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TITLE: Malaria Prevention by New Technology: Vectored Delivery of Antibody Genes

PRINCIPAL INVESTIGATOR: Gary Ketner, Ph.D.

RECIPIENT: Johns Hopkins University
Baltimore, MD 21218

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Fort Detrick, Maryland 21702-5012

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Malaria Prevention by New Technology: Vectored Delivery of Antibody Genes

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5b. GRANT NUMBER
W81XWH-15-1-0401

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Gary Ketner, Ph.D.

gketner1@jhu.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Johns Hopkins University
Baltimore, MD 21218

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Malaria, caused by parasites of the genus Plasmodium, causes between 500,000 and 1,000,000 deaths per year, mostly in sub-Saharan Africa. Malaria additionally poses a significant threat to US service personnel serving in Africa and elsewhere. No satisfactory malaria vaccine exists. Therefore, the long-term objective of the project is to assess the promise of a novel immunization technology termed vectored-immunoprophylaxis (VIP) in inducing immunity to malaria caused by P. falciparum. VIP employs adeno associated virus (AAV) derived vectors to deliver genes e ncoding protective monoclonal antibodies to animals. Mice transduced by VIP vectors engineered to produce monoclonal antibodies against the P. falciparum circumsporozoite protein (CSP) protect mice from infection by a rodent parasite that expresses P. falciparum CSP. The specific aims of this study are to optimize the VIP system for use in the Aotus nancymaae non-human primate model of P. falciparum infection and to assess the efficacy of VIP in protecting Aotus from P. falciparum infection.

15. SUBJECT TERMS-

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	15	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Malaria, caused by parasites of the genus *Plasmodium*, causes between 500,000 and 1,000,000 deaths per year, mostly in sub-Saharan Africa. Malaria additionally poses a significant threat to US service personnel serving in Africa and elsewhere. No satisfactory malaria vaccine exists. Therefore, the long-term objective of the project is to assess the promise of a novel immunization technology termed vectored-immunoprophylaxis (VIP) in inducing immunity to malaria caused by *P. falciparum*. VIP employs adeno associated virus (AAV) derived vectors to deliver genes encoding protective monoclonal antibodies to animals. Mice transduced by VIP vectors engineered to produce monoclonal antibodies against the *P. falciparum* circumsporozoite protein (CSP) protect mice from infection by a rodent parasite that expresses *P. falciparum* CSP. The specific aims of this study are to optimize the VIP system for use in the *Aotus nancymaae* non-human primate model of *P. falciparum* infection and to assess the efficacy of VIP in protecting *Aotus* from *P. falciparum* infection.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Malaria, *Plasmodium falciparum*, adeno-associated virus, AAV, *Aotus nancymaae*, monkey, vectored immunoprophylaxis, VIP, monoclonal antibody, circumsporozoite protein, CSP.

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

	Timeline (months)	
	Projected	**Completed(%)**
Goal 1: VIP vector development		
1. Prepare, purify and sequence new MAbs	1-12	25
2. Construct first-round vectors	1-18	25
3. Optimize MAb expression in new vectors	3-18	25
Milestone: Selection of candidates for mouse experiments.	12-18	20
Goal 2: Evaluate candidate vectors in mice (See also Table 2 in Project Narrative)		
1. Local IRB/IACUC Approval	Completed	
2. Assess protection by new VIP vectors; IV challenge	6-30	20
3. Assess protection by new VIP vectors; mosquito bite challenge	12-30	20
4. Determine mouse dose-responses; mosquito bite challenge	18-30	0
5. Assess protection by vector pairs; mosquito bite challenge	18-30	0
Milestones: Selection of VIP vectors for *Aotus* studies.	12, 18-24	0

	Timeline (months)	
	Projected	Completed(%)
Goal 3: Determine *Aotus* dose response	7-18	10
1. Local IRB/IACUC Approval	Completed	
Goal 4: *Aotus* challenge 1 (mAb 2A10)	13-30	0
Goal 5: *Aotus* challenge 2 (mAbs TBD)	19-36	0
Milestone: Selection of vectors to pursue in pre-clinical/Phase I trials.	36	0

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1. Major activity: Development of new VIP vectors and assessment of efficacy in mice.

a. A VIP vector encoding a new monoclonal antibody (2H8) directed against CSP was completed. Construction involved use of mass spectrometry to determine a partial amino acid sequence of a mAb prepared from a hybridoma furnished by MVI/PATH (see collaborators below), bioinformatic identification of the germline gene from which the mAb gene arose, RT-PCR-based sequencing of hybridoma mRNA to determine the complete sequence of both chains of the mAb, synthesis of synthetic DNA fragments encoding the mAb variable regions, and insertion of those fragments into the modular VIP vector (see proposal). mAb production by the vectors was confirmed in cell culture and the vectors have been used to transduce mice. These experiments, which are underway, will permit measurement of the levels of mAb produced by the vectors and assessment of protection from mosquito-vectored malaria infection.

b. Two hybridomas producing mAbs against the *P falciparum* pre-erythrocytic antigen CelTOS were obtained from Dr. Evelina Angov, WRAIR (see collaborators, below). Determination of amino acid sequence by RT-PCR based sequencing is underway.

c. The amino acid sequence of the final anti-CSP mAb has been promised by MVI/PATH; negotiations to obtain that information are ongoing. Knowledge of the sequence will eliminate the need for time- and labor-intensive sequence determination and should make vector construction for this mAb rapid.

d. In experiments conducted prior to the current grant's initiation we observed that levels of mAb expression by VIP vectors in some individual *Aotus* monkeys were lower than the levels achieved by other animals transduced with the same vectors. We hypothesized that pre-existing neutralizing antibodies to the vectors in those animals might have been responsible. Therefore, we adapted an existing protocol to use in measuring neutralizing antibody titers against VIP vectors. This assay is being used to screen candidate *Aotus* experimental subjects for potential interfering antibodies for experiments described below.

2. Major activity: VIP dose-response curve in *Aotus*.

a. Because the *Aotus* challenge model for *P. falciparum* infection has not been used in VIP studies, optimal conditions for transduction have not been established. Therefore, we have undertaken an experiment in *Aotus* intended to investigate the dose of vector required to generate sufficient antibodies for future studies, and to assess vector proficiency of two distinct vector serotypes in *Aotus*. Data from these experiments is not yet available.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report.

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

It is expected that vector development will be completed, that efficacy of new vectors in mice will have been determined, and that VIP vectors for use in the second *Aotus* challenge will have been selected. The dose-response experiment will be completed during the next reporting period, and following its completion the first Aotus challenge will begin.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*

- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. CHANGES/PROBLEMS: The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

None.

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Delays in obtaining mAb-related materials (hybridomas) and mAb sequence information despite previous written commitments by the providers have slowed vector development. Only one such commitment is still outstanding, and so this difficulty seems to have been largely solved.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

A delay in hiring staff reported in previous quarterly reports has slowed expenditures somewhat.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

None.

Significant changes in use or care of vertebrate animals.

None.

Significant changes in use of biohazards and/or select agents

None.

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to report.

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to report.

  Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year*

(international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.

Nothing to report.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Nothing to report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report .

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

  - *data or databases;*
  - *biospecimen collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Production of new VIP vectors (one completed, two in process, and a fourth anticipated) will provide material for downstream studies in this project. Information obtained from experiments underway will provide guidance for other approaches to pathogen control by VIP that might be modeled in non-human primates.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*

Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award).*

Gary Ketner Ph.D.
P.I.
6 person-months
Design of experiments, analysis and interpretation of data, organizational tasks
50% of support from Johns Hopkins University sources

Robert J. Adams. DVM
Co-inverstigator
1 person-month
Experiments in *Aotus*
Approximately 95% of support from Johns Hopkins University sources

Renuka Elizabeth Joseph, ScM
Technician
12 person-months
Design and conduct of experiments in culture and in mice; analysis of *Aotus* samples for mAb expression, construction of VIP vectors

Brendan Dolan
Graduate Student
8 person-months
Design and conduct of experiments in culture and in mice; analysis of *Aotus* samples for mAb expression, construction of VIP vectors

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations

(foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

PATH/MVI
2201 Westlake Avenue, Suite 200
Seattle, WA 98121
Furnished anti-CSP monoclonal antibody 2H8; additional mAbs pending

Walter Reed Army Institute of Research
503 Robert Grant Avenue
Silver Spring, MD 20910-7500
Furnished anti CelTOS monoclonal antibodies on a collaborative basis

Malaria Prevention by a New Technology: Vectored Delivery of Antibody Genes

PR140044

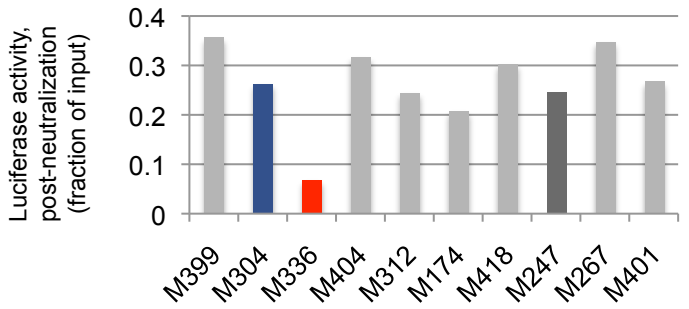
PI: Gary Ketner **Org:** Johns Hopkins University **Award Amount:** 1917485

Study/Product Aim(s)

- **Overall Goal**: Prevention of malaria sporozoite infection by vectored introduction of genes encoding monoclonal antibodies.
- **Aim1**: Identify Monoclonal Antibodies (MAbs) and MAb combinations that optimally confer protection in a mouse model of human P. falciparum sporozoite infection.
- **Aim 2:** Assess protection against sporozoite infection in a non-human primate model of P. falciparum sporozoite infection.

Approach

Newly-developed technology for inducing *in vivo* production of antibody by virus-vectored delivery of pre-formed antibody genes can result in prolonged high-level antibody production and protection from disease. Malaria infection can be prevented by antibody alone, and this technology may therefore offer a route to infection-blocking malaria immunity. The research described here will test that hypothesis in a non-human primate model.



Accomplishment: AAV neutralizing antibody screen. *Aotus* monkey sera were screened for AAV1 neutralizing antibodies by a luciferase-based assay. Neutralizing ability differed substantially among individuals. In experiments conducted prior to this grant, monkeys M304 and M336, above, were transduced with an AAV1-based vector. Monkey 304 exhibited high-level, stable MAb expression, while monkey 336 expresses briefly at a low level. *Aotus* that are candidates for the dose-response experiment have been screened and individuals with low neutralizing activity identified.

Timeline and Cost

Activities CY	1	2	3	N/A
Aim 1, Task 1. Vector development				
Aim 1, Task 2. Murine evaluation				
Aim 2, Task 1. *Aotus* dose-response				
Aim 2, Task 2. *Aotus* challenge I				
Aim 2, Task 3. *Aotus* challenge I!				
Estimated Budget ($K)	**$665**	**$732**	**$521**	**N/A**

Updated: (10/21/2016)

Goals/Milestones

- ☐**Aim 1, Task 1. Vector development**
  In progress
- ☐**Aim 1, Task 2. Murine evaluation**
  In progress
- ☐**Aim 2, Task 1. *Aotus* dose-response**
  In progress
- ☐ **Aim 2, Task 2. *Aotus* challenge I**
- ☐**Aim 2, Task 3. *Aotus* challenge I!**

Comments/Challenges/Issues/Concerns

- N/A

Budget Expenditure to Date

- $353,557.86