Lin JT Juliano JJ, Plowe CV, Silva JC, Takala-Harrison S. Association of a novel mutation in the *Plasmodium falciparum* chloroquine resistance transporter is associated with piperaquine resistance. J Infect Dis. 2017 Aug 15;216(4):468-476. PMID: 28931241; PMCID: PMC5853219.

B. Positions and Honors

Positions and Employment

1998 – 2003 Assistant Scientist, The Josephine Bay Paul Center for Comparative Molecular Biology and Evolution Marine Biological Laboratory, Woods Hole, Massachusetts

2000 – 2011 Director, Workshop on Molecular Evolution (22 instances) Marine Biological Laboratory , Centers for Disease Control and Prevention, Smithsonian Institution, and the course in Europe (Czech Republic)
2003 Guest Professor, Faculty of Biology, University of Konstanz, Germany
2003 – 2005 Visiting Associate Professor, Department of Biology, and University of Maryland Institute for Advanced Computer Studies University of Maryland, College Park
2004 – 2016 Affiliate Associate Professor, Department of Computer Science University of Maryland, College Park
2005 – 2016 Associate Professor, Department of Biology, and University of Maryland Institute for Advanced Computer Studies University of Maryland, College Park
2016 – present Professor, Department of Biology, and University of Maryland Institute for Advanced Computer Studies University of Maryland, College Park
2016 – present Affiliate Professor, Department of Computer Science University of Maryland, College Park
2018 – present Director, Center for Bioinformatics and Computational Biology University of Maryland, College Park
2018 – present Director, Master of Professional Studies in Data Science and Analytics University of Maryland, College Park

Other Experience and Professional Memberships

2001 – present Editorial Board, Molecular Phylogenetics and Evolution
2001 – 2004 Editorial Board, Systematic Biology
2004 – 2011 Subject Editor (Bioinformatics), Korean Journal of Systematic Zoology
2011 – present Reviewing Editor, Frontiers in Evolutionary and Population Genetics
2012 – present Associate Editor, Animal Systematics, Evolution and Diversity

Honors

1992 – 1995 Alfred P. Sloan Foundation Postdoctoral Fellowship in Molecular Studies of Evolution
2017 NVIDIA Global Impact Award

C. Contributions to Science

1. My research in bioinformatics has taken advantage of machine learning and statistical methods to address problems that can generally be considered regression or classification problems. For example, my researc h on genotype-phenotype relationships has focused on the novel application of tree-based statistical models (referred to as decision trees in the machine learning community), and a recent extension, random forests. I was among the first to apply these methods to sequence data to understand what sequence features are associated with functional differences among genotypes. Specific examples include studies to understand and predict variation in basic immune system functions at the molecular level, RNA editing in plant mitochondrial genomes, and drug resistance in tuberculosis. Recent research following this thread focuses on malaria parasite Plasmodium falciparum collaborative research with Shannon Takala-Harrison at the University of Maryland Medical School and other researchers (some papers listed in A. Personal Statement).
   a. Segal MR, **Cummings MP**, Hubbard AE. Relating amino acid sequence to phenotype: analysis of peptide-binding data. Biometrics. 2001 Jun;57(2):632-642. PMID: 11414594.
   b. **Cummings MP**, Myers DS. Simple statistical models predict C-to-U edited sites in plant mitochondrial RNA. BMC Bioinformatics. 2004 Sep 16;5:132. PMID: 15373947; PMCID: PMC521485.
   c. **Cummings MP**, Segal MR. Few amino acid positions in rpoB are associated with most of the rifampin resistance in *Mycobacterium tuberculosis*. BMC Bioinformatics. 2004 Sep 28;5:137. PMID: 15453919; PMCID: PMC524371.
   d. Kohlsdorf T, **Cummings MP**, Lynch VJ, Stopper GF, Takahashi K, Wagner GP. A molecular footprint of limb loss: sequence variation of the autopodial identity gene Hoxa-13. J Mol Evol. 2008 Dec;67(6):581-593. PMID: 18855040.

2. Following my longstanding interest in parallel computing, prompted in part by the computation-intensive nature of my research, I have dedicated effort toward developing and advancing the application of computer science to analyses. Quite simply, parallel computing — in various forms — has made much of my researc h practically possible. We have developed the software library BEAGLE (Broad-platform Evolutionary Analysis General Likelihood Evaluator), which speeds phylogenetic analyses dramatically. BEAGLE includes an

application programming interface (API) and library for high-performance statistical phylogenetic inference. The library implements parallelism in the likelihood calculation on important emerging computer hardware technology and can exploit hardware features included in graphics processing units (GPUs) and CPUs. The library has been used by thousands of scientists (many without even realizing it).

a. Ayres DL, Darling A, Zwickl DJ, Beerli P, Holder MT, Lewis PO, Huelsenbeck JP, Ronquist F, Swoffor d DL, **Cummings MP**, Rambaut A, Suchard MA. BEAGLE: an application programming interface and high-performance computing library for statistical phylogenetics. Syst Biol. 2012 Jan;61(1):170–173. PMID: 21963610; PMCID: PMC3243739.
b. Ayres DL, **Cummings MP**. Configuring concurrent computation of phylogenetic partial likelihoods: accelerating analyses using the BEAGLE Library. In: Ibrahim S, Choo KK, Yan Z, Pedrycz W (eds) Algorithms and Architectures for Parallel Processing. ICA3PP 2017. Lecture Notes in Computer Sc ience. 2017;10393:533–547.
c. Ayres DL, **Cummings MP**. Rerooting trees increases opportunities for concurrent computation and results in markedly improved performance for phylogenetic inference. 2018 IEEE 32nd International Parallel and Distributed Processing Workshops (IPDPSW). 2018 pg. 247–256.
d. Ayres DL, **Cummings MP**, Baele G, Darling A, Lewis PO, Swofford DL, Huelsenbeck JP, Lemey P, Rambaut A, Suchard MA. BEAGLE 3: Improved performance, scaling, and usability for a high- performance computing library for statistical phylogenetics. Syst Biol. 2019 Nov;68(6):1052–1061. PMID: 31034053 PMCID: PMC6802572.

3. Many of my publications have focused on the patterns and processes of evolution of genes and genomes, and has included studies of transposable elements, slipped-strand mispairing, satellite DNA, interorganel le sequence transfer, and phylogenetics. My research in phylogenetics falls into two broad subcategories: methodological issues, and evolution of specific taxonomic groups. The research on methodological issues have sought to answer questions such as the following. How much nucleotide sequence data is required for accurate phylogenetic inference? How do different types of sequence data compare in subsequent inferences about phylogenetic relationships? How do widely used statistical measures in phylogenetics, such as bootstrap and posterior probability values, compare? Some of my phylogenetic studies have concentrated on specific taxonomic groups including plants, algae, protists, and various invertebrate groups. Typically, in these projects my primary contributions have been in the areas of analysis, hypothesis testing, and inference, while the primary contribution of my collaborators has been in knowledge of the specific organisms studied, taxon sampling, and organismal context.
   a. **Cummings MP**, Otto SP, Wakeley J. Sampling properties of DNA sequence data in phylogenetic analysis. Mol Biol Evol. 1995 Sep;12(5):814–22. PMID: 7476127.
   b. **Cummings MP**, Handley SA, Myers DS, Reed DL, Rokas A, Winka K. Comparing bootstrap and posterior probability values in the four-taxon case. Syst Biol. 2003 Aug;52(4):477–87. PMID: 12857639.
   c. **Cummings MP**, Neel MC, Shaw KL. A genealogical approach to quantifying lineage divergence. Evolution. 2008 Sep;62(9):2411–2422. PMID: 18564377.
   d. Brown TS, Jacob CG, Silva JC, Takala-Harrison S, Djimdé AA, Dondorp AM, Fukuda M,Noedl H,Nyunt MM, Kyaw MP, Mayxay M, Hien TT, Plowe CV, **Cummings MP**. *Plasmodium falciparum* field isolates from areas of repeated emergence of drug resistant malaria show no evidence of hypermutator phenotype. Infect Genet Evol. 2015 Mar;30:318–322. PMID: 25514047; PMCID: PMC4316729.

Partial List of Published Work Available in Google Scholar:
https://scholar.google.com/citations?user=0ahvXiEAAAAJ&hl=en

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

[redacted] (PI: Cummings) 08/15/2017 – 08/14/2020

[redacted]

The goal of this project is to further develop methods for concurrent computation inherent in the calculation of phylogenetic likelihoods and implement them on both CPU (central processing units) and GPU (graphic s processing unit) devices.

[redacted] (PI: Cummings) 06/01/2020 – 05/31/2021

[redacted]

The goal of the project is to adapt the BEAGLE (Broad-platform Evolutionary Analysis General Likelihood Evaluator) library for better performance of SARS-CoV-2 data sets under BEAST (Bayesian Evolutionary Analysis Sampling Trees) v1.10 software running on NVIDIA graphics processing units (GPUs).

Completed Research Support

[redacted] (PI: Cummings) 08/01/2014 – 07/31/2018

[redacted]

The goal of this to develop systems for using parallel computation for phylogenetic analyses employed using grid, public, and GPU (graphics processing unit) computing strategies.

[redacted] (PI: Mitter) 08/01/2014 – 07/31/2018

[redacted]

The goal of this project was to infer phylogenetic relationships of major groups within Lepidoptera (moths and butterflies) using RNA-Seq data and subsequent analyses.
Role: Co-PI

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

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

NAME: Neuzil, Kathleen

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

POSITION TITLE: The Myron M. Levine, MD, DTPH, Endowed Professor in Vaccinology

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

INSTITUTION AND LOCATION	DEGREE (if applicable)	END DATE MM/YYYY	FIELD OF STUDY
University of Maryland, College Park	BS	05/1983	Zoology
Johns Hopkins School of Medicine	MD	05/1987	Medicine
Vanderbilt School of Medicine	MPH	05/1988	Public Health
Vanderbilt School of Medicine, Nashville, TN	Resident	06/1990	Internal Medicine
Vanderbilt School of Medicine, Nashville, TN	Other training	06/1991	Chief Resident
Vanderbilt School of Medicine, Nashville, TN	Fellow	06/1994	Infectious Diseases

A. Personal Statement

I have over two decades of experience in infectious diseases and vaccine science, policy and leadership. My capabilities range from proficiency in epidemiology and clinicals trials to expertise in vaccine research and development, partnerships with developing country vaccine manufacturers, and knowledge and experience with domestic and international regulatory practices. For the past two decades, my research has made major contributions to our understanding of influenza disease and prevention. This research includes pivotal epidemiologic studies of disease burden, clinical trials of vaccine candidates, and studies that employ influenza vaccine as a probe to better understand immunologic priming and the development of immune responses.

Beyond influenza, my vaccine research has been broad, and includes rotavirus, pertussis, and typhoid vaccines, among others, and has advanced science and influenced policy. These studies have ranged from designing and executing Phase 1 trials to leading pivotal clinical trials, including large multicenter field studies.

My research and leadership capabilities are complimented by over two decades of involvement in domestic and international vaccine policy, including membership on CDC's Advisory Committee on Immunization Practices (ACIP) and past and current advisory positions on World Health Organization (WHO) vaccine policy committees. I am currently the only U.S. member of the WHO Strategic Advisory Group of Experts on Immunization (SAGE).

I have a strong commitment to mentoring the next generation of vaccine scientists and leaders. This includes formal academic mentorship and participation on masters and PhD dissertation committees. I currently serve as Contact PI (Multi PI mechanism) of our NIAID T32 Training Grant in Vaccinology, as primary mentor on a K01 Award, and on the K committees for two other awardees. Beyond these specific trainee-focused grants, I work with trainees at all levels in my research. As PI or Co-PI of large multicenter initiatives funded by NIH and the Gates Foundation, I actively seek opportunities for trainees.

B. Positions and Honors

Positions and Employment

1994 - 1998 Assistant Professor, Vanderbilt University School of Medicine, Nashville, TN
1996 - 1998 Assistant Chief, Medical Service, Department of Veterans Affairs Medical Center, Nashville, TN
1997 - 1998 Acting Chief, Medical Service, Department of Veterans Affairs Medical Center, Nashville, TN
1998 - 2003 Assistant Professor of Medicine, University of Washington, Division of Allergy and Infectious Diseases, Seattle, WA
2003 - 2005 Associate Professor of Medicine, University of Washington , Division of Allergy and Infectious Diseases, Seattle, WA
2005 - 2008 Senior Technical Adviser and Clinical Director , PATH, Rotavirus Vaccine Program, Seattle, WA
2005 - 2011 Clinical Associate Professor of Medicine, University of Washington School of Medicine, Division of Allergy and Infectious Diseases, Seattle, WA
2008 - 2012 Director, Influenza Vaccine Project, PATH, Vaccine Development Global Program, Seattle , WA
2009 - 2011 Clinical Associate Professor, University of Washington, Department of Global Health

2011 - 2015	Clinical Professor, University of Washington, Department of Medicine and Global Health, Seattle, WA
2012 - 2015	Director, PATH, Vaccine Access and Delivery Global Program, Seattle, WA
2015 -	Professor of Medicine & Pediatrics and Director, Center for Vaccine Development and Global Health, University of Maryland, Baltimore, School of Medicine, Baltimore, MD
2019 -	The Myron M. Levine, MD, DTPH, Endowed Professor in Vaccinology, University of Maryland, School of Medicine, Baltimore, MD

Other Experience and Professional Memberships

2005 - 2008	Chair, Infectious Disease Society of America, Pandemic Influenza Task Force (IDSA)
2006 - 2010	Member, Centers for Disease Control, Advisory Committee on Immunization Practices
2008 - 2010	Chair, Centers for Disease Control and Prevention, Influenza Vaccine Working Group Advisory Committee on Immunization Practices
2009 - 2010	Member (Ad hoc Group of Experts on Rotavirus), World Health Organization (WHO)
2011 - 2017	Member (Working Group for Safety of Vaccines in Pregnant Women), World Health Organization (WHO), Global Advisory Committee for Vaccine Safety
2011 - 2018	IDSA Liaison Representative to Advisory Committee on Immunization Practices, Centers for Disease Control
2019 -	Voting Member, World Health Organization, Strategic Advisory Group of Experts on Immunization (SAGE)
2019 - 2022	Member, Strategic Advisory Group of Experts on Immunization (SAGE), World Health Organization (WHO)

Honors

1983	Member, Phi Beta Kappa
1987	Member, Alpha Omega Alpha Honor Medical Society
1993 - 1994	Trainee Investigator Award for Excellence in Scientific Research, Association of American Physicians, American Society for Clinical Investigation, American Federation for Clinical Research Foundation
1998	Chiron Award for Epidemiology of Infectious Diseases, International Society of Identification
2004	VA Certificate of Merit for Leading VISN 20 Smallpox Vaccination Program, US Department of Veterans Affairs
2005	Honor Award Certificate, Public Health Epidemiology and Laboratory Reserach, National Center for Infectious Diseases
2010	Joseph E Smadel Lectureship, Infectious Diseases Society of America
2014	"One of 50 Most Influential Persons in Vaccines", Vaccine Nation
2016	Distinguished Alumna Award, Vanderbilt University School of Medicine
2017	Board of Directors Elected Member, National Foundation for Infectious Diseases
2018	"Top 100 Women in Maryland Award", Maryland Daily Record

C. Contribution to Science

1. I have been a leader in the domestic and global response to COVID. As a co-PI of the IDCRC Leadership Group, I am part of the strategic team evaluating COVID vaccines and therapeutics in the US and was part of the study team who designed the first COVID-19 clinical vaccine trial in the US. I serve as the co-director of the newly constituted Coronavirus Prevention Network (CoVPN), as an ad hoc member of the ACIP working group on SARS-CoV-2 vaccines, and as a scientific advisor for the Oxford University clinical trial of the ChimpAd vectored-coronavirus vaccine. As a member of SAGE, I advise the WHO on use of COVID-19 vaccines as data become available.

a. Deming ME, Michael NL, Robb M, Cohen MS, Neuzil KM. Accelerating Development of SARS-CoV-2 Vaccines - The Role for Controlled Human Infection Models. N Engl J Med. 2020 Sep 3;383(10):e6 3. PubMed PMID: 32610006.

b. Jackson LA, Anderson EJ, Rouphael NG, Roberts PC, Makhene M, Coler RN, McCullough MP, Chappell JD, Denison MR, Stevens LJ, Pruijssers AJ, McDermott A, Flach B, Doria-Rose NA, Corbett KS, Morabito KM, O'Dell S, Schmidt SD, Swanson PA 2nd, Padilla M, Mascola JR, Neuzil KM, Bennett H, Sun W,

Peters E, Makowski M, Albert J, Cross K, Buchanan W, Pikaart-Tautges R, Ledgerwood JE, Graham BS, Beigel JH. An mRNA Vaccine against SARS-CoV-2 - Preliminary Report. N Engl J Med. 2020 Jul 14;PubMed PMID: 32663912; PubMed Central PMCID: PMC7377258.

2. My epidemiologic studies using large databases including the Tennessee Medicaid and the Veterans Affairs databases have elucidated the burden of influenza in pregnant women, children, and adults with and without chronic diseases, including HIV infection. These large population-based studies informed decisions in the United States and other countries to recommend vaccination for all pregnant women in the United States, and for young children. Likewise, our work emphasized the tremendous burden of influenza in HIV-infected individuals, particularly before the routine use of highly active antiretroviral therapy. More recently, mentoring and working with Dr. Justin Ortiz, we elucidated the contribution of influenza to respiratory failure and serious disease in the U.S., using administrative databases from several states, and to exacerbations of chronic lung disease, including cystic fibrosis.

a. Ortiz JR, Neuzil KM, Cooke CR, Neradilek MB, Goss CH, Shay DK. Influenza pneumonia surveillanc e among hospitalized adults may underestimate the burden of severe influenza disease. PLoS One. 2014;9(11):e113903. PubMed PMID: 25423025; PubMed Central PMCID: PMC4244176.
b. Neuzil KM, Coffey CS, Mitchel EF Jr, Griffin MR. Cardiopulmonary hospitalizations during influenza season in adults and adolescents with advanced HIV infection. J Acquir Immune Defic Syndr. 2003 Nov 1;34(3):304-7. PubMed PMID: 14600576.
c. Neuzil KM, Mellen BG, Wright PF, Mitchel EF Jr, Griffin MR. The effect of influenza on hospitalizations, outpatient visits, and courses of antibiotics in children. N Engl J Med. 2000 Jan 27;342(4):225-31. PubMed PMID: 10648763.
d. Neuzil KM, Reed GW, Mitchel EF Jr, Griffin MR. Influenza-associated morbidity and mortality in young and middle-aged women. JAMA. 1999 Mar 10;281(10):901-7. PubMed PMID: 10078486.

3. Having observed the considerable burden of influenza, I performed numerous clinical trials in the United States, from Phase I to large field efficacy trials, of influenza vaccines in children and adults to understand the optimal way to reduce that burden. I conducted a series of studies in infants and young children to understand immunologic priming with inactivated vaccines, and to determine how delays in administration of the second dose of vaccine, and/or the relatedness of vaccine antigens, influence immune responses. Likewise, in older children, we determined that a single dose of inactivated vaccine in children who had a prior natural infection with influenza elicited superior antibody responses when compared to two doses of vaccine in seronegative children.

a. King JC Jr, Stoddard JJ, Gaglani MJ, Moore KA, Magder L, McClure E, Rubin JD, Englund JA, Neuzil K. Effectiveness of school-based influenza vaccination. N Engl J Med. 2006 Dec 14;355(24):2523-32. PubMed PMID: 17167135.
b. Neuzil KM, Jackson LA, Nelson J, Klimov A, Cox N, Bridges CB, Dunn J, DeStefano F, Shay D. Immunogenicity and reactogenicity of 1 versus 2 doses of trivalent inactivated influenza vaccine in vaccine-naive 5-8-year-old children. J Infect Dis. 2006 Oct 15;194(8):1032-9. PubMed PMID: 16991077.
c. Walter EB, Neuzil KM, Zhu Y, Fairchok MP, Gagliano ME, Monto AS, Englund JA. Influenza vaccine immunogenicity in 6- to 23-month-old children: are identical antigens necessary for priming?. Pediatric s. 2006 Sep;118(3):e570-8. PubMed PMID: 16950948.
d. Englund JA, Walter EB, Fairchok MP, Monto AS, Neuzil KM. A comparison of 2 influenza vaccine schedules in 6- to 23-month-old children. Pediatrics. 2005 Apr;115(4):1039-47. PubMed PMID: 15805382.

4. I have led complex multicenter initiatives to develop systems and processes and collaboratively design, execute and analyze studies to answer critical scientific and policy questions. Some examples include my leadership of the Rotavirus Vaccine Project, the Influenza Vaccine Project, and currently the Typhoid Vaccine Acceleration Consortium, with typhoid conjugate vaccines trials on-going in 4 countries. The range of these initiatives has included early phase studies of new vaccines, as well as studies of licensed vaccines on alternative schedules and large cluster-randomized trials to understand total effects of vaccination.

a. Meiring JE, Gibani M. The Typhoid Vaccine Acceleration Consortium (TyVAC): Vaccine effectiveness study designs: Accelerating the introduction of typhoid conjugate vaccines and reducing the global burden of enteric fever. Report from a meeting held on 26-27 October 2016, Oxford, UK. Vaccine. 2017 Sep 12;35(38):5081-5088. PubMed PMID: 28802757.

b. Zaman K, Sack DA, Neuzil KM, Yunus M, Moulton LH, Sugimoto JD, Fleming JA, Hossain I, Arifeen SE, Azim T, Rahman M, Lewis KDC, Feller AJ, Qadri F, Halloran ME, Cravioto A, Victor JC. Effectiveness of a live oral human rotavirus vaccine after programmatic introduction in Bangladesh: A cluster-random ized trial. PLoS Med. 2017 Apr;14(4):e1002282. PubMed PMID: 28419095; PubMed Central PMCID: PMC5395158.
c. Armah GE, Sow SO, Breiman RF, Dallas MJ, Tapia MD, Feikin DR, Binka FN, Steele AD, Laserson KF, Ansah NA, Levine MM, Lewis K, Coia ML, Attah-Poku M, Ojwando J, Rivers SB, Victor JC, Nyambane G, Hodgson A, Schödel F, Ciarlet M, Neuzil KM. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: a randomised, double- blind, placebo-controlled trial. Lancet. 2010 Aug 21;376(9741):606-14. PubMed PMID: 20692030.
d. Madhi SA, Cunliffe NA, Steele D, Witte D, Kirsten M, Louw C, Ngwira B, Victor JC, Gillard PH, Cheuvart BB, Han HH, Neuzil KM. Effect of human rotavirus vaccine on severe diarrhea in African infants. N Engl J Med. 2010 Jan 28;362(4):289-98. PubMed PMID: 20107214.

5. Internationally, my research group conducted phase 1 and 2 studies and field efficacy trials of live attenuated influenza vaccines (LAIV) and inactivated influenza vaccines with and without adjuvant. These included a partnership with the developers and manufacturer of the Russian-backbone vaccine to conduct early phase studies in St. Petersburg with H5 and H7 avian vaccines, and to further elucidate our understanding of the immune response to these vaccines. My group conducted the first ever laboratory-confirmed efficacy trials using the Russian-backbone seasonal LAIV, manufactured by the Serum Institute of India, in children in Senegal and Bangladesh. In addition, we described immune responses to seasonal and pandemic LAIVs, and demonstrated that avian LAIVs induce long-term immunity in the absence of a primary antibody response.
a. Lewis KDC, Ortiz JR, Rahman MZ, Levine MZ, Rudenko L, Wright PF, Katz JM, Dally L, Rahman M, Isakova-Sivak I, Ilyushina NA, Matyushenko V, Fry AM, Lindstrom SE, Bresee JS, Brooks WA, Neuzil KM. Immunogenicity and Viral Shedding of Russian-Backbone, Seasonal, Trivalent, Live, Attenuated Influenza Vaccine in a Phase II, Randomized, Placebo-Controlled Trial Among Preschool-Aged Children in Urban Bangladesh. Clin Infect Dis. 2019 Aug 16;69(5):777-785. PubMed PMID: 30481272; PubMed Central PMCID: PMC6695509.
b. Diallo A, Victor JC, Feser J, Ortiz JR, Kanesa-Thasan N, Ndiaye M, Diarra B, Cheikh S, Diene D, Ndiaye T, Ndiaye A, Lafond KE, Widdowson MA, Neuzil KM. Immunogenicity and safety of MF59-adjuvanted and full-dose unadjuvanted trivalent inactivated influenza vaccines among vaccine-naïve children in a randomized clinical trial in rural Senegal. Vaccine. 2018 Oct 15;36(43):6424-6432. PubMed PMID: 30224199; PubMed Central PMCID: PMC6327321.
c. Brooks WA, Zaman K, Lewis KD, Ortiz JR, Goswami D, Feser J, Sharmeen AT, Nahar K, Rahman M, Rahman MZ, Barin B, Yunus M, Fry AM, Bresee J, Azim T, Neuzil KM. Efficacy of a Russian-backbone live attenuated influenza vaccine among young children in Bangladesh: a randomised, double-blin d, placebo-controlled trial. Lancet Glob Health. 2016 Dec;4(12):e946-e954. PubMed PMID: 27746226; PubMed Central PMCID: PMC5118223.
d. Victor JC, Lewis KD, Diallo A, Niang MN, Diarra B, Dia N, Ortiz JR, Widdowson MA, Feser J, Hoagland R, Emery SL, Lafond KE, Neuzil KM. Efficacy of a Russian-backbone live attenuated influenza vaccine among children in Senegal: a randomised, double-blind, placebo-controlled trial. Lancet Glob Health. 2016 Dec;4(12):e955-e965. PubMed PMID: 27746224; PubMed Central PMCID: PMC5118222.

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

T32 AI007524-21 (PI: Neuzil) 07/01/1997 – 06/30/2023
NIAID
Fellowship Training Program in Vaccinology

[redacted] (PI: Neuzil) 10/24/2016 – 10/31/2021

75N93019C00055 (PI: Neuzil) 09/16/2019 – 09/15/2026
NIAID, NIH
Collaborative Influenza Vaccine Innovation Centers (CIVICs)

Component C: Clinical Core

UM1AI148684 (PI: Stephens) 12/05/2019 – 11/30/2026
NIH, NIAID
Leadership Group for Infectious Diseases Clinical Research Consortium (IDCRC)
The formation of a Leadership Group for an Infectious Diseases Clinical Research Consortium, hereafter referred to as the Leadership Group (LG), to support the planning and implementation of clinical research that addresses the scientific priorities of NIAID in evaluating vaccines, other preventive biologics, therapeutics, diagnostic s, including prognostic and predictive markers, and devices for the treatment and prevention of infectious diseases. Role: CPI

HHSN2722013000221 (PI: Kotloff) 07/28/2017 – 09/11/2021
NIAID, Vaccine Treatment and Evaluation Unit (VTEU)
Task Area B-C.18.0010: A phase I randomized, double-blind, controlled trial in healthy adults to assess the safety, reactogenicity, and immunogenicity of a monovalent inactivated influenza A/H5N8 virus vaccine administered intramuscularly with or without AS03 or MF59 adjuvants: assessment of immunological responses and lymphocyte interplay.
Task Area FY.2017.B8C12.0080: Clinical Trials of H7N9 Vaccines.
Task area 16-0024, B1.C1.D1.0067: A Phase II Trial to Evaluate the Safety, Immunogenicity, and Efficacy of a Single Dose of Tdap on Infant Immune Responses in Pregnant Women in Mali
Role: Protocol PI

[redacted] (PI: Neuzil) 10/01/2014 – 12/31/2020
[redacted]
The purpose of this grant is to supplement the University of Maryland's Rotavirus Vaccine Impact on Diarrhea in Africa (VIDA) study by providing essential data coordination functions to ensure the availability and dissemination of high-quality data for global decision-making.

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

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

NAME: Berry, Andrea Ahn-Yee

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

POSITION TITLE: Assistant Professor

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Columbia College, Columbia University	BA	05/1998	Biochemistry
University of Pennsylvania School of Medicine	MD	05/2003	Medicine
Children's Hospital of Philadelphia	Residency	06/2006	Pediatrics
University of Maryland Medical Center	Fellowship	06/2009	Peds Infectious Disease
University of Maryland School of Medicine	Post-doc	06/2010	Vaccinology

A. Personal Statement

I am an Assistant Professor of Pediatrics and Medicine and Co-Director of the Immunoepidemiology and Pathogenesis Group within the Malaria Research Program at the Center for Vaccine Development and Global Health (CVD) within the University of Maryland School of Medicine. I leveraged my previous experience in structural biology and studies of the atomic structure of an *E. coli* fimbriae subunit [1] to focus on the antibody response to *Plasmodium* proteins [2]. Our group has extensive experience in malaria molecular epidemiology and interpretation of protein and peptide microarrays. Together with Dr. Phil Felgner at University of California, Irvine, we pioneered the high-throughput study of immune responses to diverse antigenic variants of *Plasmodium* proteins using protein microarrays [3]. Since 2014, I have led a collaboration with Roche Innovation Madison (now Nimble Therapeutics) to study diverse protein variants as overlapping peptides, which allows fine epitope mapping of the humoral immune response to malaria [3,4]. I was PI of a completed R21 to examine serologic al responses to natural Pf infection in semi-immune adults and children in Mali, West Africa, and responses to PfSPZ in North American malaria naïve volunteers (R21-AI119733) and I am currently Task Order PI of a Vaccine and Treatment Evaluation Unit (VTEU) Sub-study of antibody responses to controlled human malaria infection, natural infection, and vaccination (DMID 17-0081). In both projects, we are identifying antigen epitopes that are associated with acquisition of naturally acquired immunity and vaccine-induced protection from malaria; additional manuscripts are in preparation. My immunology experience and expertise make me well-suited to work closely with Dr. DeAnna Friedman-Klabanoff on identifying putative epitopes from peptide microarrays, as we have done for Dr. Friedman-Klabanoff's recent publication [4]. I will continue to mentor her in the design, analysis, and interpretation of peptide microarray studies.

1. **Berry AA***, Yang Y*, Pakharukova N*, Garnett JA, Lee W, Cota E, Marchant J, Roy S, Tuittila M, Liu B, Inman KG, Ruiz-Perez F, Mandomando I, Nataro JP, Zavialov AV, Matthews S. Structural insight into host recognition by aggregative adherence fimbriae of enteroaggregative Escherichia coli. PLoS Pathogens. 2014 Sep 18; 10(9):e1004404. PMC4169507 (*, authors contributed equally)
2. **Berry AA,** Gottlieb ER, Kouriba B, Diarra I, Thera MA, Dutta S, Coulibaly D, Ouattara A, Niangaly A, Kone AK, Traore K, Tolo Y, Mishcherkin V, Soisson L, Diggs CL, Blackwelder WC, Laurens MB, Sztein MB, Doumbo OK, Plowe CV, Lyke KE. Immunoglobulin G subclass and antibody avidity responses in Malian children immunized with Plasmodium falciparum apical membrane antigen 1 vaccine candidate FMP2.1/AS02A. Malaria Journal. 2019 Jan 18; 18(1):13. PMC6339315
3. Bailey JA, **Berry AA**, Travassos MA, Ouattara A, Boudova S, Dotsey EY, Pike A, Jacob CG, Adams M, Tan JC, Bannen RM, Patel JJ, Pablo J, Nakajima R, Jasinskas A, Dutta S, Takala-Harrison S, Lyke KE, Laurens MB, Niangaly A, Coulibaly D, Kouriba B, Doumbo OK, Thera MA, Felgner PL, Plow e CV. Microarray analyses reveal strain-specific antibody responses to Plasmodium falciparum apical membrane antigen 1 variants following natural infection and vaccination. Nature Scientific Reports. 2020 Mar 3; 10(1):3952. PMID:32127565. PMC7054363
4. Friedman-Klabanoff DJ, Travassos MA, Ifeonu OO, Agrawal S, Ouattara A, Pike A, Bailey JA, Adams M, Coulibaly D, Lyke KE, Laurens MB, Takala-Harrison S, Kouriba B, Kone AK, Doumbo OK, Patel JJ, Thera MA, Felgner PL, Tan JC, Plowe CV, and **Berry AA.** Epitope-specific antibody responses to a Plasmodium

falciparum subunit vaccine target in a malaria-endemic population. J Infect Dis. 2020 Sep 29 PMID: 32992328. PMC pending.

B. Positions and Honors

Positions and Employment

2003-2006 Residency, Pediatrics, Children's Hospital of Philadelphia
2006-2009 Fellowship, Pediatric Infectious Diseases, University of Maryland School of Medicine
2009-2010 Research Associate, University of Maryland School of Medicine
2010-present Assistant Professor, University of Maryland School of Medicine

Other Experience and Professional Memberships

2003-2010 Member, American Academy of Pediatrics
2006- Pediatrics Infectious Disease Society
2006- Infectious Disease Society of America
2007- American Society of Tropical Medicine and Hygiene
2010- Fellow, American Academy of Pediatrics
2013-2016 Member, Vaccine Advocacy Committee, Pediatrics Infectious Disease Society
2013- Reviewer: *Clinical and Vaccine Immunology, PLOS Neglected Tropical Diseases, Human Vaccines & Immunotherapeutics, Pharmacoepidemiology and Drug Safety, Journal of the Pediatric Infectious Disease Society, Nature Scientific Reports, Clinical Infectious Diseases, BMC Immunology, Malaria Journal, American Journal of Tropical Medicine and Hygiene*
2014, 2019 Ad hoc reviewer, Centers for Disease Control and Prevention, Special Emphasis Panel
2017- Member, Research Affairs Committee, Pediatric Infectious Disease Society
2018- Planning committee, Global Health Session at St. Jude's/PIDS Annual Meeting
2019 Symposium Chair, American Society of Tropical Medicine and Hygiene Annual Meeting
2020 Ad hoc reviewer, United Kingdom Research and Innovation, Medical Research Council

Honors

1997 Summer Undergraduate Research Fellowship, Columbia College
1998 Phi Beta Kappa
1998 Magna Cum Laude, Columbia College
1998 21st Century Endowed Scholarship at University of Pennsylvania School of Medicine
2001 Howard Hughes Medical Institute Medical Student Research Fellowship
2016 UMSOM Passano Foundation Clinical Investigator Award

C. Contribution to Science

1. **Studies of the humoral immune response to malaria infection and vaccination on protein and peptide microarrays**

   My research in malaria has focused on the humoral immune response to malaria infection and vaccination with the aim of identifying cross protective responses and specific epitopes that elicit those responses in order to inform vaccine design. My colleagues and I have been using protein and peptide microarrays to study individuals' humoral immune responses to thousands of malaria antigens simultaneously. Unlike others in our field who have studied antigens from a single laboratory strain (3D7), we have uniquely focused on studying antigenic diversity by including diverse variants of key *P. falciparum* antigens derived from field sample DNA [a-b,d]. As we initially predicted, we have observed some advantages of diversity arrays over traditional arrays, including the ability to correlate acquisition of immunity with seroreactivity to increasing numbers of antigenic variants.

   a. Bailey JA, Pablo J, Niangaly A, Travassos MA, Ouattara A, Coulbaly D, Laurens MB, Takala-Harrison SL, Lyke KE, Skinner J, **Berry AA**, Jasinskas A, Nakajima-Sasaki R, Kouriba B, Thera MA, Felgner PL, Doumbo OK, Plowe CV. Seroreactivity to a large panel of field-derived Plasmodium falciparum Apical Membrane Antigen 1 and Merozoite Surface Protein 1 variants reflects seasonal and lifetime acquired responses to malaria. Am J Trop Med Hyg, 2015 Jan:92(1):9-12. PMC4347399.
   b. Zhou A, **Berry AA**, Bailey JA, Pike A, Dara A, Agrawal S, Stucke EM, Ouattara A, Coulibaly D, Lyke KE, Laurens MB, Adams M, Takala-Harrison S, Pablo J, Jasinkas A, Nakajima R, Niangaly A, Kouriba B, Kone AK, Rowe JA, Doumbo OK, Thera MA, Patel JJ, Tan JC, Felgner PL, Plowe CV, Travassos MA.

Antibodies to peptides in semi-conserved domains of RIFINs and STEVORs correlate with malaria exposure. mSphere 2019 Mar 20;4(2). PMID: 30894432. PMC6429043

c. Bailey JA, **Berry AA**, Travassos MA, Ouattara A, Boudova S, Dotsey EY, Pike A, Jacob CG, Adams M, Tan JC, Bannen RM, Patel JJ, Pablo J, Nakajima R, Jasinskas A, Dutta S, Takala-Harrison S, Lyke KE, Laurens MB, Niangaly A, Coulibaly D, Kouriba B, Doumbo OK, Thera MA, Felgner PL, Plowe CV. Microarray analyses reveal strain-specific antibody responses to Plasmodium falciparum apical membrane antigen 1 variants following natural infection and vaccination. Nature Scientific Reports. 2020 Mar 3; 10(1):3952. PMID:32127565. PMC7054363

d. Friedman-Klabanoff DJ, Travassos MA, Ifeonu OO, Agrawal S, Ouattara A, Pike A, Bailey JA, Adams M, Coulibaly D, Lyke KE, Laurens MB, Takala-Harrison S, Kouriba B, Kone AK, Doumbo OK, Patel JJ, Thera MA, Felgner PL, Tan JC, Plowe CV, and **Berry AA.** Epitope-specific antibody responses to a Plasmodium falciparum subunit vaccine target in a malaria-endemic population. J Infect Dis. 2020 Sep 29 PMID: 32992328. PMC pending.

2. Pediatric vaccine studies principal investigator

As Head of the Pediatric Clinical Studies Unit of the Center for Vaccine Development from 2010-2017, I studied vaccines including Rotavirus (Rotateq and Rotarix in mixed sequential schedules), HPV (immunogenicity when given at non-recommended intervals in adolescent girls), H3N2v (a potential pandemic influenza strain), seasonal influenza (full- vs. half- dose for infants age 6-35 months; adjuvanted quadrivalent vaccine), MMR, meningococcal vaccination in infants, and DTaP and DTaP-combination vaccines. These studies inform public health policy and directly lead to licensure of vaccines directed at important pediatric illnesses. As per routine practice at the CVD, I also participate in other adult studies in varying roles. This experience was an opportunity for me to manage projects and collaborate with others. Although I have shifted away from this role in order to focus my research efforts on malaria projects, we are just finishing an industry sponsored clinical trial of a chimpanzee-adenovirus vectored RSV vaccine in seropositive 12-23 month old children, which I led. The leadership and project management skills I developed in this role have transferred to my management of malaria projects, work with T32 trainees, and expertise for VTEU projects.

a. **Berry AA**, Abu-Elyazeed R, Diaz-Perez C, Mufson MA, Harrison CJ, Leonardi M, Twiggs JD, Peltier C, Grogg S, Carbayo A, Shapiro S, Povey M, Baccarini C, Innis BL, Henry O. Two-year antibody persistenc e in children vaccinated at 12-15 months with a measles-mumps-rubella virus vaccine without human serum albumin. Hum Vaccin Immunother. 2017 May 8:1-7. [Epub ahead of print] PubMed PMID: 28481690.

b. Jackson LA, Campbell JD, Frey SE, Edwards KE, Keitel WA, Kotloff KL, **Berry AA**, Graham I, Atmar RL, Creech CB, Thomsen IP, Patel SM, Gutierrez AF, Anderson EL, El Sahly HM, Hill H, Noah DL, Bellamy AR. Serologic Responses to Varying Dosages of a Monovalent H7N9 Influenza Vaccine Given With and Without AS03 and MF59 Adjuvants: A Randomized Clinical Trial . JAMA, 2015 Jul 21;314(3):237 -46, PMC unavailable.

c. Libster R, McNeal M, Walter EB, Shane AL, Winokur P, Cress G, **Berry AA**, Kotloff KL, Sarpong K, Turley CB, Harrison CJ, Pahud BA, Marbin J, Dunn J, El-Khorazaty J, Barrett J, Edwards KM; VTEU Rotavuris Vaccine Study Work Group. Safety and Immunogenicity of Sequential Rotavirus Vaccine Schedules. Pediatrics 2016 Feb;137(2):1-10. PMCID: PMC4732359.

d. Munoz FM, Anderson EJ, Bernstein DI, Harrison CJ, Pahud B, Anderson E, Creech CB, **Berry AA**, Kotloff KL, Walter EB, Atmar RL, Bellamy AR, Chang S, Keitel WA. Safety and immunogenicity of unadjuvanted subvirion monovalent inactivated influenza H3N2 variant (H3N2v) vaccine in children and adolescents. Vaccine 2019, Aug 23; 37(36):5161-5170. PMID: 31375440

3. Malaria clinical trials expertise

UMSOM has the capacity to conduct controlled human malaria infections (CHMI) by mosquito bite or by direct venous inoculation [a]. CHMI is an essential tool for evaluating malaria vaccine candidates prior to larger, more costly field studies. I have served as co-investigator for several malaria vaccine studies [b-c], many of which have included CHMI, as well as a CHMI proof of concept [d] and an immunology study (U01AI110852). I have direct experience with screening and enrolling healthy adult volunteers, vaccination, CHMI, reading blood smears and interpreting qPCR, and safety follow up of inpatient and outpatient CHMI volunteers.

a. Friedman-Klabanoff DJ, Laurens MB, **Berry AA**, Travassos MA, Adams M, Strauss KA, Shrestha B, Levine MM, Edelman R, Lyke KE. The Controlled Human Malaria Infection Experience at the University of Maryland. Am J Trop Med Hyg. 2019 Jan 21. PMID: 30675854.
b. Ishizuka AS, Lyke KE, DeZure A, **Berry AA**, Richie TL, Mendoza FH, Enama ME, Gordon IJ, Chang LJ, Sarwar UN, Zephir KL, Holman LA, James ER, Billingsley PF, Gunasekera A, Chakravarty S, Manoj A, Li M, Ruben AJ, Li T, Eappen AG, Stafford RE, K C N, Murshedkar T, DeCederfelt H, Plummer SH, Hendel CS, Novik L, Costner PJ, Saunders JG, Laurens MB, Plowe CV, Flynn B, Whalen WR, Todd JP, Noor J, Rao S, Sierra-Davidson K, Lynn GM, Epstein JE, Kemp MA, Fahle GA, Mikolajczak SA, Fishbaugher M, Sack BK, Kappe SH, Davidson SA, Garver LS, Björkström NK, Nason MC, Graham BS, Roederer M, Sim BK, Hoffman SL, Ledgerwood JE, Seder RA. Protection against malaria at 1 year and immune correlates following PfSPZ vaccination. Nat Med. 2016 May 9. PubMed PMID: 27158907.
c. Lyke KE, Ishizuka AS, **Berry AA**, Chakravarty S, DeZure A, Enama ME, James ER, Billingsley PF, Gunasekera A, Manoj A, Li M, Ruben AJ, Li T, Eappen AG, Stafford RE, Natasha KC, Murshedkar T, Mendoza FH, Gordon IJ, Zephir KL, Holman LA, Plummer SH, Hendel CS, Novik L, Costner PJM, Saunders JG, Berkowitz NM, Flynn BJ, Nason MC, Garver LS, Laurens MB, Plowe CV, Richie TL, Graham BS, Roederer M, Sim BKL, Ledgerwood JE, Hoffman SL, Seder RA. Attenuated PfSPZ Vaccine induces strain-transcending T cells and durable protection against heterologous controlled human malaria infection. PNAS. 2017 February 22, 2711-2716.
d. Laurens MB, **Berry AA**, Travassos MA, Strauss K, Adams M, Shrestha B, Li T, Eappen A, Manoj A, Abebe Y, Murshedkar T, Gunasekera A, Richie TL, Lyke KE, Plowe CV, Kennedy JK, Potter GE, Deye GA, Sim B, Hoffman SL. Dose dependent infectivity of aseptic, purified, cryopreserved Plasmodium falciparum 7G8 sporozoites in malaria-naive adults. J Infect Dis. 2019 Aug 16.

4. Atomic model and pathogenesis studies of *E. coli* fimbriae

With a goal of understanding the molecular structure of vaccine candidate antigens in order to inform rational vaccine design, my pediatric infectious disease fellowship focused on enteroaggregative E. coli fimbriae (AAF) and their contribution to host recognition and virulence. My role was to use NMR to solve the structure of AafA. Through determining the atomic structure of AAF subunits, we were able to study fimbriae binding and recognize that AAF bind fibronectin through electrostatic interactions, a mechanism that had not previously been reported for bacterial adhesion to abiotic surfaces. The structural information gained from this work informs vaccine development against pathogenic E. coli. Elements of the molecular biology and protein chemistry I learned from this project are directly applicable to my current work: tertiary protein structure determines antibody binding sites, and an understanding of amino acid structure is essential to identifying amino acids that are most likely to be associated with cross reactivity on protein and peptide microrarrays.

a. Yang Y, **Berry AA**, Lee W-C, Garnett JA, Marchant J, Levine JA, Simpson PJ, Fogel SA, Varney KM, Matthews SJ, Nataro JP, and Inman KG. Complete 1H, 13C and 15N NMR assignments for donor-strand complemented AafA, the major pilin of aggregative adherence fimbriae (AAF/II) from enteroaggregative E. coli. J Biomol NMR Assign, 4; 2010. PMCID unavailable
b. **Berry AA***, Yang Y*, Pakharukova N*, Garnett JA, Lee W, Cota E, Marchant J, Roy S, Tuittila M, Liu B, Inman KG, Ruiz-Perez F, Mandomando I, Nataro JP, Zavialov AV, Matthews S. Structural insight into host recognition by aggregative adherence fimbriae of enteroaggregative Escherichia coli. PLoS Pathogens. 2014 Sep 18; 10(9):e1004404. PMC4169507 (*, authors contributed equally)

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/andrea.berry.1/bibliography/43391448/public/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

K23AI125720 (PI: Berry) 02/06/2017 – 01/31/2022
NIH/NIAID
Protein microarray antibody responses to *P. falciparum* in a human challenge model
Research goal: Identify antibody responses of malaria naïve volunteers following controlled human malaria infection on protein and peptide microarrays

N01AI80001 (PI: Kotloff) 08/25/2017 – 05/31/2021
NIH/NIAID
Evaluation of control measures against infectious diseases other than AIDS (VTEU)
Research goal: Clinical testing of vaccines and related products in human subjects.
I am Principal Investigator for Task Order within this contract, "A sub-study to evaluate epitope mapping of antibodies elicited by controlled human malaria infection, vaccination, and natural infection with *Plasmodium falciparum* malaria in 14-0040, 15-0052, and 13-0088" Task order dates: 9/2017 to 5/31/2021
Role: Task Order Principal Investigator

U01AI110852 (PI: Williamson) 04/01/2015 – 02/28/2021
NIH/NIAID
A systems biology approach to malaria immunity
Research goal: Evaluate immune response to repetitive P. falciparum infection administered by infectious mosquito bite in a Controlled Human Malaria Infection study.
Role: Co-investigator

R01AI141900 (PI: Carneiro da Silva) 01/15/2019 – 12/31/2023
NIH/NIAID
Genome-wide sieve analysis and immunological validation to identify targets of protective efficacy in field trials of a whole-organism malaria vaccine
Research goal: use comparative genomics and immuno-proteomics to identify parasite proteins that are the target of PfSPZ Vaccine-induced protection.
Role: Co-Investigator

Completed Research Support

R01-AI093635-01A1 (PI: Plowe) 02/02/2012 – 01/31/2017
NIH/NIAID
Immuno-epidemiological epitope mapping of a blood stage malaria vaccine antigen
Research goal: Characterize antibody responses against apical membrane antigen-1 (AMA1) to inform the selection of optimal AMA1 epitopes for inclusion in a multi-allelic, broadly protective malaria vaccine.
Role: Co-investigator

R21AI119733 (PI: Berry) 07/01/2016 – 06/30/2019
NIH/NIAID
Ultra-dense peptide array analysis of naturally acquired and vaccine-induced P. falciparum immunity
Research goal: Identify specific malaria peptides that are targeted by antibodies that protect against both natural and experimentally-induced malaria in humans. Such correlates of protection help us to understand and predict natural and vaccine-induced protection, and to identify vaccine candidate antigens.
Role: Principal investigator

[redacted] (PI: Berry) 03/17/2017 – 03/31/2020
GSK
[redacted]
Research goal: Phase 1/2 safety and immunogenicity study; first in children
Role: Local PI

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

BIOGRAPHICAL SKETCH

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

NAME: Adams, John H.

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

POSITION TITLE: Distinguished University Professor

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

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Hendrix College, Conway, Arkansas	B.A.	1978	Biology
University of Illinois at Urbana-Champaign	M.Sc.	1982	Vet. Med. Sci.
University of Illinois at Urbana-Champaign	Ph.D.	1986	Vet. Med. Sci.

A. Personal Statement

My research interests focus on two broad areas in malaria parasite biology: host-parasite interactions and understanding the critical metabolic processes important for infection and pathogenesis. These interests serve to guide basic and translational discovery projects to improve antimalarial drugs and vaccines targeting different stages of *Plasmodium falciparum* and *P. vivax*. To support this K23 project, we have successfully developed significantly more efficient *in vitro* culture methods for *P. vivax* and *P. falciparum* liver stages, using a 384-well primary human hepatocyte culture platform for complete liver stage development with breakthrough to blood-stage infection, which is suitable for evaluating either drugs or vaccines against infecting sporozoites or liver stage development (1). A critical breakthrough that led to these more highly efficient LS assays was discovery of methods to manipulate sporozoite activation to enhance infectivity (2). Subsequently we have adapted the platform for *P. cynomolgi*, using primary NHP hepatocyte cultures, for similar assays with the non-human primate malaria (3). In other projects, my group led development of a whole genome forward genetic screen to define most essential and dispensable genes in the *P. falciparum* genome (4). Altogether, contributions from my research program have improved our knowledge of parasite biology and enhanced development of antimalarial therapies.

1. Roth A*, Maher SP*, Conway A, Ubalee R, Chaumeau V, Andolina C, Kaba SA, Vantaux A, Bakowski MA, Thomson Luque R, Adapa SR, Singh N, Barnes SJ, Cooper C, Rouillier M, McNamara CW, Mikolajczak SA, Sather N, Witkowski B, Campo B, Kappe SHI, Lanar DE, Nosten F, Davidson S, Jiang RHY, Kyle DE, **Adams JH**. (2018) A comprehensive model for assessment of liver stage therapies targeting *Plasmodium vivax* and *Plasmodium falciparum*. *Nature Communications*, 9:1837. DOI: 10.1038/s41467-018-04221-9. *Contributed equally. PMCID: PMC5993793
2. Roth A, Adapa SR, Saxena V, Liao X, Ubalee R, Zhang M, Goffe R, Li S, Saggu GS, Pala ZR, Garg S, Davidson S, Jiang RHY, **Adams JH**. (2018) Unraveling the *Plasmodium vivax* sporozoite transcriptional journey from mosquito vector to human host. *Scientific Reports*, 8:12183. doi:10.1038/s41598-018-30713- 1.
3. Vanachayangkul P, Im-erbsin R, Tungtaeng A, Kodchakorn C, Roth A, **Adams J**, Chasatit C, Saingam P, Sciotti RJ, Reichard GA, Nolan CK, Pybus BS, Black CC, Lugo LA, Wegner MD, Smith PL, Wojnarski M, Vesley BA, Kobylinski KC. Safety, pharmacokinetics, and liver-stage *Plasmodium cynomolgi* effect of high-1 dose ivermectin and chloroquine in Rhesus Macaques. *AAC*, Aug 20;64(9):e00741-20. doi: 10.1128/AAC.00741-20. PMID: 32660993. PMCID: PMC7449176.
4. Zhang M*, Wang CCQ*, Otto TD, Oberstaller J, Liao X, Adapa SR, Udenze K, Li S, Bronner IF, Casandra D, Mayho M, Brown J, Li S, Swanson J, Rayner JC, Jiang RHY, **Adams JH**. (2018) Uncovering the essential genome of the human malaria parasite *Plasmodium falciparum* by saturation mutagenesis. *Science,* 360, eaap7847. DOI: 10.1126/science.aap7847. PMCID: PMC6360947. *Contributed equally.

B. Positions and Honors

Positions and Employment

1976, 1978 Teaching Assistant, Department of Biology, Hendrix College, Conway, Arkansas
1979 – 1986 Graduate Assistant, Department of Veterinary Pathobiology, University of Illinois at Urbana-Champaign

1986 – 1987	Post-doctoral Research Fellow, Department of Parasitology, University of Queensland, Australia
1987 – 1991	Senior Staff Fellow (1989 - 1991), Staff Fellow (1987 - 1989), Malaria Section, Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, Maryland
1991 - 2007	Professor (2005-2007), Associate Professor (1998 - 2005), Assistant Professor (1991 - 1998), Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana.
2007 – Present	Distinguished University Professor, Center for Global Health & Infectious Diseases Research, College of Public Health, University of South Florida, Tampa, Florida.

Other Experience, Professional Memberships, and Honors

1978	Honorable Mention, National Science Foundation Graduate Fellowship.
1981	University of Illinois List of Teachers Ranked as Excellent by Their Students.
1986	University of Queensland Postdoctoral Research Fellowship.
1997	Burroughs Wellcome Fund New Investigator Award in Molecular Parasitology.
1998 – 2004	Chair & Member, Scientific Advisory Committee, MR4 at ATCC.
2000	Program Officer, Annual Midwestern Conference of Parasitologists-52, Notre Dame
2000 – 2004	ASP Committees: R. Barclay McGhee Memorial Lecture; Ashton Cuckler Award.
2000 – 2009	Editorial Board, *Infection and Immunity*.
2002 – 2006	Founding President, American Committee of Molecular, Cellular, & Immuno-Parasitology (ACMCIP) of the American Society of Tropical Medicine & Hygiene (ASTMH)
2006 – 2007	Chair, American Society of Parasitologist Tellers Committee.
2006 – 2010	Member, Pathogenic Eukaryotes Study Section (NIH).
2006 – 2014	*Trends in Parasitology*, Advisory Editorial Board.
2008	21st Century World Class Scholar, State of Florida Board of Governors
2008	Hendrix College Odyssey Award for Research
2008 – 2012	PATH Malaria Vaccine Initiative's Vaccine Science Portfolio Advisory Council
2009	FDA Blood Products Advisory Committee Meeting.
2009	Organizer, *Vivax malaria research III: 2009 and beyond*, Gamboa, Panama.
2009 – 2011	CRIMALDI Scientific Advisory Committee
2009 – present	Member, PlasmoDB Scientific Advisory Committee
2009 – 2019	Editor, *Infection and Immunity*
2011 – 2017	Mentor, PRIDE – Functional and Applied Genomics of Blood Disorders, NHLBI-funded Summer Institute Training Programs for Junior Faculty at Georgia Health Sciences University
2012 – 2013	Visiting Professor, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand
2012 – 2013	Visiting Professor, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand
2012, 2016	Site visit review of research program: Laboratory of Emerging Pathogens, FDA CBER.
2013	*ad hoc* consultant, NIH Board of Scientific Counselors, site review of LMIV, LMVR & LPD
2015	Co-Chair, ASTMH Basic Science Pre-meeting Course, October 25, 2015.
2017	Fellow of AAAS (Section on Biological Sciences)
2017 – 2021	Member, Clinical Research and Field Studies in Infectious Diseases Study Section
2017	Fellow of ASTMH

Patents Issued:

US Patent No. 5,198,347 (March 30, 1993). DNA encoding *Plasmodium vivax* and *Plasmodium knowlesi* Duffy receptor. Inventors: LH Miller, JH Adams, DC Kaslow, X Fang. Duffy Binding protein as a vaccine.

US Patent No. 6,120,770 (September 19, 2000). *Plasmodium* Proteins Useful for Preparing Vaccine Compositions. Inventors: JH Adams, SHI Kappe and JP Dalton. *Plasmodium* MAEBL as a vaccine.

US Patent No. 7,932,088 (April 26, 2011). High Efficiency Transformation of *Plasmodium falciparum* by the Lepidopteran Transposon, piggyBac. Inventors: JH Adams, MJ Fraser, Jr., B Balu, and DA Shoue. The use of *piggyBac* transposable element as a tool for functional profiling of the *Plasmodium* genome.

US Patent No. 9,123,690 (September 1, 2015) Synthetic Antigen Based on the Ligand Domain of the Plasmodium vivax Duffy Binding Protein. Inventors: JH Adams, FB Ntumngia, JL Schloegel, SJ Barnes, AM McHenry, P Chootong.

C. Contribution to Science

1. **Identifying and characterizing Duffy binding protein (PvDBP) family**. My career in malaria researc h began in Louis Miller's lab at NIH with the task to isolate and characterize the gene encoding the *P. knowlesi* DBP (PkDBP). At the time *P. knowlesi* was the primary model for studying *Plasmodium* merozoite invasion as well as a model for studying *P. vivax*. Characterizing the genes encoding the PkDBP and its paralogs generated two seminal findings: i) the merozoite's micronemes sequester *Plasmodium* ligands for host cell invasion; ii) gene duplication and diversity leads to ligand heterogeneity. Further studies in my research led to determining the key characteristics of *ebl* Erythrocyte Binding Proteins with Region II as the predicted ligand domain, identifying MAEBL and other homologs, and eventually the significance of Fy^a/Fy^b heterogeneity (a.k.a., Duffy antigen receptor for chemokines or DARC).
   a. **Adams JH,** Hudson DE, Torii M, Ward GE, Wellems TE, Aikawa M, Miller LH. (1990). The Duffy receptor family of *Plasmodium* knowlesi is located within the micronemes of invasive malaria merozoites. *Cell* . 63:141-153. PMID:2170017.
   b. **Adams JH,** Sim BKL, Dolan SA, Fang X, Kaslow DC, Miller LH. (1992). A family of erythrocyte binding proteins in malaria parasites. *PNAS (USA).* 89: 7085-7089. PMCID: PMC49650
   c. Kappe SHI, Noe AR, Blair PL, Fraser T, and **Adams JH.** (1998). A chimeric family of erythrocyte binding proteins of malaria parasites. *PNAS (USA).* 95: 1230-1235. PMCID: PMC18728
   d. King CL, **Adams JH**, Xianli J, Grimberg B, McHenry A, Greenberg L, Siddiqui A, Howes R, da Silva-Nunes M, Ferreira MU, Zimmerman PA. (2011). Fy^a/Fy^b Polymorphism in Human Erythrocyte Duffy Antigen Affects Susceptibility to *Plasmodium vivax* Malaria. *PNAS (USA),* 108(50):20113-8; PMCID: PMC3250126

2. **Defining immune selection as an underlying mechanism of allelic variation in *Plasmodium* ligands**. My initial studies as an independent PI led to the discovery *P. vivax* DBP ligand domain was under immune selective pressure generating most of the allelic diversity in ligand domain. After establishing *P. vivax* infections could elicit functionally inhibitory anti-DBP antibodies, we experimentally validated that allelic diversity could alter antigenic character of DBP. Extensive site-directed mutation analysis of DBP established that variable residues flanked residues critical for RBC binding in a pattern similar to other microbial ligands. These findings supported my hypothesis that allelic diversity was driven by immune selection and this was the underlying mechanism for strain-specific anti-DBP immunity. Differential screening with inhibitory vs. non-inhibitory anti-DBP antibodies confirmed that variant residues comprise the dominant Bc epitopes. Subsequent structural studies by my collaborator Niraj Tolia confirmed the importance of epitopes' locations for DBP function where binding antibody would prevent the required DBP-DARC dimerization. More recently we have developed human monoclonal antibodies to conserved neutralizing epitopes and defined the 3D structures (unpublished).
   a. Tsuboi T, Kappe S, Al-Yaman F, Prickett MD, Alpers MP, **Adams JH.** (1994). Natural variation within the principal adhesion domain of the *Plasmodium vivax* Duffy binding protein. *Infection and Immunity* . 62**:** 5581-5586. PMCID: PMC303305
   b. VanBuskirk KM, Sevova E., **Adams JH.** (2004). Conserved residues in the *Plasmodium* vivax Duffy- binding protein ligand domain are critical for erythrocyte receptor recognition. *PNAS (USA*), 101, 15754- 15759. PMCID: PMC524844.
   c. Chootong P*, Ntumngia FB*, Vanbuskirk KM*, Xianli J, Cole-Tobian JL, Fraser TS, King CL, **Adams JH**. (2010). Mapping epitopes of the *Plasmodium vivax* Duffy binding protein with naturally acquired inhibitory antibodies. *Infection & Immunity,*78(3), 1089-1095. PMCID: PMC2825952. *Contributed equally.
   d. Ntumngia FB†, Thomson-Luque R, Torres L, Gunalan K, Carvalho L, **Adams JH†**. (2106). A Novel Erythrocyte Binding Protein of *Plasmodium vivax* provides an Alternate Invasion Pathway into Duffy Positive Reticulocytes. *mBio*, 7(4): e01261-16. doi:10.1128/mBio.01261-16. PMCID: PMC4999553. †Corresponding authors.

3. **Immunity to *P. vivax* DBP**. A better understanding of parasite biology will enhance our ability to make more effective long-lasting therapies to control and eliminate malaria. Experimental studies of DBP have benefited from *in vitro* functional assays to guide evaluation and functional characterization of DBP. Functional analysis of the DBP is now focused on it use as a possible blood-stage vaccine. My research program has helped (i) define the determinants for receptor recognition, (ii) identify epitope targets for immune antibody neutralization, (iii) engineer a better DBP immunogen, and (iv) create enabling technologies for discovery research.

a. Chootong P*, Ntumngia FB*, Vanbuskirk KM*, Xianli J, Cole-Tobian JL, Fraser TS, King CL, **Adams JH**. (2010). Mapping B-Cell Epitopes associated with naturally acquired inhibition of the *Plasmodium vivax* Duffy binding protein. *Infection & Immunity,*78(3), 1089-1095. PMCID: PMC2825952. *Contributed equally.
b. Changrob S, McHenry AM, Nyunt MH, Sattabongkot J, Han ET, **Adams JH**, Chootong P. (2018) Persistence of Long-lived Memory B Cells specific to Duffy Binding Protein in individuals exposed to *Plasmodium vivax*. *Scientific Reports*. 2018 May 29;8(1):8347. doi: 10.1038/s41598-018-26677-x.
c. Carias L, Dechavanne S, Barnes SJ, Suon S, Sreng S, Amaratunga C, Fairhurst RM, Dechavanne C, Nicolete V, Ferreira MU, Chen E, Tolia N, **Adams JH**, King CL. (2019) Identification and characterization of functional human monoclonal antibodies to *Plasmodium vivax* Duffy binding protein. *The Journal of Immunology*, ji1801631: doi:10.4049/jimmunol.1801631. PMID: 30944159. PMCID: n/a.
d. Urusova D, Carias L, Huang Y, Nicolete VC, Popovici J, Roesch C, Salinas ND, Witkowski B, Ferreira MU, **Adams JH**, Gross ML, King CL, Tolia NH. (2019) Structural basis for neutralization of *P. vivax* by naturally-acquired human antibodies that target DBP. *Nature Microbiology*, doi.org/10.1038/s41564- 019-0461-2. PMID: n/a. PMCID: n/a.

4. **Engineered vaccine to overcome DBP strain-specific immunity**. Our knowledge gained from immunochemical and the structure-function studies of DBP enabled us to engineer an immunogen, termed DEKnull, to provide broadly neutralizing strain-transcending antibodies. The 3D structure OF DEKnull solved by my collaborator Niraj Tolia's group, confirmed DEKnull changes were limited to the DEK epitope. Our studies underway with DEKnull-2, are intended to design the optimal immunization strategy to elicit long- term memory Bc as occur naturally in individuals with protective immunity after infections.
   a. Ntumngia FB, **Adams JH**. (2012). Design and Immunogenicity of a Novel Synthetic Antigen based on the Ligand Domain of the *Plasmodium vivax* Duffy Binding Protein. *Clinical and Vaccine Immunology* , 19(1): 30; PMCID: PMC3255949
   b. Ntumngia FB, Schloegel J, McHenry A, Barnes SJ, George MT, Kennedy S, **Adams JH**. (2013). Immunogenicity of single versus mixed allele vaccines of *Plasmodium vivax* Duffy binding protein region II. *Vaccine* 31(40):4382-4388. PMCID: PMC4497540.
   c. Chen E, Salinas ND, Ntumngia FB, **Adams JH**, Tolia NH. (2015). Structural analysis of the synthetic DBP antigen DEKnull relevant for *Plasmodium vivax* malaria vaccine design. *PLoS NTD*, 9(3):e000364 4. doi: 10.1371/journal.pntd.0003644. PMCID: PMC4368114.
   d. Ntumngia FB, Pires CV, Barnes SJ, George MT, Thomson-Luque R, Kano FS, Alves JRS, Urusova D, Pereira DB, Tolia N, King CL, **Adams JH**. (2017). An engineered mutant of the *Plasmodium vivax* Duffy binding protein enhances induction of broadly neutralizing antibodies. *Scientific Reports,* 7: 13779. doi:10.1038/s41598-017-13891-2. PMCID: PMC5653783.

5. **Functional genomics of *P. falciparum***. My lab has developed methods for whole genome functional analysis of *P. falciparum*, using *piggyBac* transposon mutagenesis. Our studies based on random *piggyBac* integration in the parasite genome has defined the essential and dispensable genes and GO pathways required for asexual blood-stage growth under ideal *in vitro* culture conditions. Importantly, these studies highlighted the vital nature of nucleic acid metabolism, especially RNA post-transcriptional mechanisms regulating gene expression. Parallel phenotyping has been used to classify drug activities and decipher drug-gene interactions related to drug mechanisms of action. My group worked closely with Julian Rayner, Thomas Ott, and Rays Jiang to develop the tools for quantitative forward genetic screens of *P. falciparum* mutants.
   a. Pradhan A, Siwo G, Singh N, Martens B, Balu B, Button-Simons K, Tan A, Zhang M, Udenze K, Jiang RHY, Ferdig MT, **Adams JH***, Kyle DE*. (2015) Chemogenomic profiling of *Plasmodium falciparum* as a tool to aid antimalarial drug discovery. *Scientific Reports*, 5, 15930; doi: 10.1038/srep15930 (in press). *Co-corresponding authors. PMCID: PMC4635350
   b. Bronner IF*, Otto TD*, Zhang M*, Udenze K, Wang CCQ, Quail MA, Jiang RHY, **Adams JH†**, Rayner JC†. Quantitative Insertion-site Sequencing (QIseq): A new tool for high throughput phenotyping of transposon mutants. *Genome Research*, ePub May 10, 2016; doi:10.1101/gr.200279.115. PMCID: PMC4937560 *Contributed equally to this manuscript; †Co-corresponding authors.
   c. Zhang M*, Wang CCQ*, Otto TD, Oberstaller J, Liao X, Adapa SR, Udenze K, Li S, Bronner IF, Casandra D, Mayho M, Brown J, Li S, Swanson J, Rayner JC, Jiang RHY, **Adams JH**. (2018) Uncovering the

essential genome of the human malaria parasite *Plasmodium falciparum* by saturation mutagenesis. *Science,* 360, eaap7847. DOI: 10.1126/science.aap7847. *Contributed equally.

d. Hart KJ, Oberstaller J, Walker MP, Kennedy MF, Padykula I, **Adams JH**, Lindner SE. *Plasmodium* Transmission is Critically Regulated by CAF1/CCR4/NOT Deadenylase Complex Assembly and Function. *PLoS Pathogens,* Jan 31 2019; 15(1):e1007164. PMCID: PMC6355032

Complete List of Published Work in My Bibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/john.adams.1/bibliography/40755598/public/?sort=date&direction=descending or ORCID http://orcid.org/0000-0003-3707-7979

D. Research Support

Ongoing Research Support

R01AI064478 (PI: Adams) 08/01/2006 – 03/31/2024
NIH/NIAID
Immunological characterization of the P. vivax DBP
The goal of this project is to promote anti-vivax vaccine development by defining Duffy binding protein ligand domain (DBPII) epitopes capable of eliciting a strain-transcending antibody inhibition.

R01AI117017 (MPI: Adams, Ferdig) 02/10/2015 – 01/31/2021
NIH/NIAID
Chemogenomic Profiling of Plasmodium Falciparum Drug Responses and Resistance
This project will use a chemogenomic systems approach to define critical pathways linked to ART-R, understand mechanisms of action of ART and other antimalarial partner drugs, and predict drug combination therapies with optimal synergistic anti-parasite activity to minimize the emergence of resistance.

R01AI130171 (PI: Adams) 06/15/2018 – 05/31/2022
NIH/NIAID
Discovering the essential genome of Plasmodium falciparum
This project, will identify genes essential for blood-stage growth and sexual development to prioritize therapeutic targets and provide a large collection of genetic mutant to the research community.

R01AI137162 (PI: Goldberg) 02/01/2018 – 01/31/2023
Washington University/NIAID
Structural vaccinology and design of novel immunogens for malaria vaccine development
Aims: 1) Design pre-erythrocytic and transmission-blocking vaccines, 2) immunogens to focus responses to neutralizing epitopes in blood-stage parasites, 3) design a multi-stage, cross-species protective immunogen.
Role: Co-Investigator

R21AI149730 (PI: Adams) 05/22/2020 – 04/30/2022
NIH/NIAID
Evaluation of ivermectin as an antimalaial therapy against P. falciparum liver stage
The objective of this study is to determine if Ivermectin MDA, when combined with other antimalarial drug treatments, could be effective in eliminating *P. falciparum* transmission, aiding in the malaria eradication initiative.

[redacted] (PI: Adams, Roth, Vega-Rodriguez) 09/01/2019 – 08/31/2021
Tres Cantos Open Lab Foundation
[redacted]
This project offers an advance in antimalarial drug discovery by targeting pre-erythrocytic stages to block malaria infection.

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

PHS 398 OTHER SUPPORT

LAUFER, MIRIAM K.

ACTIVE:

U01AI089342 (PI: Laufer) 08/01/2011 – 07/31/2021 (NCE)
NIH/NAID $[redacted]
Clinical trial of trimethoprim-sulfamethoxazole or chloroquine in adults on ART
This project will guide Malawi health policy and ultimately affect the health of millions of ART recipients by providing evidence to determine whether to stop TS prophylaxis in person on ART and the importance of preventing HIV-associated opportunistic infections and malaria in persons who are stable on ART.

R01AI117734 (PI: Pasetti) 02/01/2015 – 01/31/2021 (NCE)
NIH NIAID $[redacted]
Vaccines and maternally acquired immunity to prevent shigellosis in children
This study seeks to understand the mechanisms by which maternal immunity prevents shigellosis in young infants and identify a novel broadly protective vaccine for toddlers and young children.
Role: Co-Investigator

K24AI114996 (PI: Laufer) 01/11/2016 – 12/31/2020
NIH $[redacted]
Mentoring and patient-oriented research in malaria
This grant is designed to support mentorship of the next generation of translational, patient-oriented malaria researchers in the United States and Malawi and also to allow the applicant to acquire new skills and knowledge to expand her research capacity. With the benefit of this training and support, the applicant and her trainees will develop new strategies to help prevent, treat and eventually eradicate malaria.

D43TW010075 (MPI: Laufer and Mathanga) 05/10/2016 – 02/29/2021
NIH $[redacted]
Interdisciplinary malaria research training in Malawi
This proposal will train the essential key experts who are necessary to establish an interdisciplinary team of investigators capable of leading scientifically innovative and public health-relevant malaria research in Malawi.

U01HD092308 (PI: Cairo/Laufer) 06/07/2017 – 05/31/2022
NIH $[redacted]
The impact of in utero HIV exposure on infant T and B cell responses in Malawi
This study is a longitudinal analysis of T and B cell subsets in HUE infants from birth to 9 months of age, to assess the relationship between viremia below detectable levels before conception and immunologic alterations in infants.

U19AI089683 (PI: Taylor) 07/01/2017 – 03/31/2024
NIH/Michigan State University Subaward $[redacted] (subaward)
Malawi International Center of Excellence in Malaria Research
The Intransigence of Malaria in Malawi: Understanding Hidden Reservoirs, Successful Vectors and Prevention Failures.
This award seeks to understand the persistence of malaria in Malawi. Pertinent to the research proposed are studies to systematically characterize human to mosquito transmission of P. falciparum.
Role: Co-Investigator

R61HD103066 (PI: Laufer/Gladstone) 07/01/2020 – 06/30/2025
NIH/NICHD $[redacted]

"Long-term neurocognitive outcomes of HIV-exposed uninfected children". We will follow infants born to mothers with HIV infection to determine the impact of HIV and antiretroviral exposure on neurocognitive development with the goal of identifying the children at highest risk of neurocognitive delay in long term follow up. Our results will guide the future development of interventions to improve the well-being of children with HIV- exposure throughout the world.

R01HD100235 (PI: Laufer) 07/01/2020 – 06/30/2025
NIH/NICHD $[redacted]
Neurocognitive development of HIV-exposed and uninfected infants in Malawi
This study will provide detailed evidence to identify the highest risk children within this group and to guide the future development of interventions to improve the well-being of children who are HIV-exposed in sub-Saharan Africa and throughout the world.

PENDING:
R21AI156297 (PI: Laufer/Mzilahowa) 09/01/2020 – 08/31/2022
NIH/NIAID $[redacted]
Characterizing malaria vector competence among natural populations of Anopheles
This study aims at determining the natural variation of malaria vectors Anopheles *funestus* and Anopheles *arabiensis* in modulating *Plasmodium falciparum* development.

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

PHS 398 OTHER SUPPORT

TAKALA-HARRISON, SHANNON

ACTIVE:

R01AI125579 (PI: Takala-Harrison) 02/03/2017 – 1/31/2022
NIH $[redacted]
Identification and validation of molecular markers of piperaquine resistance
Objectives: To identify specific genes and polymorphisms within these regions of the *P. falciparum* genome and to validate these candidate resistance markers in the field and in the laboratory.

1R01AI145852 (PI: Harrison/O'Connor) 04/01/2020 – 03/31/2025
NIH/NIAID $[redacted]
Genomic and geospatial analysis of malaria parasite migration to inform elimination
The association between estimated local human travel patterns and parasite migration patterns will be assessed and will facilitate identification of segments of the travel network that coincide with regions of high parasite migration that can be used to define geographical units for targeting elimination interventions.

R01AI141900 (PI: Carneiro Da Silva) 01/15/2019 – 12/31/2023
NIH/NIAID $[redacted]
Genome-wide sieve analysis and immunological validation to identify targets of protective efficacy in field trials of a whole-organism malaria vaccine
In the proposed work, we will generate and compare parasite whole genome sequence data from the vaccine and control arms of field efficacy trials of PfSPZ Vaccine to identify parasite loci that are the target of the vaccine-induced protective efficacy, and to determine the potential impact of geography and time on vaccine efficacy.
Role: Co-Investigator

U19AI110820A (PI: Rasko) 04/15/2014 – 03/31/2024
NIH/ NIAID $[redacted]
Genomics-based investigation of the determinants of polymicrobial infectious disease outcomes
Objectives: Genomic studies of the impact of external factors on parasite development and disease outcome
This project examines the impact of co-infection on parasite development and disease outcome for tropical parasitic diseases.
Role: Co-Investigator

HHSN272201300022I (PI: Kotloff) 08/25/2014 – 05/31/2021
NIH/NIAID $[redacted]
Vaccine Treatment and Evaluation Unit (VTEU) Overall Administration, Clinical Operations Support and Concept and Protocol Development, Implementation and Assays
Task Order DMID 17-0081FY.2018.A1B1C1D1.0077
Role: Co-Investigator

[redacted] (PI: Kotloff) 10/10/2018 – 09/30/2022
Bill & Melinda Gates Foundation $[redacted]
[redacted]
Purpose: To assess the child mortality impact of various azithromycin intervention packages to inform future program design in Mali and sub Saharan Africa
Role: Co-Investigator

PENDING:

R21AI151459 (PI: Valim) 09/01/2020 – 08/31/2022
NIH/NIAID $[redacted]
Longitudinal Antibody Profile Correlated with Protection from Malaria in Malawi
This study seeks to understand the impact of parasite genetic diversity on the development of functional antibody responses in a cohort of participants in Malawi.

Role: Co-Investigator

R21AI156297 (PI: Laufer/Mzilahowa) 09/01/2020 – 08/31/2022
NIH/NIAID $[redacted]
Characterizing malaria vector competence among natural populations of *Anopheles*
This study aims at determining the natural variation of malaria vectors A*nopheles funestus* and *Anopheles arabiensis* in modulating *Plasmodium falciparum* development.
Role: Co-Investigator

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

PHS 398 OTHER SUPPORT

CUMMINGS, MICHAEL P.

ACTIVE

[redacted] (PI: Cummings) 08/15/2017 – 07/31/2021
NSF $[redacted]
[redacted]
This project is for continued development of the BEAGLE library, a high-performance likelihood calculation platform for evolutionary models. This research reformulates the BEAGLE library and its API to focus on consolidating more capabilities into a single BEAGLE instance through several research initiatives.

[redacted] (PI: Cummings) 06/01/2020 – 02/28/2021
NSF $[redacted]
RAPID: Accelerating Phylodynamic Analyses of SARS-CoV-2
[redacted]

PENDING

21010156 (PI: Cummings) 10/01/2020 – 09/30/2023
UMB/NIH $[redacted]
Assessing patterns of seroreactivity for antigens of SARS-CoV-2 and other human coronaviruses
The objective of the project is to measure IgG and IgA antibody responses to SARS-CoV-2 epitopes and assess the degree of cross-reactivity amongst seasonal coronaviruses and SARS-CoV-2 variants.

20116204 (PI: Cummings) 04/01/2021 – 03/31/2026
UMB/NIH $[redacted]
Identification and Characterization of multi-stage vaccine targets with potential for cross-species protection against two major *Plasmodium* species
The objective of the project is to use data analytical approaches to identify potential vaccine candidates for two parasite species causing malaria, and to evaluate the top candidates in the laboratory experiments.

[redacted] (PI: Pop) 02/01/2021 – 01/31/2024
NSF $[redacted]
[redacted]
The objective is to develop program that exposes students to research within bioinformatics and computational biology, called BRIDGE (**B**ioinformatics **R**esearch **I**n **D**ata science for **Ge**nomics). Ten students will receive training and mentorship for ten weeks each summer and will be jointly advised by members of the University of Maryland Center for Bioinformatics and Computational Biology (CBCB).
Role: Co-Investigator

BUDGET JUSTIFICATION

KEY PERSONNEL

DeAnna Friedman-Klabanoff, M.D., Principal Investigator (9.6 calendar months). Dr. Friedman-Klabanoff is a pediatric infectious disease specialist and has been conducting malaria research looking at vaccine-induced and naturally acquired immunity to *Plasmodium falciparum* in both clinical and epidemiological studies for the past 3 years. She will be an Assistant Professor at the University of Maryland School of Medicine, Department of Pediatrics, Division of Infectious Diseases and Tropical Pediatrics, and faculty member in the Malaria Research Program in the Center for Vaccine Development and Global Health.

Dr. Friedman-Klabanoff will be responsible for overall conduct of study activities, including protocol development and implementation, microarray design, management of research staff, analysis of peptide microarray data, and performance and analysis of *in vitro* pre-erythrocytic assays. Dr. Friedman-Klabanoff will additionally assist with study activities and progress of upcoming ICEMR cohorts through field visits to Malawi and maintain regular communication with the study team and collaborators through teleconference calls and emails. Dr. Friedman-Klabanoff will present the results of this project at national and international meetings.

Fringe benefits: Fringe benefits are calculated at 25.1% of salary for faculty.

RESEARCH SUPPORT EXPENSES

SUPPLIES

The research supplies budget includes the costs of data collection supplies, laboratory supplies, sample shipping, protein expression, purchase of adjuvant, rabbit immunizations, *in vitro* liver assays, server fees, bioinformatics support, and software usage. These costs are budgeted based on an approximate range of $[redacted] per year. Microarray design and probing costs are not included in the budget because these are being funded through other sources.

TRAVEL

Malawi. Dr. Friedman-Klabanoff will travel to Malawi once per year for one month in Years 1-5 with support from this grant to work with field study teams for sample and data collection and meet with Dr. Don Mathanga and other collaborators to discuss the progress and results of this study, as well as discuss manuscripts and next grant applications. ($[redacted] in Years 1-5).

University of South Florida. Dr. Friedman-Klabanoff will travel to Tampa one month in Year 2 and one month in Year 3 with support from this grant for training in performing and analyzing the *in vitro* pre-erythrocytic assays described in the Research Strategy. Two trips are needed by nature of the assays: the first would be to learn to perform and analyze the *in vitro* assay through early liver stages and the second would be to learn to perform the assay through blood stage breakthrough ($[redacted] in Years 2&3).

ASTMH annual conference. The travel budget will support attendance of the annual American Society of Tropical Medicine and Hygiene conference each year. Dr. Friedman-Klabanoff will present ongoing results of the project at this conference. The duration of each trip is estimated to be five days. ($[redacted] per year).

Additional conferences. We are also requesting funds for the Gordon Research Conference in Years 1 and 3. (GRC $[redacted] in Years 1 and 3)

Leadership training. The budget also includes funding for Dr. Friedman-Klabanoff to attend the AAMC Early Career Women Faculty Leadership Development Seminar and the Advanced Course of Vaccinology in Year 5. ($[redacted] for AAMC and $[redacted] for ADVAC in Year 5)

OTHER

Publication costs. Funding is requested to cover publication costs in scientific peer-reviewed journals. ($ [redacted] in Years 3 and 4, $ [redacted] in Year 5)

Computer and software. Funding is requested for a laptop computer in Year 1 and a desktop computer in year 4, including relevant software ($[redacted] in Years 1 and 4).

Textbooks. We are requesting funds for textbooks and online and in-print journal subscriptions. ($ [redacted] per year).

Tuition and fees. Funding is requested for the coursework described in the Career Development Plan, to occur at UMB, ADVAC, AAMC, and online. ($[redacted] in Year 1; $931 in Year 2; $[redacted] in Year 5).

F&A COSTS

An MTDC rate of 8% has been applied, as allowed in the K23 grant mechanism.

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OMB Number: 0925-0001

Expiration Date: 02/28/2023

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

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

● Yes ○ No

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

2. *Program Income Section

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

○ Yes ● No

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

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

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3. Human Embryonic Stem Cells Section

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

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

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

Cell Line(s) (Example: 0004):

4. Human Fetal Tissue Section

*Does the proposed project involve human fetal tissue obtained from elective abortions? ❍ Yes ● No

If "yes" then provide the HFT Compliance Assurance

If "yes" then provide the HFT Sample IRB Consent Form

5. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ❍ Yes ❍ No

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

*Previously Reported: ❍ Yes ❍ No

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:

PHS 398 Career Development Award Supplemental Form

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

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	PHS_Career_IntroductionToApplication.pdf
Candidate Section	
2. Candidate Information and Goals for Career Development	PHSCareer_CandidateInformationAndGoals.pdf
Research Plan Section	
3. Specific Aims	PHS_Career_SpecificAims.pdf
4. Research Strategy*	PHS_Career_Res_Strategy.pdf
5. Progress Report Publication List (for Renewal applications)	
6. Training in the Responsible Conduct of Research	PHS_Career_Training_Resp_Conduct_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)	PHS_Career_Mentor_Statements_Letters.pdf
9. Letters of Support from Collaborators, Contributors, and Consultants	PHS_Career_SupportLtrs.pdf
Environment and Institutional Commitment to Candidate Section	
10. Description of Institutional Environment	PHS_Career_Inst_Environment.pdf
11. Institutional Commitment to Candidate's Research Career Development	PHS_Career_Inst_Commitment.pdf
12. Description of Candidate's Contribution to Program Goals	
Other Research Plan Section	
13. Vertebrate Animals	PHS_Career_VertebrateAnimals.pdf
14. Select Agent Research	
15. Consortium/Contractual Arrangements	
16. Resource Sharing	PHS_Career_Resource_Sharing_Plan.pdf
17. Authentication of Key Biological and/or Chemical Resources	PHSCareer_KeyBioAndOrChemResources.pdf
Appendix	
18. Appendix	

PHS 398 Career Development Award Supplemental Form

Citizenship*:

19. U.S. Citizen or Non-Citizen National?* ● Yes ❍ No

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

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

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

Tracking Number: GRANT13242170 Funding Opportunity Number: PA-20-205. Received Date: 2020-11-11T16:27:19.000-05:00

INTRODUCTION

I am pleased to submit a thoroughly revised proposal of K23 AI155838 entitled "Serological markers of natural immunity to *Plasmodium falciparum* infection" that addresses each of the key concerns raised by the reviewers, with overarching critiques addressed below. In addition, significant revisions have been indicated with a line on the left margin of the proposal.

Critiques from Resume and Summary of Discussion:

Critique: Addition of an immunologist to the mentoring team is needed. Response: I have described in more detail the pertinent expertise of Dr. Berry. I have also added Dr. John Adams to my advisory committee, a recognized expert in the evaluation of the functional capacity of pre-erythrocytic antibodies to *Plasmodium* spp. using *in vitro* liver model assays (details in Career Development Plan).

Critique: With regard to the machine learning component, the applicant should acquire and run validation and testing sets before moving on to the mouse model. Response: The Random Forest method I will use integrates separate training and testing sets by the nature of the bootstrap sampling (details in Research Strategy). I have also added verification of the model into Future Directions.

Critique: Prioritization of positive candidates…missing from the research plan. Response: The training plan and approach for Aim 2 now include rabbit immunization, ELISA, and *in vitro* assays to assess the functionality of antibodies to pre-erythrocytic epitopes to inform prioritization (details in Training Plan & Research Strategy).

Critique: Appropriate controls…missing from the research plan. Response: Additional detail about the control samples was added. Control samples are from malaria-naïve volunteers who serve as controls for many protein and peptide microarray projects to allow for comparison and control of interarray variability (details in Research Strategy). While I agree that it would be preferable to have the control samples come from the same population as the participants, no population in Malawi can be considered malaria naïve.

*Critique: Inadequate description of the study site and population in Malawi...*Response: Additional details have been added to the Research Strategy, Facilities and Other Resources, and Institutional Environment.

Critique: word "may" was problematic in resource sharing plan. Response: This was removed.

Critiques from Individual Reviewers:

Critique (Rev. 1): Not completely clear how many peptides will be tested. Define exactly what a broadly protective response is. Sample size is defended, though…taking this into account may be needed and a larger sample size. Response: Additional details regarding the peptide array, definitions, additional methods of analysis and considerations for increasing sample size have been added to the Research Strategy.

Critique (Rev. 1): Is there a fold change…that would be considered biologically significant? Response: Currently, the literature shows no specific antibody level that correlates with protection from malaria. However, because of the sample size and number of peptides tested on the peptide array, I will generally be detecting large differences in antibody responses.

Critique (Rev. 2): Several of the candidate antigens may be immunogenic…but seem implausible as targets…for protective vaccines. Response: I have edited the list of proteins included on the peptide array based on this critique to remove proteins such as DNA ligases, DNA repair proteins, etc.

Critique (Rev. 2): Biosketches not included for advisors. No documentation of the pertinent immunology expertise of Dr. Berry. Response: Biosketches are now included for Drs. Berry, Neuzil and Adams. Additional documentation of Dr. Berry's pertinent expertise has been added to the Career Development Plan.

Critique (Rev. 3): The collection of sufficient samples appears to be a potential issue…time-consuming…may affect timeline. Response: The first round of mosquito collections is complete, and analysis is pending to determine whether I have the desired sample size. Additional human and entomological samples are possible if additional participants are needed (details in Timeline).

Critique (Rev. 3): Different variables and controls that may change the results are not introduced. Response: A more detailed description of covariates and controls were added to the Research Strategy.

Critique (Rev. 2 and 3): Antibodies to epitopes that are post-translationally modified could…be undetected….there is little consideration of alternative approaches. [O]nly linear protective epitopes will be identified…the role of cell-mediated immunity will not be characterized…[and] may also play a role. Response: I have added additional detail to the limitations and added alternative approaches into the Approach for Aim 1 in the Research Strategy. Future directions will incorporate cell-mediated immunity in collaboration with our immunology colleagues.

Critique (Rev. 2 and 3): Publication record. Response: My manuscript describing peptide array analyses I performed (see Preliminary data) has been published in *Journal of Infectious Diseases*, supporting the feasibility of finding significant differences in humoral responses due to natural immunity using peptide array technology.

CANDIDATE STATEMENT

The research described in this proposal builds on skills I developed during my previous training and will help me to continue toward **my long-term career goal**: to become an independent clinical and translational researcher dedicated to the development of a highly efficacious malaria vaccine.

Prior Research Experience

In college, I pursued a B.S. in medical microbiology and immunology and gained experience in basic laboratory bench research. I first characterized *E. coli* heat shock proteins and then characterized genes involved in *Toxoplasma* cyst formation to generate non-cyst forming strains as candidate vaccine antigens. After gaining experience in basic laboratory research, I wanted to translate laboratory findings into clinically relevant applications through clinical research. During medical school, I conducted an epidemiological study in Mbarara, Uganda, on vitamin D deficiency in adults with HIV and HIV-TB coinfection. Through this work, I gained experience in recruiting study staff, designing data collection tools, and analyzing and interpreting study findings. My experiences in college and in Uganda motivated me to pursue a career in pediatric infectious disease and global health research. To gain additional intensive experience in tropical infectious diseases after residency, I spent a year in Rwanda attending on wards and co-coordinating the medical student pediatrics rotation. After seeing so many children suffer devastating complications from malaria, I was motivated to pursue malaria vaccine research. In summary, my interest in vaccinology and infectious disease research in resource-limited settings grew from my bench research experience, my experience doing research in Uganda, and my clinical experience in Rwanda, and led me to apply for pediatric infectious disease fellowship.

Current Position and Research

In my current research, I work with Dr. Matthew Laurens to lead a Phase I trial of a candidate malaria vaccine that contains full length, recombinant circumsporozoite protein (rCSP). Through this project, I developed skills in study design, protocol development, and clinical trial implementation. However, I recognize the unique challenges and complexities of leading large international trials and want to expand my skills in study design, leadership and implementation. I have been working with Dr. Miriam Laufer on professional development skills since 2016 and chose her as a primary mentor for this project given her extensive expertise leading translational and clinical malaria research activities in Malawi.

During fellowship, I also developed a sub-study to assess vaccine-induced epitope-specific antibody responses elicited by the rCSP vaccine using peptide microarrays. To gain experience with the analysis of peptide microarray data, I analyzed responses to CSP in a small sample of children and adults in Mali, and the manuscript describing this work is in press at *Journal of Infectious Diseases*. While analyzing this small segment of a large data set, I became interested in the application of regression models and data science approaches to large data sets with limited sample sizes. This led me to ask Drs. Shannon Takala Harrison and Michael Cummings to serve as co-mentors for this proposal because of their expertise in genomic epidemiology and computational biology, respectively. As more research has also pointed to the importance of not only the epitope specificity but also the function of the antibodies in immunity to malaria, I have also developed an interest in learning techniques to evaluate antibody function. Dr. Harrison introduced me to Dr. John Adams, an expert in the use of *in vitro* models to study pre-erythrocytic immunity to *Plasmodia* sp., who will train me to use the models and will be on my advisory committee.

During the last three years, I have also pursued formal didactic research training to support my hands-on experience with my mentors. I am pursuing a Master of Science in Clinical Research and have completed nine courses toward this degree. With the support of this career development award, I plan to finish the coursework and prepare my Master's thesis in Spring of 2022.

Academic/Professional Achievements/Honors

During my fellowship, I compiled and analyzed data from the 47-year experience of Controlled Human Malaria Infection (CHMI) at the University of Maryland CVD. This work led to a poster presentation at the 2018 National Foundation for Infectious Diseases Annual Conference on Vaccinology Research, an oral presentation at the 2018 American Society of Tropical Medicine and Hygiene Annual Meeting, and a publication in the *American Journal of Tropical Medicine and Hygiene.*[1] Due to the complexity of and delay in the start of the rCSP vaccine trial, we are just now at the stages of interim analysis. A manuscript describing the interim safety and immunogenicity results from the rCSP vaccine trial is currently under review. I presented the results of my work describing humoral responses to CSP in children and adults in Mali in an oral presentation at the 2019 ASTMH Annual Meeting and a manuscript describing this work is currently in press in *Journal of Infectious Diseases*.

With mentorship from Dr. Laufer, I secured funding to lay the groundwork for this proposal. I successfully competed for two national foundation awards: a Burroughs-Wellcome/ASTMH Post-doctoral Fellowship in

Tropical Infectious Diseases and the Pichichero Family Foundation Vaccines for Children Initiative Research Award. I have now been able to travel to Malawi to visit the field sites and participate in protocol development.

The training and mentorship detailed in this K23 application will provide me with the additional training and skills necessary to lead international projects, analyze large datasets using biostatistics and data science approaches, and use *in vitro* assays to evaluate the function of antibodies to pre-erythrocytic *P. falciparum* proteins. With these skills, I will develop a unique research niche and transition to independence. I will have 75% of my time committed to the research proposed in this application, with the remaining 25% of my time split between clinical service and further clinical and translational vaccinology projects.

CAREER GOALS AND OBJECTIVES

My long-term career goal is to become an independent investigator dedicated to the development of a highly efficacious malaria vaccine, combining clinical and translational research skills to inform vaccine development and utilizing both domestic and international trials in pursuit of this goal. Through my prior research experiences and formal research training, I have a strong background in domestic clinical research, small international observational studies, and experience analyzing smaller peptide microarray data sets. However, to progress into a productive and innovative career, I require skills in implementing and leading larger international projects, utilizing computational biology and *in vitro* functional assays to complement our clinical research. For this proposal, I have the following **short-term goals**:

1. Implementing and leading international projects, training and supervising diverse international teams
2. Applying computational biology techniques to the analysis of peptide microarray data
3. Using biostatistical methods such as multivariable regression to explore the effects of covariates on clinical outcomes
4. Acquiring skills and expertise in assessment of pre-erythrocytic antibody function for *Plasmodium* sp.
5. Developing skills needed for a successful academic research career, including grant-writing, manuscript preparation, scientific presentation skills, and leadership skills

I will gain this expertise through a combination of structured coursework, independent study, and instruction and feedback from my mentors, as detailed in my Career Development Plan.

CAREER DEVELOPMENT PLAN

Formal Coursework and Leadership Training

Formal coursework in clinical and translational research methods will be taken for credit towards the Master of Science in Clinical Research program administered by the Department of Epidemiology and Public Health at the University of Maryland. Proposed coursework will be completed through the University of Maryland, Baltimore, and the University of Maryland, College Park, and are listed in **Table 1.** This didactic instruction will advance my ability to conduct and analyze clinical and translational research.

Table 1: Proposed coursework

Course number	Title	Term	# Credits	Objectives	Related Goal(s)
PREV 721	Regression Analysis	Fall 2021	2	Improve ability to utilize and interpret regression models	3
BISI 678Z	Rotations in Biological Sciences	Fall 2021	3	Learn the theory and practice of machine learning and computational biology	2
PREV 801	Longitudinal Data Analysis	Spring 2022	3	Learn to use marginal and mixed effects models for data analysis	3
PREV 718	Programming for Bioinformatics	Spring 2022	1	Improve skills in scripting and programming in bioinformatics	2
CIPP 909	Responsible Conduct of Research	Spring 2023	1	Stay current on topics related to responsible conduct of research	1, 5

Mentorship

Primary mentors and co-mentors **(Table 2):**

Dr. Miriam Laufer will be my primary mentor for this project. She is the Director of the Malaria Research Program (MRP) at the CVD and Professor of Pediatrics, Medicine, Epidemiology and Public Health. Dr. Laufer is internationally recognized for her work in malaria epidemiology and elimination, has worked in Malawi since 2002 and is known for her excellent mentorship. Dr. Laufer and I have already had a productive mentoring relationship that has led to multiple grant awards that have supported the planning phases of the proposed research in Malawi. We will meet weekly during the award period, either in person or via videoconferencing, to discuss research progress and my career development goals. Dr. Laufer and I will also work closely together to write

manuscripts and propose new research with the data generated from this research and additional upcoming ICEMR cohort studies.

Dr. Shannon Takala Harrison will serve as a co-mentor on my project. She is the Head of the Genomic Epidemiology Unit in MRP at the CVD and Associate Professor of Medicine, Epidemiology and Public Health. Dr. Harrison is an internationally recognized expert in understanding how malaria parasite diversity affects drug resistance and vaccine effectiveness. We will meet monthly in Year 1 to discuss data analysis, career development goals, and my progress in my coursework. Beginning in Year 2, we will meet biweekly to discuss my progress in data analysis, my findings, and the conclusions I am making based on the findings. Dr. Harrison will also provide intensive mentoring and guidance on scientific writing.

Dr. Michael Cummings will also serve as a co-mentor on my project. He is the Director of the Center for Bioinformatics and Computational Biology and Professor of Biology and Advanced Computer Studies and has expertise in the use of informatics to identify features associated with clinical characteristics and drug resistance in malaria and to predict variation in basic immune system functions at the molecular level. Dr. Cummings will provide me with training and mentoring in machine learning and computational biology. In the first semester of the award, I will complete an independent study course with Dr. Cummings. Specific training components and activities include background on theory and underlying analytical methods, experimental/analytical design activities, assessment of technical competencies, and extensive practice with analyses and interpretation. We will meet biweekly throughout the project, initially focusing on my progress on the independent study, then transitioning to mentoring on analysis of the data from the proposed project.

To synthesize feedback from all my mentors, we will meet as a team twice yearly to discuss the progress of the project and next steps. The experience and expertise of these three mentors will help to ensure my success in my planned research and my research career overall.

Advisory Committee **(Table 2):**

Dr. Kathleen Neuzil will serve as an advisor. She is Director of the CVD, the Myron M. Levine Professor in Vaccinology, and Professor of Medicine and Pediatrics. We will meet at least four times per year – twice individually and twice as part of the advisory committee meetings – to discuss my career development.

Dr. Andrea Berry will also serve as an advisor. She co-leads the Immunoepidemiology and Pathogenesis Unit in the MRP at the CVD and Assistant Professor of Pediatrics and Medicine and is one of only a handful of malaria researchers with expertise in designing, analyzing, and interpreting peptide array studies. She led the design and piloting of the diversity reflecting peptide microarray that our lab now utilizes. Dr. Berry has been mentoring me on peptide microarray project design and analysis since 2016 and will continue to do so throughout this award. We will meet at least monthly to discuss microarray design and analysis.

Dr. John Adams will serve as an advisor and provide training in the use of novel *in vitro* liver models to study antibodies to the pre-erythrocytic stages of *Plasmodium* sp for epitope identification and vaccine optimization. He is Distinguished University Professor in the College of Public Health's Center for Global Health and Infectious Disease Research at the University of South Florida (USF). I will complete two month-long rotations in Dr. Adams' lab at USF to learn to perform, analyze and interpret the *in vitro* assays through early liver stages and then through blood stage breakthrough. We will meet at least monthly to discuss study design and plan the rotation and then data analysis and interpretation when the data is available.

Table 2: Proposed mentors and advisors, training, and timetable

Mentor/Advisor	Training	Meeting frequency	Related Goal(s)
Miriam Laufer, M.D., M.P.H.	Project implementation and leadership, ensuring adequate data collection at field sites, training and supervising diverse international teams, scientific writing	Weekly individual meetings. Biannual mentor group meetings (Q1 and Q3). Biannual advisory committee meetings (Q2 and Q4)	1, 5
Shannon Takala Harrison, Ph.D.	Molecular epidemiology methods, including regression models, scientific writing	Monthly individual meetings initially, then biweekly starting in Y2. Biannual mentor group meetings (Q1 and Q3). Biannual advisory committee meetings (Q2 and Q4)	3, 5
Michael Cummings, Ph.D.	Computational biology and machine learning techniques	Biweekly individual meetings. Biannual mentor group meetings (Q1 and Q3). Biannual advisory committee meetings (Q2 and Q4)	2, 3

Kathleen Neuzil, M.D., M.P.H.	Career development, leadership and networking, research portfolio management, scientific writing	Biannual individual meetings (Q1 and Q3). Biannual advisory committee meetings (Q2 and Q4)	1, 5
Andrea Berry, M.D.	Peptide microarray design and analysis, interpretation of results	Monthly individual meetings. Biannual advisory committee meetings (Q2 and Q4)	2, 3
John Adams, Ph.D.	*In vitro* functional antibody studies, scientific writing	Monthly individual meetings. Biannual advisory committee meetings (Q2 and Q4)	4, 5

My overall career development advisory board will meet twice yearly for feedback regarding the project design and analysis, career development, project timelines, project management, and future project development. They will evaluate my progress and proficiency in my career development goals through review of my research progress, progress in coursework, manuscripts, and presentations.

Additional Professional Development

During the period of this award, I will attend the K Club series, which focuses on tips for transitioning from a K to an R, time management, how to give an elevator talk, etc. I will also participate in our Scientific Writing Accountability Group to increase my writing productivity as a junior faculty member. Data generated from this proposal will build toward an R01 that will further characterize epitopes of interest identified as potential next-generation vaccine candidates. To assure the success of my R01 award proposal, I will utilize resources available at the University of Maryland, such as the Grant Writing workshops within our Office of Research Career Development and the “Writing Your First R01” series to assemble a competitive proposal. In Year 5 of this proposal, I will attend the Association of American Medical Colleges (AAMC) Early Career Women Faculty Leadership Development seminar to gain leadership skills in academic medicine, including communication, strategic planning, negotiation. I will also attend the Advanced Course of Vaccinology (ADVAC) in Year 5 to acquire leadership and decision-making skills in the field of vaccinology and network with leaders in the field.

Meetings and Conferences

As part of my planned research project, I anticipate that I will publish a first author manuscript for each of the aims and write 1-2 review articles. Scientific seminars and institutional meetings will include the weekly Malaria Research Program meetings, as well as in the weekly Immunoepidemiolgy and Pathogenesis Unit meetings, where I will present updates on my research project, discuss results, refine plans for analyses, and develop further research plans at least biannually. I will also prepare abstracts to present at the American Society of Tropical Medicine and Hygiene and Infectious Disease Society of America annual meetings, both held annually in the fall, as well as the Gordon Research Conference in Y1 and Y3 and the Molecular Approaches to Malaria conference in Y4, allowing me to network with other researchers in malaria vaccinology.

TIMELINE

Formal coursework will be completed in Years 1 and 2, and leadership training will happen in Year 5. *In silico* analysis will happen in Years 1 and 2 and immunologic assays will be conducted in Years 2 and 3. Data analysis and dissemination of results will occur in Years 2-5 of this study. I will preparation an R01 in Years 3 and 4, and plan for possible resubmission Year 5 (indicated by the lighter gray shaded boxes), if needed.

Task	Year 1		Year 2		Year 3		Year 4		Year 5	
Formal coursework										
Leadership training										
Specific Aim 1										
Mosquito collection										
Human infection follow up										
Identification of study subjects										
Microarray analysis										
Dissemination and manuscripts										
Specific Aim 2										
In silico analysis										
In vitro analysis										
Data analysis										
Dissemination and manuscripts										
Transition to an Independent Researcher										
Preparation of R01									If needed	
Submission of R01										If needed

SPECIFIC AIMS

Developing an effective vaccine against *Plasmodium falciparum*, the most common and deadly cause of malaria, is a global priority. After a *Plasmodium*-infected *Anopheles* mosquito bite, sporozoites travel to the liver, invade and replicate. They then emerge into the circulation to invade erythrocytes, causing blood-stage infection and disease. In most high transmission settings, individuals acquire immunity to blood-stage infection after repeated exposure to the parasite. A major challenge in malaria vaccine development is an incomplete understanding of naturally acquired immunity to the early, pre-erythrocytic stages of *P. falciparum* infection.

Several limitations have impaired prior efforts to characterize naturally acquired pre-erythrocytic immunity. One obstacle lies in the difficulty of accurately classifying exposure in an endemic region. Because exposure to parasite-infected mosquito bites is heterogeneous, even within small geographic areas, epidemiological studies cannot distinguish individuals who are exposed to an infectious mosquito bite and protected from blood-stage infection from unexposed individuals. We also have little information about immunity to diverse parasite proteins.

P. falciparum surface proteins have evolved extensive genetic diversity to evade the immune system. Many prior studies of humoral immunity have focused on one laboratory-adapted strain, 3D7, which is not representative of the parasite diversity found in natural infections. Another challenge is distinguishing precise epitopes associated with protection. Many *P. falciparum* surface proteins have immunodominant regions that elicit high antibody titers but correlate more strongly with exposure than with protection. Using whole protein methods may result in high antibody binding to immunodominant regions at the expense of masking lower but differential binding to protective epitopes. A better understanding of naturally acquired immunity to diverse epitopes associated with protection from infection could lead to improved or novel vaccine candidates.

The **overall research goal** of this proposal is to use novel high-throughput tools to characterize naturally acquired humoral immunity to diverse pre-erythrocytic epitopes. With help from my mentoring team, I have designed a study combining individual level *P. falciparum* exposure and infection data collected from an NIH- funded study with original, diversity-reflecting peptide microarray analysis, *in silico* epitope prediction, and *in vitro* functional assays to identify functional epitopes associated with protection from *P. falciparum* infection.

Aim 1: Identify serologic responses associated with natural protection against *P. falciparum* infection.
Hypothesis: Sera from malaria-exposed children protected from blood-stage *P. falciparum* infection will recognize pre-erythrocytic epitopes that are not recognized by children who develop blood-stage infection.
Approach: Our ongoing cohort study in southern Malawi includes repeated household mosquito collection: speciated mosquitoes are assessed for blood meals and *P. falciparum* infection, and blood meal DNA microsatellite analysis identifies which humans in the household were bitten. Exposed children, those bitten by a *P. falciparum*-infected mosquito, will be classified as infected or protected based on active surveillance data. Serum from the day of exposure will be probed on a high-throughput diversity-reflecting peptide microarray that includes alleles from diverse, field-derived sequences to characterize pre-exposure immunity to pre-erythrocytic antigens. Using a combination of statistical approaches, including machine learning algorithms such as Random Forest, we expect to identify antibodies associated with protection from blood-stage infection.

Aim 2: Assess the function of antibodies targeting *P. falciparum* pre-erythrocytic antigens of interest.
Hypothesis: *In silico* epitope prediction tools and *in vitro* liver models will identify functional epitopes in pre-erythrocytic proteins that overlap with epitopes of interest identified in the microarray analysis.
Approach: *In silico* analysis using ABCPred and the Immune Epitope Database will be performed to identify B- and T-cell epitopes in pre-erythrocytic proteins to identify potential functional epitopes. Identified epitopes will be compared to results from Aim 1 and *P. falciparum* genomes from a global database to select ten proteins with potential functional and conserved epitopes for expression, generation of polyclonal antibodies, and functional studies in an *in vitro* liver model to evaluate effects on liver stage development and blood-stage breakthrough.

Expected outcomes include: 1) a method to identify *P. falciparum* pre-erythrocytic epitopes associated with protection from infection in a malaria-endemic setting and 2) a list of potential functional and conserved epitopes associated with naturally acquired immunity. The **overall impact** will be to apply the novel insight into the basis of naturally acquired humoral immunity to malaria to identify promising new vaccine candidates.

Through mentorship and training in international project management and leadership, analytical approac hes for large datasets including data science approaches, and *in vitro* functional antibody studies, I will be well positioned to achieve my overall career goal to become an independent global health researcher dedicated to the development of a highly-effective malaria vaccine, using clinical research and immunology to inform and optimize vaccine development. These results will build toward an R01 to further characterize identified epitopes of interest using targeted *in vitro* and *in vivo* functional studies to select the most promising novel malaria vaccine candidates for further development.

SIGNIFICANCE

Malaria remains a disease of substantial public health importance, causing approximately 228 million cases of illness and 405,000 deaths worldwide in 2018.[1] An effective malaria vaccine has the potential to make a pivotal change in malaria control and eradication and is a key priority for NIAID.[2,3] One challenge in the development of an effective malaria vaccine is the complex lifecycle of the parasite. First, a *Plasmodium*-infected *Anopheles* mosquito bites a person and injects sporozoites. The sporozoites travel to the liver, where they invade hepatocytes and replicate. Merozoites exit the liver into the bloodstream to invade erythrocytes, leading to blood- stage infection, which can be symptomatic or asymptomatic. People with asymptomatic infection can serve as reservoirs of ongoing transmission, as they do not seek care and do not get treated for malaria.

Barriers to vaccine development. For a vaccine to contribute significantly to malaria eradication, it must act on the early, pre-erythrocytic, part of the lifecycle, to block both symptomatic disease and asymptomatic infection. However, our understanding of naturally acquired immunity to the pre-erythrocytic stages of *P. falciparum* infection is limited due to several critical barriers:

Distinguishing protection from lack of exposure: *P. falciparum* exposure occurs when an individual is bitten by a *Plasmodium*-infected female *Anopheles* mosquito; quantifying exposure in individuals requires intensive surveillance. However, exposure to infected bites of *Anopheles* is not uniform, even within small geographic areas.[4] So, assuming that exposure is uniform throughout a community can lead to misclassification of participants as protected from infection when, in fact, they were never exposed.

Identifying correlates of protection: ELISA is generally limited to assessment of a few antigens and the signal seen is the cumulative signal over the entire antigen examined. But some malaria proteins have repetitiv e regions that induce high reactivity but correlate more with exposure to malaria than protection from infection. [5] For example, circumsporozoite protein (CSP) has an immunodominant central repeat region, but studies have identified radiation attenuated sporozoite vaccine-induced antibodies to the junction between Region 1 and the central repeat region that show potent neutralizing activity.[6,7] Studying humoral responses to small peptides within the protein will allow us to differentiate responses to multiple epitopes and could reveal antibody responses to non-dominant epitopes that correlate better with protection than the immunodominant responses seen with whole protein methods.

Genetic diversity of P. falciparum: *P. falciparum* surface proteins have evolved extensive genetic diversity as a means of evading the host immune response. Many prior studies examining humoral immunity have examined responses to only one variant of diverse antigens, usually the laboratory-adapted 3D7 parasite strain.[8]

Overcoming barriers. To address the first barrier, we will utilize samples from a cohort study in Malawi that included collection of blood-fed mosquitoes with mosquito blood meal matching to participants using human microsatellite markers. Children in the study were followed using monthly active and passive case detection, so we can determine which participants developed blood-stage infection after exposure to an infected mosquito. In this way, we will characterize exposure and subsequent blood-stage infection on an individual level. We will then examine humoral immunity to peptides spanning pre-erythrocytic antigens on the day of exposure on a peptide microarray designed using diverse, field derived sequences to address the last two barriers, identifying responses that are associated with protection from blood-stage infection.

Prioritization of epitopes: B- and T-cell epitope prediction tools can be used to examine the functional roles of proteins and/or epitopes of interest, and have been used for some malaria proteins and vaccine candidates.[9-12] The most advanced malaria vaccine candidate to date, RTS,S, is a truncated version of the 3D7 CSP that includes part of the central repeat region, as well as the C-terminal region, which has known T-cell epitopes. RTS,S has only modest efficacy in field trials, and this efficacy wanes over time.[13,14] A recent study found that predicted class II T-cell epitopes in the C-terminal region of 3D7 were clustered in the highly variable Th2R region, which correlates with the allele-specific efficacy seen in the Phase 3 trial of RTS,S/AS01 in children.[9,15]

Evidence is mounting that functional antibodies to *P. falciparum* pre-erythrocytic antigens are key to protection from malaria infection.[16-19] First-generation *in vitro* assays to evaluate functional inhibition of pre- erythrocytic stages suffered from significant variability and subjective manual counting.[20] Recently, researchers have developed a standardized high-throughput pre-erythrocytic assay with automated image analysis that uses commercially available materials.[21] By combining *in silico* epitope prediction tools and *in vitro* functional assays with the results from our peptide array analysis, we can validate potential protective epitopes and prioritize candidates for further *in vitro* and *in vivo* studies.

Rigor of prior work: Prior studies of naturally acquired humoral immunity have not yet found antibodies consistently associated with protection from infection. A study done in children in Western Kenya found no significant association between humoral responses to each of 163 proteins on a protein microarray and time to

infection after treatment.[22] Two other studies using weekly active surveillance done in Western Kenya found a prolonged time to and decreased risk of infection with malaria after treatment in adults and decreased risk of clinical malaria after treatment in children associated with high levels of antibodies to three pre-erythrocytic antigens by ELISA: the central repeat region of CSP, the repeat region of liver-stage antigen-1 (LSA-1), and thrombospondin-related adhesive protein (TRAP).[23,24] These studies examined responses to the 3D7 proteins only, which does not account for the parasite genetic diversity seen in the field, and assumed uniform exposure throughout the community, which is likely not the case.

Humoral immunity to pre-erythrocytic proteins has also been studied in the context of trials of whole sporozoite vaccines that used controlled human malaria infection (CHMI) to evaluate efficacy. These vaccines cause liver stage infection but cannot cause blood stage infection. In studies of immunity induced by whole sporozoite vaccines, up to 192 proteins have been associated with protection from infection after CHMI.[25-27] However, in these studies, the protein array consisted of entire proteins or large segments of 3D7-like proteins, not specific epitopes. In addition, the findings of these vaccine trials, which were conducted in malaria-naïv e individuals, may not be generalizable to malaria-experienced populations in endemic areas.

Using proteins identified in these studies to down select for potential protective pre-erythrocytic humoral responses, we will create a customized peptide microarray. We will include reference strains and previously sequenced, field-derived samples from Mali, Malawi, and Southeast Asia on the peptide array. We will probe sera from the day that children were bitten by an infected *Anopheles* mosquito and compare responses in those who are protected from blood-stage infection to those who are susceptible to blood-stage infection. This approach will allow us to identify naturally acquired humoral responses to epitopes associated with protection from infection.

The public health impact of this proposal will be to identify pre-erythrocytic targets of antibodies that prevent *P. falciparum* blood-stage infection to inform future vaccine development. Utilizing samples and data from a well-characterized cohort, we will identify *P. falciparum* pre-erythrocytic epitopes associated with protection from *P. falciparum* blood-stage infection (Aim 1) and use *in silico* and *in vitro* tools to identify the functional potential of these epitopes of interest (Aim 2). If successful, this research will yield possible new pre-erythrocytic vaccine candidates with the potential to protect both travelers and endemic populations from malaria.

INNOVATION

Our approach offers an innovative integration of field-derived data with serological assays to inform our understanding of naturally acquired immunity to *P. falciparum*. **Using blood meal matching, we will define individual-level exposure to malaria in a field setting**. We will also use a **unique, diversity-reflecti ng peptide array** containing *P. falciparum* antigens and their naturally occurring genetic variants from over 400 field-derived parasite isolates from sites in Africa and Asia, as well as laboratory strains. Finally, we will use ***in silico* and novel *in vitro* methods to identify and validate epitopes of interest** to identify promising pre- erythrocytic epitopes for further characterization as potential new vaccine candidates.

APPROACH

PRELIMINARY DATA

Use of peptide microarray data and epitope prediction tools to characterize vaccine-induced antibody responses: Our research group has experience using peptide microarrays to quantify antibody responses to >170,000 peptides, representing >50 antigens and their variants.[28-30] In 40 serum samples from Mali probed on this diversity-reflecting peptide array, the median coefficient of variation between the replicates was less than 2% and the mean Pearson and Spearman correlation was greater than 97% (Andrea Berry, under review). Using this array, our group evaluated antibody responses to the vaccine antigen apical membrane antigen-1 (AMA1) in children participating in an AMA1-based vaccine trial. This analysis showed robust antibody responses to the vaccine-type cluster 1 loop (c1L) epitope post-vaccination, but not to the c1L epitope from heterologous strains **(Figure 1),** consistent with allele-specific efficacy observed in the trial **(Figure 1)**.[29,31,32]

Our group also used *in silico* epitope prediction tools to identify epitopes for detailed sieve analyses of this AMA vaccine.[12] *In silico* methods identified eight “strong” binder epitopes. Sequences from parasites isolated from trial participants during follow-up were compared with the vaccine strain at these epitopes. Sequences from parasites isolated from participants with clinical malaria in the vaccine group (i.e. those that escaped the vaccine) had more mutations that differed from the vaccine sequence compared to strains isolated from control vaccine recipients in one epitope in the c1L region, providing additional evidence that polymorphisms in this c1L region impacted the efficacy of this AMA1 vaccine. These findings support our ability to identify differential serologic al responses among individuals with potential clinical implications even within a high transmission setting and use *in silico* methods to help identify immunologically relevant epitopes.

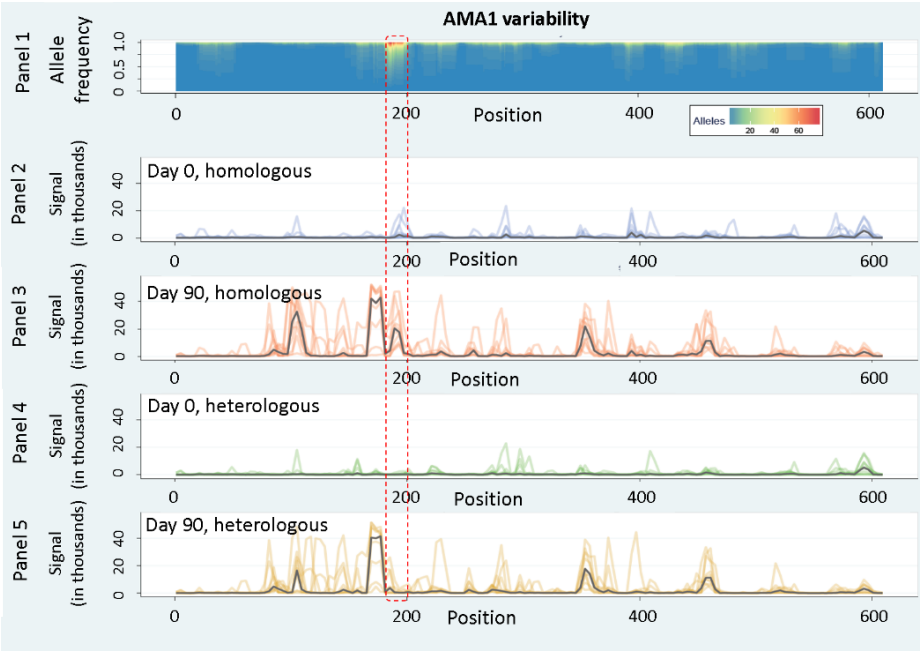

Figure 1. Allele specific humoral responses in an AMA1 hyper-variable region. **Panel 1**, visual depiction of the variability of the 331 AMA1 haplotypes included on the peptide array using a gradient color scale. **Panels 2-5,** seroreactivity of 10 children who received AMA1 vaccine to 16-aa peptides overlapping by 15-aa that cover AMA1. Median seroreactivity for each group of n=10 indicated in black. **Panels 2 and 3**, seroreactivity to vaccine strain peptides, prior to vaccination (**Panel 3**) and 30 days following last vaccination (Day 90) (**Panel 4**). **Panels 4 and 5**, seroreactivity to peptides representing a heterologous AMA variant, prior to vaccination (**Panel 4**) and 30 days following last vaccination (Day 90) (**Panel 5**). The red box denotes the c1 loop (c1L) which has been associated with vaccine escape.[31] Antibody responses induced by the vaccine reacted only to amino acids corresponding to the homologous c1L haplotype, consistent with the allele-specific vaccine efficacy observed in the clinical trial.[32]

Naturally acquired responses to pre-erythrocytic proteins on peptide microarray: *P. falciparum* CSP is the predominant surface protein and a major malaria subunit vaccine target. Pilot implementation of RTS,S/AS01 is occurring in three African countries[33] and recent studies have detailed protective antibodies to the junction between Region 1 and the central repeat region (R1-NANP junction) of CSP induced by an irradiated sporozoite vaccine,[6,7] but little is known about naturally acquired immunity to precise and diverse CSP epitopes (CSP schematic in **Figure 2A**). I examined data available from our peptide array experiments to address this gap in knowledge. Adults showed greater magnitude and breadth of antibody responses in the R1-NANP junction and the central repeat region, but not to the 3D7-like sequence in the Th2R epitope, which is included in RTS,S **(Figure 2B).**[30] Children acquired short-lived immunity to the R1-NANP junction and a Th2R epitope during the season but not the central repeat region **(Figure 2C).**[30] These results show that we can find differential responses to pre-erythrocytic proteins between subjects and within the same subjects over time in a high transmission setting.

In summary, our preliminary results show that peptide arrays can provide meaningful information about both vaccine-induced and naturally acquired immunity to *P. falciparum.* Therefore, we believe the diversity - reflecting peptide microarray will provide important insights into naturally acquired humoral immunity to diverse pre-erythrocytic epitopes.

Optimization of Mosquito Blood Meal Matching. The assays were optimized using *Anopheles gambiae* mosquitoes that fed on donor blood. Mosquito and human blood spots were sent to Michigan State University for blood meal matching. Laboratory staff performing the matching were blinded to the source of blood meal for each mosquito. DNA was amplified in 28 of 30 mosquitoes killed up to 12 hours after feeding and all human samples. Twenty-five of the 28 mosquitoes with amplified DNA were correctly matched to the blood meal source.

RESEARCH DESIGN AND METHODS

We will use samples collected as part of the community-based household surveillance cohort conducted by the Malawi ICEMR to identify participants who are exposed to female blood-fed *Anopheles* mosquitoes. The study was designed to characterize *P. falciparum* infection, disease, and potential for transmission from humans to mosquitoes. This cohort included 96 households in 16 clusters in each of two health center catchment areas, Machinga and Balaka districts, high transmission areas where approximately 33% of children < 5 years of age have parasitemia detected by microscopy.[34] They are both located in southern Malawi on the border with Lake Malawi. For identification of sampling clusters, the two sites were divided into four areas to ensure capture varied eco-geographic zones, and four index households were chosen from the population census done in 2018. The five nearest households were offered enrollment with replacement of any refusals or migrations with the next nearest households. All members of each household were invited to participate in the study. At enrollment, we collected a blood sample from every participant to identify unique, non-coding microsatellite markers in human DNA that will enable matching to mosquito blood meals. Each month, the team collected data on malaria symptoms status and antimalarial use for each participant. Finger prick blood samples are collected from study

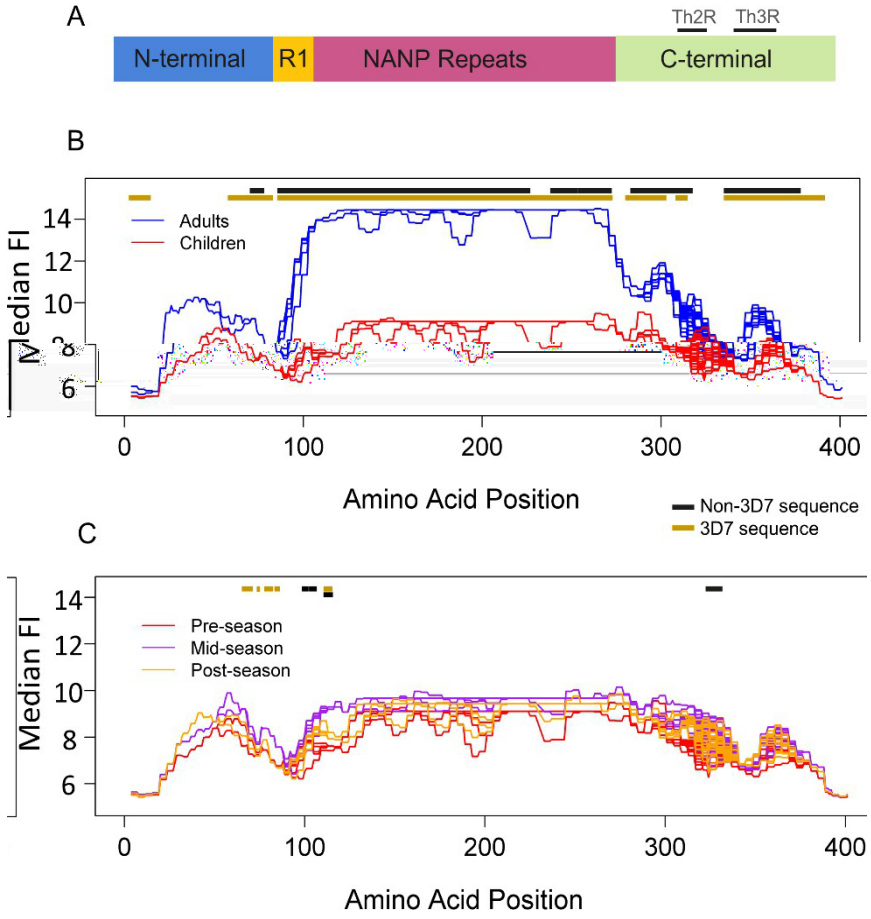

Figure 2: A). Schematic of the circumsporozoite protein (CSP). The four regions of CSP include the N-terminus, Region I, the central repeat or NANP repeat region, and the C-terminus. **B). Differential seroreactivity Malian adults v. children:** X-axis: amino acid position along the protein, y-axis: median smoothed log2 f luorescence intensity (FI) to 73 CSP haplotypes. Overlap occursin areas of sequence conservation. Median smoothed log2 FI of adults (blue) and children (red). Gold line above the line graph: areas where seroreactivity to the 3D7-like variant was significantly greater in adults compared to children, black line: areas where seroreactivity to non-3D7variants was significantly greater in adults compared to children (P<0.05 with Benjamini-Hochberg [BH] correction). At no position along the protein did children have significantly greater seroreactivity than adults. **C). Seroreactivity in Malian children over the course of a malaria season:** Median smoothed log2 FI of children pre-season (red) compared to mid-season (purple) and post-season (yellow). Gold line above the line graph: areas where seroreactivity to the 3D7-like variant was significantly greater in children mid-season compared to pre-season, black line: areas where seroreactivity to the non-3D7 variants was significantly greater mid-season compared to pre-season (P<0.05 with BH correction). No significant differences existed between seroreactivity post- to mid-season or post- to pre-season.

participants for active detection of malaria infection. Passive case detection of malaria illness occurs through continuous surveillance in the local health center.

During the rainy season, the study team visited each household every two weeks to collect mosquitoes using pyrethroid spray catches. Mosquitoes were collected in the early morning to try to obtain mosquitoes that fed overnight and were resting indoors. The success of recovery of human DNA from the human blood in the mosquito midgut decreases significantly after 36 hours,[35] so we only selected mosquitoes for further analysis that had obvious signs of having taken a recent blood meal. Human microsatellites identified in blood from the midguts of fed female *Anopheles* mosquitos will be compared with participant microsatellites to identify the source of the blood meal. This method has successfully identified the specific sources of mosquito blood meals by Dr. Ned Walker, who will be conducting these analyses for the ICEMR, as well as other groups.[36,37] To determine if the mosquito was infected, the mosquito salivary glands will undergo PCR for detection of sporozoites. Monthly active surveillance samples will undergo PCR for the *P. falciparum* 18S ribosomal RNA gene and any positive PCR will be defined as blood-stage malaria infection. By identifying the source of the blood meals and monitoring for blood-stage malaria infection using active and passive surveillance, we will discriminate between individuals who are *P. falciparum*-exposed and protected from blood-stage infection from those exposed and infected.

Aim 1: Identify serologic responses associated with natural protection against *P. falciparum* infection.

Hypothesis: Sera from malaria-exposed children who are protected from blood-stage P. falciparum infection will recognize pre-erythrocytic epitopes that are not recognized by children who develop blood-stage infection.

The study population will be drawn from participants aged 6 months to 15 years of age who were the sole source of a blood meal of a *P. falciparum*-infected mosquito. We will exclude children who have been treated for symptomatic malaria in the month prior to the bite or have malaria infection at the time of the exposure (**Figure 3**). *P. falciparum*-exposed participants who do not develop blood-stage malaria infection within one month based on monthly active surveillance samples and passive surveillance at the local health center will be classified as exposed and protected. Participants who develop blood-stage malaria infection (with or without symptoms) within the next month or who presented to the local health center for treatment of malaria between one week and one

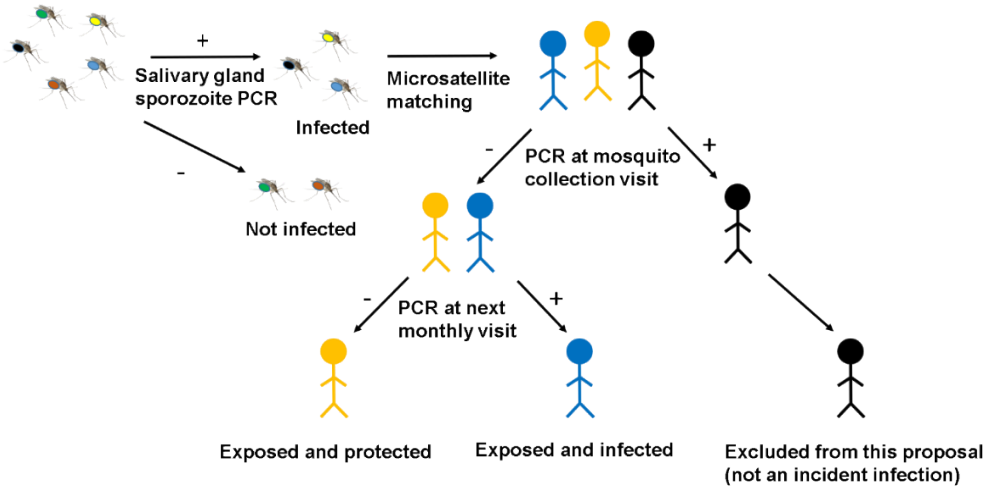

Figure 3. Classification of participants as infected at the time of blood meal, exposed and protected or exposed and infected.

month after the exposure will be classified as exposed and *P. falciparum* infected. We will use the diversity-reflecting peptide microarray to characterize the breadth and depth of serologic al responses to pre-erythrocytic epitopes on the day of *P. falciparum* exposure, as assessed by mosquito blood meal analysis. We anticipate that participants who do not develop blood-stage infection after exposure will have different serologic responses to pre-erythrocytic epitopes compared to those who become infected.

The peptide array will be fabricated *in situ* with a Roche Sequencing Solutions Maskless Array Synthesizer.[38] Peptides are synthesized directly onto the slide using light-directed synthesis. The peptide array is incubated with the antibody of interest and fluorophore-labeled secondary antibody.[39] Nimble Therapeutic s uses proprietary quality control (QC) metrics, including amino acid QC, synthesis drift, background signal and signal uniformity, and all arrays must pass these QC metrics prior to data being used for analysis. They also incorporate thousands of QC peptides on each array to monitor and limit inter-array variability. These methods ensure reproducibility of the results. The array can include up to 392,000 peptides and will be designed from a database of peptides derived from at least 125 proteins previously associated with protection from infection after vaccination or in naturally acquired immunity (including proteins listed in **Table 1**) that have 1-321 variants each, excluding proteins unlikely to be viable vaccine candidates such as DNA ligases and DNA repair proteins. This database is based on publicly available sequences and over 400 field samples from Mali, Malawi, and Southeast Asia sequenced by our research team. Each variant antigen is represented by 16-amino acid peptides with 15-amino acid overlap covering the length of the sequence. Epitopes usually range from 4-12 amino acids long, and previous research determined that 16-amino acid peptides are the optimal length for serum profiling when considering signal robustness versus peptide synthesis.[40,41] Peptides are printed on the array in triplicate to ensure quality of the replicates. Eight malaria-naïve negative control samples from Baltimore volunteers will be probed on the array. These samples are controls used for all protein and peptide microarray studies from our lab and help evaluate interarray variability. Median age of the volunteers is 34 years, 4 are male, 6 are white and non-Hispanic and 2 are Black.

Table 1: Proteins associated with protection from malaria infection after vaccination[25-27] and those also associated with naturally acquired immunity[23-27] in prior studies.

Proteins associated with protection from malaria infection after vaccination	
Protein name	**Gene ID**
merozoite surface protein MSA180[25,26]	PF3D7_1014100
zinc finger protein[25,26]	PF3D7_1433400
falstatin[25]	PF3D7_0911900
heat shock protein 70[27]	PF3D7_0818900
conserved protein, unknown function[27]	PF3D7_1141100
conserved protein, unknown function[27]	PF3D7_0206500
protein kinase[26,27]	PF3D7_0203100
zinc finger protein[27]	PF3D7_0409800
histone acetyltransferase GCN5[26,27]	PF3D7_0823300
transcription factor with AP2 domain(s)[26,27]	PF3D7_1139300
RNA lariat debranching enzyme, putative[27]	PF3D7_1340600
conserved membrane protein, unknown function[26,27]	PF3D7_1021700
rhoptry neck protein 3[27]	PF3D7_1252100
ADP-ribosylation factor GTPase-activating protein[27]	PF3D7_1244600
asparagine/aspartate rich protein[27]	PF3D7_1233600
parasite-infected erythrocyte surface protein[27]	PF3D7_0501200
gamma-glutamylcysteine synthetase[26,27]	PF3D7_0918900
Proteins associated with both naturally-acquired immunity and protection after vaccination	
Protein name	**Gene ID**
LSA1[23,24,26]	PF3D7_1036400
CSP[23,24,26,27]	PF3D7_0304600
thrombospondin-related anonymous protein (TRAP)[23-25,27]	PF3D7_1335900

Analysis plan and data interpretation: Fluorescence intensities for all peptides will be normalized using no peptide control spots. **To ensure rigor and reproducibility**, we will assess degree of variability between pairwise replicates using coefficient of variation and correlation assessments, including Pearson correlation and linear regression slope and intercept. Agreement between triplicates will also be assessed using pairwise scatterplots. Mean log2 FI will be calculated for replicate peptides. Because overlapping peptides can contain the same epitope, a sliding window-based average smoothing procedure will be used to increase the signal-to-noise ratio by leveraging information between neighboring peptides.[42] Each amino acid position along the protein sequence will be represented by a smoothed log2 FI equal to the average FI for all peptides with a midpoint six positions before or after the given amino acid position. For example, the amino acid at position 100 will have a

corresponding smoothed log2 FI equal to the average of the all peptides with midpoint positions at 94 through 106. Peptides will be defined as "serorecognized" when the fluorescence intensity is >2.5 times the mean of negative controls. For serorecognized peptides, we will use a Wilcoxon rank sum test to compare the median number of peptides recognized in protected participants compared to *P. falciparum* infected participants. We will use Fisher's exact test to determine whether more of the protected children serorecognized each peptide as compared to infected children and a chi-square test to compare proportions of variants serorecognized at each position between the two groups. To compare the intensity of seroreactivity to each serorecognized peptide, we will use a Bayes regularized t-test comparing children protected from infection to those who develop infection. We will explore the effect of covariates such as age and sex of participant, household cluster, species of mosquito and mosquito salivary gland sporozoite densities on the median number of peptides recognized, proportion of variants serorecognized at each position and seroreactivity to each serorecognized peptide. Any variables associated with the predictor or outcome of interest will be incorporated into regression models. The limited sample size and large number of peptides tested necessitates an estimation of the false discovery rate, for which we will use a permutation-based method.[43]

We will also use machine learning algorithms, such as Random Forest, to identify which peptide or combination of peptides best predicts infected versus protected phenotypes. Our general approach will be to treat the problem as one of classification, creating statistical/machine learning models that predict infected versus protected individuals. More generally, from a machine learning perspective, this problem is one of hypothesis generation rather than hypothesis testing. Consequently, our focus is directed toward analyzing features (variables) that might best distinguish infected versus protected individuals, rather than determining statistical significance. We will analyze serorecognition as a binary variable and seroreactivity as a continuous variable. The Random Forest method we will use integrates separate training and testing sets using bootstrap sampling of observations performed before constructing each tree in the forest. Each individual model is built using ~0.632 of the observations, some of which are sampled multiple times, and tested on the remaining ~0.368 of the data. This provides a cross-validation-like procedure to prevent overfitting. For methods in which such testing-traini ng sets are not structurally included, we will divide the data into training, testing, and validation subsets and through a cross-validation design. Peptide positions with the largest proportion of serorecognized variants will be considered cross-reactive. Regions of cross-reactive peptides that correlate with a "protected" phenotype suggest possible conserved/cross-protectiv e epitopes.

Consideration of sex as a biological variable: We will include both female and male participants in our study. We will examine the effects of sex on the responses seen in the peptide array analysis using bivariate and regression models.

Sample size and power: The proposed sample size of 50 exposed children who develop *P. falciparum* blood-stage infection and 50 exposed children who do not develop infection will give 80-97% power to detect a moderate effect size of the mean intensity of seroreactivity between the two groups by t-test. This sample size would also give greater than 85% power to detect a 30% difference in the proportion of peptide variants recognized at a position (i.e., potential cross-reactive epitopes) between the two groups by chi-square test.

Analysis of the >6,000 collected female *Anopheles* mosquitoes is ongoing. A study using similar methods found approximately 7-9% of *Anopheles* mosquitoes in Malawi are positive for *P. falciparum*.[44] (McCann et al, under review) and in our preliminary analysis our results show an even higher infection rate. Conservatively, we assume 90% of mosquitoes will have a human source blood meal,[45] and that 50% will be successfully matched to a single human source in a study household.[35] Therefore, for each mosquito collection time point, we estimate that we will have 100-130 sporozoite-positive mosquitoes that have fed on a child enrolled in the study.

Expected outcomes: We expect that we will identify differential serorecognition and seroreactivity to distinct peptides and regions of pre-erythrocytic proteins between protected and *P. falciparum* infected children. These results will inform our vaccine development pipeline. We will determine peptides with few variants (suggesting conserved regions), as well as regions of proteins with the highest number of variants recognized, suggesting cross-reactivity, for further epitope characterization. Through the analysis of this data, I will also ensure that I have gained skills in utilizing machine learning techniques to analyze peptide microarray data and use methods such as multivariable regression to explore the effect of covariates on clinical outcomes.

Potential obstacles and alternative strategies: It is possible that we did not collect enough infected mosquitoes for our desired sample size given the proportion of mosquitoes expected to be sporozoite-posit ive. However, we collected >6,000 mosquitoes that are currently undergoing analysis. If the samples from the ICEMR cohort are not enough for our desired sample size, future ICEMR cohorts are planned that will collect similar human and entomological samples to those collected in this cohort and will be able to provide additional samples.

In addition, we will not know whether an exposure is homologous or heterologous to prior exposures. We are likely to miss serological responses that protect against unique or rare variants, but this is not the goal of our study - our goal is to identify broadly protective serological responses (responses to conserved regions or that cross-react with all variants on the array). We also recognize that bites happened on days other than our mosquito collection days. But, children who remain protected from infection after a known exposure, regardless of how many other exposures they have in that month, have immunity that is either cross-protective or broader in breadth or depth compared to those who go on to develop infection after a known bite or another bite around the same time that we did not capture. This immunity represents the kind of immunity that we would like to replicate with a vaccine.

The peptide array, which consists of linear peptides, may not capture responses to discontinuous epitopes. However, the peptides are flexible and can exhibit conformations, so conformational epitopes could still be captured. It is also likely that at least some protective epitopes are linear, so we will still gain important information from this approach. Antibodies to post-translationally modified epitopes could also go undetected. If we do not find significant differences between groups using peptide array methods, we can work with long-term collaborators to develop protein microarrays to examine differences in responses between our defined groups. We are also exploring ways to incorporate post-translationally modified proteins on the protein array. Because our approach focuses on serological responses, we will not characterize the important contribution of cell- mediated immunity. Although we recognize that serological assessments only describe a part of the complexity of the immune responses to malaria infection, prior trials have found differential humoral responses between those with asymptomatic and those with symptomatic infection and studies of the RTS,S vaccine suggest that humoral immunity plays an important role in protection against malaria.

Aim 2: Assess the function of antibodies targeting *P. falciparum* pre-erythrocytic antigens of interest.

Hypothesis: In silico epitope prediction tools and in vitro liver models will identify functional epitopes in pre- erythrocytic proteins that overlap with epitopes of interest identified in the microarray analysis.

In-silico epitope prediction. To validate the antigenic potential of epitopes associated with protection in Aim 1, we will use *in silico* B- and T-cell epitope prediction tools to find predicted epitopes in pre-erythrocytic proteins and their variants included on the peptide microarray. We will use ABCPred to predict linear B-cell epitopes, which utilizes a trained recurrent neural network based method and can predict continuous B-cell epitopes.[46,47] We will set the epitope length to 12 amino acids and use a score threshold of 0.8 to identify the most promising B-cell epitopes.[46] A higher score equates to a higher probability of being an epitope, so selecting 0.8 as the threshold increases the specificity of the method. For T-cell epitope prediction, we will use the Immune Epitope Database (IEDB) and the most common human leukocyte antigen (HLA) frequencies in Malawi.[9,48,49] IEDB utilizes a consensus method to predict MHC-I and MHC-II binding.[50-52] We will search for epitopes 10 amino acids in length and use a cutoff of a binding strength (IC_{50}) of ≤50 nM, which corresponds to high affinity binding.[53] A protein sequence-based distance matrix that gives higher values for biochemically dissimilar amino acids will then be used to examine amino-acid similarity between peptide variants at similar amino acid positions that are identified as strong binders, which identify potential conserved regions.

All pre-erythrocytic proteins represented on the peptide array will be analyzed for predicted epitopes using ABCPred and IEDB. Epitopes will be ranked based on strength of association with protection in Aim 1 and probability of being a B- and/or T-cell epitope in Aim 2 and compared to our database and published databases of *P. falciparum* sequences to evaluate for sequence diversity. The proteins containing the top ten most promising epitopes from Aims 1 and 2 with relatively little sequence diversity will be chosen for further evaluation using *in vitro* assays.

***In vitro* functional assays.** The proteins containing the top ten most promising epitopes will be expressed using protocols established at the Burnet Institute that we are adopting at UMB.[54] Briefly, genes will be codon- optimized for expression in mammalian cells. HEK293 Freestyle™ cells will be cultured and transfected and, six days later, expressed protein will be harvested by centrifuging the cells and collecting the supernatant, which will be purified using Ni affinity columns and gel filtration. Protein expression will be evaluated using SDS-PAGE and Western blot analyses. This expressed protein will then be combined with cationic adjuvant formulation 09 (CAF09) adjuvant, which has been used in other pre-clinical studies of malaria vaccines and induces robust antibody, Th1, and $CD8^+$ T-cell responses.[55,56] New Zealand white rabbits will be used to generate polyclonal antibodies through a contract our core facility has with GenScript. Three rabbits for each antigen will be immunized with 0.1 mg/mL of protein plus CAF09 adjuvant on days 0, 14, 42, and 56. Control rabbits will receive buffer plus CAF09 on the same schedule. On days 28, 56, and 72, rabbits will be bled to assess antibody-antigen binding specificity by ELISA. IgG will be purified with protein A/G to eliminate serum components that could result

in *in vitro* inhibition. Some antigens chosen for expression may not result in good quality protein for immunization and some immunizations may not result in high quality antibody production, so we will choose the five best constructs to move on to *in vitro* testing based on the quality of the antibody responses seen on ELISA.

Liver model. Dr. Adams' team developed an *in vitro* liver model to study pre-erythrocytic development of *Plasmodia* sp. using commercially available 384-well plates and reagents to promote primary human hepatocytes (PHH) to reacquire primary cell characteristics.[21] Infection rates are significantly higher than in previous models, and the model can support complete liver stage development through blood stage breakthrough. The model uses collagen-treated 384-well microplates and methods that provide a suitable environment for cryopreserved PHH to form liver lobules and begin active metabolism of bile within days of culture.[21] The plates have optically clear bottoms to be able to evaluate infection and development using immunohistochemistry and high-content image analysis and/or fluorescent biomarkers. PHH lots are screened prior to bulk use to ensure their ability to support parasite development.

Inhibition of liver stage development assay (ILSDA). We will use methods established by Dr. Adams' lab and previously published to test the ability of the polyclonal serum to inhibit liver stage infection and development. [21] Briefly, *P. falciparum* sporozoites will be incubated overnight with serial dilutions (1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256) of test serum, monoclonal antibodies for positive controls or control rabbit serum or culture medium for negative controls. Sporozoites will be added to each well and allowed to invade overnight before washing. Media will be changed every two days until fixation. Plates will be fixed at four time points (days 4, 8, 9, 12) to evaluate effects on cell cycle (early versus late liver stage versus blood stage breakthrough). Fresh human red blood cells will be added around day 7-8 to examine the ability of the polyclonal sera to inhibit blood stage breakthrough. The wells will be fixed with 4% paraformaldehyde and washed. They will then be incubated in blocking buffer with mouse anti-GAPDH mAb overnight. Then, the wells will be washed three times and incubated with anti-rabbit or goat anti-mouse secondary antibody and Hoechst then washed and filled with PBS for imaging and storage. Imaging and analysis will be performed using the Operetta Imaging System or CellInsight CX7 as described previously.[21] Hepatocyte nuclei will be counted using the DAPI channel and parasites will be counted using the FITC channel capturing anti-GAPDH and identified by area, mean intensity, maximum intensity and cell roundness. Percent inhibition of ILSDAs are calculated using total populations counts and parasite size, normalizing to the negative controls: % Inhibition = $100 - [(X / \text{ICtrl}) \times 100]$, where X is the measured inhibition by the antibody or immune sera and ICtrl is the average of the negative control replicates.[21]

Expected outcomes: We expect to identify pre-erythrocytic epitopes that are significantly associated with the "protected" phenotype in Aim 1, are strong binders on *in silico* analysis, are relatively conserved with few variants, and have functional activity in at least one pre-erythrocytic stage in the *in vitro* assays.

Potential obstacles and alternative strategies**:** A limitation of the *in silico* approaches is their accuracy. When compared to known epitopes, ABCPred has approximately 65% accuracy, and IEDB has an area under the curve of 0.71 to 0.76.[46,57] The combination of clinical data with the *in silico* analysis will increase our confidence that we are identifying epitopes that are likely functional. Some epitopes that are, in fact, functional may be excluded by this method. However, the purpose of using the technique is to validate peptide array results and down select the most promising candidates for *in vitro* testing. If we have a small number of epitopes of interest, we can lower the score threshold to increase the sensitivity of the method and/or include other *in silico* methods that can identify discontinuous epitopes.

If no promising epitopes of interest that differ between the "protected" group and the "infected" group can be identified as described in Aim 1, additional *in silico* analysis methods will be used to confirm the findings from ABCPred and IEDB to identify the most promising epitopes of interest for *in vitro* assays. The proteins and variants represented on our peptide microarray are unique in that the variants are derived from field samples and therefore represent the diversity found in nature. By using these methods, new potential vaccine candidates can be identified for further study from samples representing the diversity of circulating strains in malaria- endemic regions from the *in silico* analysis alone, if necessary.

FUTURE DIRECTIONS

Preliminary data from this study will be used to apply for further funding to validate the model developed in Aim 1 with additional cohorts studying both natural and vaccine induced immunity and to evaluate whether any of the epitopes seem to exhibit a threshold level associated with protection from infection. We will also apply for further funding to conduct *in vitro* and *in vivo* studies of promising antigens, evaluating both humoral and cell-mediated immunity and protection from malaria infection to further the development of novel malaria vaccine strategies.

Training in the Responsible Conduct of Research

I appreciate the importance of research integrity and the responsible conduct of research (RCR) to the quality of the data that I obtain and am committed to staying current in these fields. Therefore, I have completed the following courses and have constructed the following training plan:

Completed Courses:

Introduction to Clinical and Translational Research at UMB
Format: In-person, lectures and discussions involving a diverse group of UMB faculty
Duration: 1 week **Frequency:** Once, completed August 2017
Topics covered: Identifying RCR issues including conflict of interest (COI), data management and fraud, authorship, and human research protections

Health Insurance Portability and Accountability Act Training (HIPAA 125 & HIPAA 201)
Format: Online module with assessment
Duration: Two sessions **Frequency:** Once, completed July 2017
Topics covered: An introduction to HIPAA, what constitutes protected health information and how to handle it, security awareness, HIPAA principles as they apply to research

UMB PREV 633: Application of Legal and Regulatory Issues in Clinical Research
Format: Didactics, and interactive small group discussions facilitated by UMB faculty
Duration: One semester (1 credit) **Frequency:** Once, completed December 2018
Topics covered: Informed consent, privacy and confidentiality, quality management, data safety monitoring plans, financial disclosure and COI, the institutional review board (IRB), and biobanking

UMB CIPP 909: Research Ethics
Format: Small group discussions facilitated by UMB faculty
Duration: One semester (1 credit) **Frequency:** Once, completed May 2018
Topics covered: Scientific ethics, conflicts of interest, authorship, peer review, mentor/mentee responsibilities, data management, scientific misconduct, collaborative research and intellectual property, ethical use of animals in research and human subjects research

Training Plan:

Collaborative IRB Training Initiative (CITI) Course for Biomedical Research Investigators
Format: Online module with assessment
Duration: 18 modules over two weeks initially, 14 modules for the refresher course **Frequency:** Every 3 years, last completed August 2020
Topics covered: Avoiding group harms, special populations (including children and pregnant women), COI, IRB process, informed consent, genetics research, and special considerations for international studies

UMB CIPP 907: Responsible Conduct of Research
Format: Lectures and seminar discussions led by a diverse group of UMB faculty, and class exercises
Duration: One semester (1 credit) **Frequency:** Once, planned Spring 2022
Topics covered: Scientific integrity; research ethics; animal and human research; authorship; peer review; conflicts of interest; data handling and management; fraud and misconduct; genetics and reproduction; ownership of data and intellectual property; and the role of the scientist in society

Global Health Training Centre Research Ethics Training produced by the World Health Organization
Format: Online modules with assessment
Duration: 14 modules completed over a semester, 1-2 hours per week **Frequency:** Once, planned Fall 2022
Topics covered: Research ethics, vulnerability, engaging with communities, privacy and confidentiality, informed consent, biobanking and genetic research, and special populations including people with disabilities, women, and research in public health emergencies with a special focus on international research

Individual Mentoring
Format: In person, 1:1 meetings with Dr. Miriam Laufer and advisory committee meetings
Duration: Ongoing **Frequency:** At least twice yearly with Dr. Laufer, twice yearly with my advisory committee
Topics covered: The unique opportunities and challenges of pediatric and international research, including the informed consent process in areas with low literacy and translation issues, getting assent from children, community engagement, loss to follow-up

DESCRIPTION OF THE INSTITUTIONAL ENVIRONMENT

University of Maryland School of Medicine

The University of Maryland School of Medicine (UMSOM) was established in 1807 and is the oldest public medical school. The SOM consistently ranks in the top 10% of public medical schools and is recognized as a leader in biomedical and public health research with grants and contracts totaling over $542 million in FY19. The Department of Pediatrics has over 100 faculty members in 21 divisions. A letter of institutional commitment from the Department Chair, Dr. Steven Czinn, is included in Dr. Friedman-Klabanoff's application.

Center for Vaccine Development and Global Health

Originally created in 1974 as the Center for Vaccine Development, in 2018, it was renamed the Center for Vaccine Development and Global Health (CVD) to encompass its global health reach. Dr. Kathleen M. Neuzil, MD, MPH, FIDSA was named the Director. The CVD offers a multi-disciplinary approach to global health research and vaccine development, including basic molecular biology; cell biology and immunology research; microbial pathogenesis; development of novel vaccine candidates; Phase I, II, III, and IV clinical trials; epidemiological field studies; sophisticated applied immunology laboratories; biostatistics; and data management. Because of the CVD's international mission, it has established extensive resources to support international research, including a dedicated international regulatory affairs specialist and an administrative team that manages grants and subcontracts to a wide range of institutions throughout the world.

Malaria Research Program

The Malaria Research Program (MRP), led by Professor Miriam Laufer, MD, MPH, conducts a broad range of research including malaria epidemiology, drug resistance, immunology, and vaccine development with support from NIH, the Bill and Melinda Gates Foundation, and others. The Program is home to 10 faculty members with grants and contracts totaling more than $20 million. The MRP has international programs in Malawi, Mali, and Myanmar. Domestically, MRP is comprised of a Genomic Epidemiology Unit led by Shannon Takala- Harrison, PhD; a Malaria Vaccine and Challenge Unit led by Kirsten Lyke, MD; an International Clinical Studies Unit led by Matthew Laurens, MD MPH; and an Immunoepidemiology and Pathogenesis Unit co-led by Andrea Berry, MD and Mark Travassos MD MSc. MRP's genomics work is also supported by Dr. Joana Carneiro Da Silva, faculty in the Institute for Genome Sciences (IGS), whose specialty is in malaria genomics. The IGS is an NIAID-sponsored Genomic Sequencing Center for Infectious Disease, which includes a focus on integrated genomics research in tropical parasitic diseases, led by Dr. Da Silva and with co-investigators from the MRP and CVD. Regarding vaccine development, the MRP is one of only three academic institutions in the United States capable of conducting human malaria challenges to test vaccine efficacy.

Malaria Alert Center (MAC), University of Malawi College of Medicine

The University of Malawi (UM) was founded just after Malawi gained independence in 1962, with the College of Medicine formed in 1991 as a constituent college. The UM Malaria Alert Center provides postgraduate training, provision of laboratories to support for malaria research, and access to field sites where observational and intervention studies can be conducted. Drs. Don Mathanga, Professor in the Faculty of Public Health at UM, and Dr. Terrie Taylor, University Distinguished Professor in the Department of Osteopathic Medical Specialties at Michigan State University, are the co-principal investigators of the International Center for Excellence in Malaria Research (ICEMR) grant from which the samples will be obtained. Dr. Friedman-Klabanoff will interact with Drs. Mathanga and Taylor at national conferences and when she travels to Malawi during her work.

Opportunities for intellectual interactions and career development

Topical journal clubs, dissertation defenses, and invited lectures occur regularly within CVD and across the SOM. Annually, the Frontiers in Vaccinology Visiting Professorship Symposium is held, in which CVD and affiliate faculty members give presentations on current research activities to a visiting Professor who provides feedback and discussion. Other divisions where CVD faculty participate in educational activities include Microbiology and Immunology, Infectious Diseases, Pediatric Infectious Diseases, Epidemiology, IGS, and the Institute for Human Virology.

Specific support for junior faculty is provided through the Office of Research Career Development, which offers an extensive array of workshops and seminars covering grant writing, identifying funding sources, and scientific leadership, as well as scientific writing accountability groups. There is a specific group for Career Development Awardees, the K Club, designed to support junior faculty as they transition to independence with seminars on time management, giving an elevator talk, and transitioning from a K to an R, among others. Included in this is a specific seminar series on "Writing Your First R01."

PHS Human Subjects and Clinical Trials Information

OMB Number: [redacted]

Expiration Date: 02/28/2023

Use of Human Specimens and/or Data

Does any of the proposed research in the application involve human specimens and/or data *

● Yes ○ No

Provide an explanation for any use of human specimens and/or data not considered to be human subjects research.

Are Human Subjects Involved

● Yes ○ No

Is the Project Exempt from Federal regulations?

○ Yes ● No

Exemption Number

❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8

Other Requested Information

2020-11-11T16:27:19.000-05:00

Human Subject Studies

Study#	Study Title	Clinical Trial?
1	Serological markers of natural immunity to Plasmodium falciparum infection	No

Section 1 - Basic Information (Study 1)

OMB Number: [redacted]

Expiration Date: 02/28/2023

1.1. Study Title *

Serological markers of natural immunity to Plasmodium falciparum infection

1.2. Is this study exempt from Federal Regulations * ❍ Yes ● No

1.3. Exemption Number ❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8

1.4. Clinical Trial Questionnaire *

1.4.a. Does the study involve human participants?	● Yes	❍ No
1.4.b. Are the participants prospectively assigned to an intervention?	❍ Yes	● No
1.4.c. Is the study designed to evaluate the effect of the intervention on the participants?	❍ Yes	● No
1.4.d. Is the effect that will be evaluated a health-related biomedical or behavioral outcome?	❍ Yes	● No

1.5. Provide the ClinicalTrials.gov Identifier (e.g. NCT87654321) for this trial, if applicable

Section 2 - Study Population Characteristics (Study 1)

2.1. Conditions or Focus of Study

- Malaria

2.2. Eligibility Criteria

Inclusion criteria:
- Children 6 months to 15 years of age who had data and samples collected as part of U19AI089683-08
- Participant had serum samples collected as part of U19AI089683-08 available for analysis with adequate volume remaining to conduct peptide microarray analysis
- Participant was DNA matched with a sporozoite-positive Anopheles mosquito blood meal
- Active and passive surveillance data for the participant is available for the month after the matched mosquito blood meal

Exclusion criteria:
- Plasmodium falciparum PCR positive on the day of mosquito collection
- Received antimalarial treatment in the month prior to the mosquito collection timepoint

2.3. Age Limits	Min Age: 6 Months	Max Age: 15 Years
2.3.a. Inclusion of Individuals Across the Lifespan	HS-1-InclusionofIndividualsAcrossLifespan.pdf	
2.4. Inclusion of Women and Minorities	HS-1-Inclusionofwomenandminorities.pdf	
2.5. Recruitment and Retention Plan	HS-1-RecruitmentAndRetentionPlan.pdf	
2.6. Recruitment Status	Not yet recruiting	
2.7. Study Timeline	HS-1-StudyTimeline.pdf	
2.8. Enrollment of First Participant	07/01/2021	Anticipated

INCLUSION OF INDIVIDUALS ACROSS THE LIFESPAN

The samples for the proposed research will be collected in a cohort study conducted by the Malawi International Center of Excellence in Malaria Research Program. A total of ninety-six households in each of two catchment areas will be recruited and all individuals in the household will be approached for enrollment in the study. In Malawi, an average household size is 4.5 members, about half of which are under age 15. This proposed study will only use samples from children 6 months through 15 years of age whose parents have consented to future use of stored specimens. Only children will be included because adults have more robust immunity to malaria, and this could confound the results of the study.

INCLUSION OF WOMEN AND MINORITIES

The samples for the proposed research will be collected in a cohort study conducted by the Malawi International Center of Excellence in Malaria Research Program. A total of ninety-six households in each of two catchment areas will be recruited and all individuals in the household will be approached for enrollment in the study. In Malawi, an average household size is 4.5 members, about half of which are under age 15. The study will recruit participants without regard to gender. All participants will be Malawians of African descent.

RECRUITMENT AND RETENTION PLAN

All samples for the proposed research were collected as part of a cohort study coordinated by the Malawi International Center of Excellence in Malaria Research (ICEMR) Program. No additional recruitment of participants will take place for the proposed work. We aim to enroll a subset of 100 children from the ICEMR cohort study for the proposed work, as described in the Research Strategy. Recruitment occurred at two sites in Malawi: Machinga District and Balaka District. Machinga District covers an area of approximately 3,582 km^2 and has a population density of 205 persons per km^2.[1] Balaka District borders Machinga District to the west, with approximately 68 km between the district capitals. It has an area of approximately 2,142 km^2 and a population density of 205 persons per km^2.[1] Both districts have similar household size (Balaka 4.3, Machinga 4.5) and percent of population over age 18 (Balaka 47%, Machinga 45%).[1]

The recruitment and retention plan for the ICEMR household cohort study can be found below.

We aim to enroll all members of 96 households in each health center catchment area. The size of the sampling frame will be chosen such that identifying sufficient households will not be a barrier to enrollment. Enrollment visits will be conducted over the course of one month.

Individuals will be followed monthly with active surveillance, and passive surveillance will be conducted at the local health center.

Monthly individual visits: Participants will be asked to come to an assigned, easily accessible, central location monthly for an interview and sample collection. Local leaders and study team members will disseminate reminders to participants. At each monthly visit, an interviewer will conduct an individual survey.

If participants do not turn up for monthly visits, study staff or community volunteers will visit their home to remind them. Participants who do not attend three consecutive monthly visits without a known explanation will be considered to have been lost to follow up. However, should participants return to the study site, they may resume participation. If a household withdraws, is lost to follow-up, or moves out of the study area, enrollment will be offered to the nearest non-enrolled neighboring household. If households move within the study area, new household data will be collected, and the household will continue to be followed.

1. National Statistical Office. *2018 Malawi Population & Housing Census.* Zomba, Malawi2018.

2.7 Study Timeline

The regulatory approvals for the Malawi International Centers of Excellence in Malaria Research (ICEMR) cohort study were obtained from the University of Malawi College of Medicine Research and Ethics Committee, and from the Michigan State University Institutional Review Board (IRB). We will obtain the required regulatory approvals from the University of Maryland, Baltimore IRB for the study proposed in this application. This step will take 1-3 months.

The cohort study from which the samples will be collected has been discussed with village headmen, chiefs, and health surveillance assistants to obtain permission to conduct the study. Community sensitization meetings were held to provide education about malaria and information about the study.
Staff training on study protocol, SOP's, and specimen collection was completed as a part of the ICEMR cohort study.

Enrollment of participants into the ICEMR cohort study and mosquito collection for the first malaria season is completed. Blood meal matching and sporozoite PCR are currently underway. If the collected mosquitoes were not enough to meet enrollment goals, we anticipate that enrollment goals will be met in Year 1 with mosquito collection during the next malaria season through subsequent ICEMR cohort studies based on household size and composition, known biting behaviors of mosquitoes and parasitemia rates in the area.

The serum samples will be shipped to the University of Maryland at the beginning of Year 2, and immunologic assays will be conducted in the US in Years 2 and 3 of the study. *In silico* analysis will happen in Year 2. Data analysis and dissemination of Aim 1 results will be done in Years 2-3 of this study. *In silico* analysis will happen concurrently in Years 1 and 2 and *in vitro* assays will be performed in Years 2 and 3. Data analysis and dissemination of Aim 2 results will be done in Years 3-5 of this study. Preparation of an R01 will happen in Years 3 and 4, with preparation of a resubmission planned in Year 5 (indicated with the lighter gray shaded boxes), if needed.

Task	**Year 1**		**Year 2**		**Year 3**		**Year 4**		**Year 5**	
Formal coursework										
Leadership training										
Specific Aim 1										
Mosquito collection										
Human infection follow up										
Identification of study subjects										
Microarray analysis										
Dissemination and manuscripts										
Specific Aim 2										
In silico analysis										
In vitro analysis										
Data analysis										
Dissemination and manuscripts										
Transition to an Independent Researcher										
Preparation of R01									If needed	
Submission of R01										If needed

2.9. Inclusion Enrollment Reports

IER ID#	Enrollment Location Type	Enrollment Location
Study 1, IER 1	Foreign	Household/Community

Inclusion Enrollment Report 1

1. Inclusion Enrollment Report Title* : Serological markers of natural immunity to Plasmodium falciparum infection

2. Using an Existing Dataset or Resource* : ❍ Yes ● No

3. Enrollment Location Type* : ❍ Domestic ● Foreign

4. Enrollment Country(ies): MWI: MALAWI

5. Enrollment Location(s): Household/Community

6. Comments: The samples for the proposed research will be collected in a cohort study coordinated by the Malawi International Center of Excellence in Malaria Research (ICEMR). No additional recruitment of patients will take place as part of the proposed work.

Planned

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	0
Asian	0	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	50	50	0	0	100
White	0	0	0	0	0
More than One Race	0	0	0	0	0
Total	50	50	0	0	100

Cumulative (Actual)

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	0	0	0	0	0	0	0	0	0	0
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	0	0	0	0	0	0	0	0	0	0

Section 3 - Protection and Monitoring Plans (Study 1)

3.1. Protection of Human Subjects	HS-1-ProtectionOfHumanSubjects.pdf
3.2. Is this a multi-site study that will use the same protocol to conduct non-exempt human subjects research at more than one domestic site?	❍ Yes ● No ❍ N/A
If yes, describe the single IRB plan	
3.3. Data and Safety Monitoring Plan	
3.4. Will a Data and Safety Monitoring Board be appointed for this study?	❍ Yes ● No
3.5. Overall structure of the study team	

3.1 PROTECTION OF HUMAN SUBJECTS

3.1.1. Risks to Human Subjects

a) Human Subjects Involvement, Characteristics, and Design

This study involves characterization of humoral responses to *Plasmodium falciparum* before and after exposure to infectious bites of mosquitoes. The human and entomological samples for the proposed research will be collected as part of a Malawi International Center of Excellence in Malaria Research (ICEMR) Program cohort study that began early 2019.

Samples for Aim 1 will be serum from children aged 6 months to 15 years eligible for the proposed research. Samples will be identified only by study number. Laboratory staff performing the peptide microarrays will be blinded to clinical data. Only those analyzing the final data will have access to data from the ICEMR study, such as age, bed net usage, geographic location, and symptomatic malaria episodes, associated with samples through the study number.

The activities related to this study are specifically probing the serological specimens on the peptide microarray. Participants for the proposed study will be selected among children ages 6 months to 15 years who are present at the time of a mosquito collection. We will include only children who were present at the time of mosquito collection, had specimens collected at the time of mosquito collection and the subsequent active surveillance visit, and whose parents consented to future use of specimens when providing consent for the ICEMR cohort study. We will exclude children who have been treated for symptomatic malaria in the month prior to mosquito collection and children who have a positive qPCR at the time of mosquito collection. For our proposed study, dried blood spots on filter paper will be used to extract parasite DNA and test for malaria. The serum samples will be used for peptide microarray analysis. The proposed research will pose no additional physical risk to the volunteers or produce additional discomfort, since it will involve assays on specimens that are already being collected as part of another study.

Human subjects involvement, characteristics and study design for the ICEMR cohort study are described below.

Title: *Intransigence of malaria in Malawi: Understanding hidden reservoirs, successful vectors, and prevention failures. Project 002 (224): Transmission - Gametocyte and Entomology cohorts.*

This study is a household-based cohort study to characterize the sources of *Anopheles* blood meals and identify which groups of humans harbor infections with the greatest potential for transmission to mosquitoes in two health center catchment areas in Machinga and Balaka District, Malawi. Households in the catchment area will be mapped in a census described as a component of a separate protocol. Ninety-six households in each district will be randomly selected and offered enrollment.

Prior to the initiation, the study was discussed with village headmen, chiefs, and health surveillance assistants. Community meetings were held to provide education about malaria and information about the study. In each selected household, consent will be sought from the head of each household and all adults living in the household. One parent or guardian will also be requested to provide consent for each child (persons <18 years old) living in the house. Assent will be sought from children age 13-17 years. Eligible participants are members of selected households with routine use of the study health center who give consent/assent. Exclusion criteria include use of cotrimoxazole prophylaxis for HIV infection.

b) Study Procedures, Materials, and Potential Risks

The longitudinal cohort study will include:

Monthly individual visits: Participants will be asked to come to an assigned, easily accessible, central location monthly for an interview and sample collection. Reminders will be disseminated by local leaders and study team

members. At each monthly visit an interviewer will conduct an individual survey and collect blood samples by finger prick from participants six months and older.

Household visits: Study teams will visit each household twice yearly and conduct a household survey and entomologic sampling that included mosquito collection in and around the household.

Passive case detection: Passive surveillance for malaria cases will be conducted at the local health center. Episodes of clinical malaria, including testing and treatment, will be documented by study or clinic staff.

After data collection, specimen processing, and data cleaning are complete, all personal identifiers will be removed. The de-identified data will be shared through publicly available databases appropriate for the data type.

At the first monthly visit in the ICEMR cohort study (or at 6 months of age if participant enrolled when <6 months old) non-coding DNA microsatellite testing will be performed for determining which humans were bitten by mosquitoes. This data will be used in the proposed study to identify which humans were bitten by mosquitoes that harbored sporozoites to follow them over time and determine which develop blood stage infection and which do not. The genetic information does not cover any coding regions and does not provide any sensitive genetic data about the individual.

Samples used will not be fully anonymized so that genetic data can be linked with clinical and demographic data at the individual level. When identifiable information can be linked to study samples, there is always a potential risk of breach of confidentiality. Blood samples and nucleic acids from study participants are coded by investigators at each trial site, and codes are not available to University of Maryland investigators. No sensitive information about study participants will be generated.

3.1.2. Adequacy of Protection Against Risks

a) Informed Consent and Assent

This study involves analysis of humoral responses from blood samples collected from an already planned cohort study conducted in Malawi, as described above. No additional recruitment will be done as part of the proposed research. Only children whose parents consented to future use of samples in the process of informed consent for the ICEMR cohort study will be enrolled in the proposed research. Approval to perform further analysis on clinical samples will be obtained from the University of Maryland, Baltimore IRB prior to conducting the proposed research.

b) Protections Against Risk

For samples used in this proposed study, the data generated from each study sample will be linked to the coded, unique identifier provided by local investigators on the original sample container, as well as any clinical or demographic information necessary to determine associations with treatment outcome or adjust for confounding in statistical models. Codes linked to identifying information are maintained by local investigators and will not be provided to the investigators at University of Maryland. Study data will be stored in a REDCap database with limited and password-protected access to delegated team members. Participants will not be identified in any publications resulting from the study.

The non-coding DNA microsatellite analysis could provide paternity data, therefore, all genetic data will be kept confidential and only linked by the coded, unique identifier.
Subject confidentiality is held in strict trust by the investigators, the study staff, and the study sponsor. Confidentiality is extended to cover testing of biological samples, in addition to the clinical information relating to participants. The study protocol, documentation, data, and all other information generated will be held in strict

confidence. No information concerning the study, or the data will be released to any unauthorized third party without prior approval of the sponsor.

The study monitor, regulatory authorities, IRBs, or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator. The clinical study staff will permit access to such records.

Records will be stored in a locked facility or through a password-protected database indefinitely. Access to records will be limited to study personnel and authorized authorities. Written permission from the sponsor will be sought prior to destruction of any source documents or databases.

If maps are used to display information about malaria prevalence, household GPS data will be aggregated such that individual households are not identifiable.

c) Vulnerable Subjects

Children. This is an observational study that includes vulnerable subjects: children. In Malawi, children are the group at highest risk of contracting clinical malaria, and therefore, are a major target group that would benefit from a highly effective malaria vaccine. The proposed study aims to better characterize the humoral responses associated with protection from blood stage malaria infection in children to inform further malaria vaccine development. As we are only enrolling children whose parents consented to future use of samples in the process of informed consent for the ICEMR cohort study, the proposed study is a MINIMAL RISK study. Children old enough to understand the study provided assent in addition to parent or guardian consent for the ICEMR cohort study. The study procedures meet all the HHS regulatory requirements for research involving children as specified in https://www.hhs.gov/ohrp/regulations-and-policy/regulations/45-cfr-46/index.html#subpartd

3.1.3. Potential Benefits of the Proposed Research to Research Participants and Others

Participants in the ICEMR cohort study will directly benefit from enrollment in the study because if they are ill they will be referred or transported to the local health facility for treatment. The ICEMR study will ensure that malaria rapid diagnostic test capacity and the current nationally recommended malaria treatment is readil y available at the local health center.

No additional direct benefits will be gained by the study participants as a result of the analyses described in this proposed work. However, if efforts to identify and characterize new potential vaccine candidate antigens are successful, this will have great public health benefit for the region, as well as for other malaria endemic areas worldwide.

3.1.4. Importance of the Knowledge to be Gained

If pre-erythrocytic antigens associated with protection from blood stage malaria infection can be identified and characterized, new or improved candidate malaria vaccines could be developed, which will aid in malaria control and elimination efforts in the region and globally.

Section 4 - Protocol Synopsis (Study 1)

4.1. Study Design

4.1.a. Detailed Description

4.1.b. Primary Purpose

4.1.c. Interventions

Type	Name	Description

4.1.d. Study Phase

Is this an NIH-defined Phase III Clinical Trial? ❍ Yes ❍ No

4.1.e. Intervention Model

4.1.f. Masking ❍ Yes ❍ No

❑ Participant ❑ Care Provider ❑ Investigator ❑ Outcomes Assessor

4.1.g. Allocation

4.2. Outcome Measures

Type	Name	Time Frame	Brief Description

4.3. Statistical Design and Power

4.4. Subject Participation Duration

4.5. Will the study use an FDA-regulated intervention? ❍ Yes ❍ No

4.5.a. If yes, describe the availability of Investigational Product (IP) and Investigational New Drug (IND)/ Investigational Device Exemption (IDE) status

4.6. Is this an applicable clinical trial under FDAAA? ❍ Yes ❍ No

4.7. Dissemination Plan

Delayed Onset Studies

Delayed Onset Study#	Study Title	Anticipated Clinical Trial?	Justification
The form does not have any delayed onset studies			

VERTEBRATE ANIMALS

Generation of polyclonal antibodies in rabbits – GenScript via UMB BIORESCO
New Zealand white rabbits will be immunized with proteins adjuvanted with CAF09. CAF09 contains *N,N*-dimethyl-*N,N*-dioctadecylammonium, monomycolyl glycerol, and Poly:IC in cationic liposomes. Whole blood will be drawn and polyclonal antibodies will be obtained from the plasma. Polyclonal antibodies will be assessed for functional activity in multiple, stage-specific *in vitro* assays.

Description of Procedures (species, strain, sex, numbers): Polyclonal antibodies will be generated by GenScript (Piscataway, NJ, USA) under a service contract through the University of Maryland BIORESCO core facility. Antibodies will be assessed for functional activity against malaria parasites.

- **New Zealand white rabbits (NZW)**: For these studies, we will use the common laboratory rabbit *Oryctolagus cuniculus*. All strains employed for this study will be first filial generation rabbits.
- **Assays:** All the proposed experiments will be carried out under the oversight of a specialist at GenScript. *In vivo* studies are proposed that involve vaccination of rabbits with recombinant protein antigens and CAF09 adjuvant. The rabbits will be immunized with 0.1 mg/mL of protein plus CAF09 adjuvant on days 0, 14, 42, and 56 (three rabbits for each antigen). Control rabbits will receive buffer plus CAF09 on the same schedule. On days 28, 56, and 72, rabbits will be bled (10 mL from each rabbit at each time point) to assess antibody-antigen binding specificity by ELISA. These studies will all be performed at GenScript in collaboration with UMB.
- **Number of rabbits**: Female and male rabbits aged <1 year will be used for generation of antibodies. A total of approximately 20 rabbits will undergo vaccination during this project.
- **Veterinary care of animals:** All animals are housed in GenScript's fully AALAC accredited Animal Care facility in accordance with IACUC guidelines with unrestricted access to food and water. Rabbits are observed daily and are maintained in micro-isolation under pathogen-free conditions.
- **Sampling:** Live rabbits will have periodic blood draws to assay for serum antibodies, including specificity and avidity. Polyclonal antibodies collected will be used in *in vitro* functional assays.

Justification: The use of animals is necessary for the proposed study, as *in vitro* models do not mimic the complex cellular and molecular interactions involved in antibody maturation. Studying the impact of adjuvant co-administration on antibody avidity and specificity requires the use of intact animals. Moreover, decades of studies on the rabbit immune system provide a vast base of knowledge and context, which aid in the design, methodology, and interpretation of our experiments. Rabbits are preferred because they yield large serum volumes required for testing in multiple assays, and rabbit IgG can interact with human complement and Fcγ-receptors on immune cells. Every attempt will be made to minimize pain and suffering of the rabbits and the minimum number of rabbits will be used to answer the proposed questions.

Minimization of Pain and Distress: Animals will be restrained briefly for the vaccination and blood draws causing discomfort, which is necessary to conduct these studies. All animal handling will be conducted by trained staff and vaccinated rabbits will be monitored at least twice on the day of vaccination and thereafter at least three times per week for signs of illness, including scruffy coat, hunched posture, and reduced activity. Rabbits displaying any of these features will be euthanized.

Rabbits will be euthanized using an overdose of chemical anesthetic (isoflurane). The methods of euthanasia utilized have been chosen to minimize animal suffering and are consistent with the current recommendations of the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals.

RESOURCE SHARING PLAN

Data sharing plan. The final research data will be shared by open access publication in peer-reviewed journals in compliance with the NIH Data Sharing Policy, and through presentations at scientific meetings such as annual meetings of the American Society of Tropical Medicine and Hygiene, Gordon Research Conferences, etc. The raw peptide array data will be shared with collaborators and others directly or through appropriate repositories after publication in peer-reviewed journals.

Sharing model organisms. N/A

Genomic Data Sharing. We will use previously sequenced pre-erythrocytic antigens, including antigenic variants derived from genomic data from field studies in Malawi and other global regions, for the design of a custom peptide array. If not already available, sequences used for the design of these arrays will be shared with collaborators and others directly and through GenBank after publication in peer-reviewed journals.

AUTHENTICATION OF KEY BIOLOGICAL RESOURCES

Peptide microarrays: Peptide microarrays are fabricated, verified, probed, and scanned by Nimble Therapeutics in accordance to standardized SOPs. The peptide synthesis is digitally controlled and completely automated, which eliminates variability resulting from biological processes or human error. The systematic synthesis of peptides allows all the overlapping peptides for a specific protein to be randomized on the surface of the peptide array. Sera will be bound to arrays, followed by commercially available secondary antibodies. All arrays pass proprietary QC metrics including amino acid QC, synthesis drift, background signal, and signal uniformity. Arrays are scanned and signal is extracted from the images with internally developed software. Signal data is formatted and output to plain-text files via version-controlled R scripts.

Sample tracking and validation: Blood samples were collected into tubes pre-labeled with barcodes that are pre-linked to the human participants. Sample custody and time from sample collection in the field to storage in the site office is tracked in an Access database. Receipt in the molecular lab in Blantyre is documented in Freezerworks. Each sample barcode is scanned into the Freezerworks database with a time stamp and the freezer location information (box, rack, shelf) is entered at that time. Freezer temperatures are tracked. When samples are chosen for microarray probing, the Freezerworks database will be used to find the appropriate sample, which will then be shipped to University of Maryland, Baltimore, on dry ice via courier. The samples will be checked to verify accuracy and completeness upon arrival to UMB, aliquoted, and the aliquot shipped on dry ice via courier to Nimble Therapeutics for peptide microarray probing.

Reagents. A highly immunogenic liposomal adjuvant (CAF09) will be used to immunize rabbits. This adjuvant will be purchased from Statens Serum Institut. Standard analytical methods for bio-macromolecules, including, but not limited to, dynamic light scattering for particle size and surface charge, differential scanning calorimetry or membrane stability and HPLC for component analysis will be used for validation of the adjuvant formulations. Other reagents will be purchased from a reliable commercial vendor that provides certificate of analysis for every lot. Wherever possible, samples will be processed in a single batch to avoid batch effects.

Antibodies. Antibodies for high content imaging will be validated by confirming the expected immunofluorescence pattern. These reagents are obtained through public repositories, such as MR4/BEI Resources or the European Malaria Reagent Repository. Other secondary antibodies or monoclonal antibodies will be sourced from reputable commercial suppliers. Lot/batch number will be recorded, and specificity confirmed by immunoblot and reference ELISA.

Recombinant proteins. Protein expression will be performed using protocols established at the Burnet Institute and transferred to UMB. Genes will be codon-optimized for mammalian cell expression and include a 6xHis tag. HEK293 Freestyle™ cells will be transfected and expressed protein will be collected in the supernatant and purified using Ni affinity columns and gel filtration to obtain purified monomeric proteins. Proteins will be evaluated by SDS-PAGE and western blotting (antibody to 6xHis tag).

Primary human hepatocytes. Primary human hepatocyte cell lines will be purchased from BioIVT, formerly BioreclamationIVT. To ensure reproducibility of experiments, several samples of hepatocyte lots will be selected and screened against already validated cell lot aliquots. Cell lots are selected based on those that have been found to be plateable, exhibit biomarkers previously determined to correlate with optimal infection of human malaria parasites and confirmed susceptibility to malaria.

Parasites. The *Plasmodium falciparum* line to be used for liver stage studies will be NF54, which is a well characterized culture-adapted parasite line often used for sporozoite challenge and liver stage infection studies. It is the parent line from which the *P. falciparum* genome reference strain 3D7 was cloned. This NF54 line was previously cloned and sequenced (Zhang et al., (2018) *Science*). *P. falciparum* (strain NF54)-infected *Anopheles stephensi* will be purchased from Johns Hopkins University Malaria Research Institute insectary and parasitology core and shipped live to USF for aseptic dissection of the salivary glands.