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PRINCIPAL INVESTIGATOR: Natasa Strbo

CONTRACTING ORGANIZATION: University of Miami

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Fort Detrick, Maryland 21702-5012

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14. ABSTRACT
The innovative approach taken by our laboratory, relies on secreted gp96-Ig chaperoning antigenic proteins that are efficiently taken up by activated APCs and cross presented via MHC I to CD8 CTL, thereby stimulating an avid, antigen specific, cytotoxic CD8 T cell response. Here we developed malaria vaccine that relies on secreted gp96-Ig chaperoning Plasmodium falciparum antigenic sporozoite proteins CSP and AMA1. The generation of a powerful, cytotoxic anti sporozoite CD8 CTL response by the vaccine is expected to provide prophylactic immunity for malaria by removing infected liver cells before sporozoites can replicate and spread to the erythrocyte stage causing parasitemia.
In the fourth year, we completed all proposed mouse immunogenicity experiments that addressed the effect of secondary 293-gp96-Ig $_{PfAMA1\text{-}PfCSP}$ immunization and induced memory responses as well as we compared the immunogenicity of the 293-gp96-Ig$_{PfAMA1\text{-}PfCSP}$ vaccine to the immunogenicity of NMRC-M3V-D/Ad-PfCA vaccine. We found that gp96-Ig vaccination provided stronger antigen specific CD8 T cell responses in the liver and uterus compared to NMRC vaccine. Since we have already completed manufacturing of GMP-grade vaccine material, we are ready for non-human primate studies.

15. SUBJECT TERMS
Malaria, Plasmodium Falciparum, circumsporozoite protein (CSP), apical membrane antigen-1, vaccine (AMA1), heat shock proteins, gp96-Ig, cytotoxic T cells, cell mediated immunity

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1. Introduction

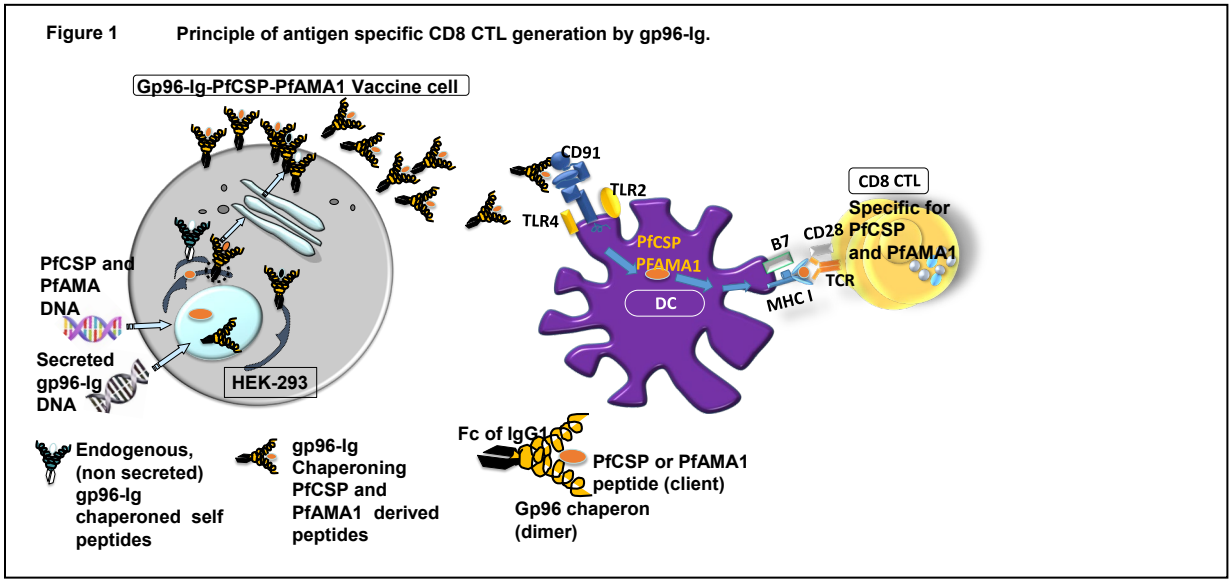
Proposed vaccine principle relies on cell-based secreted gp96-Ig chaperoning PfCSP and PfAMA1 proteins that are efficiently taken up and cross presented by activated DC via MHC I to CD8 CTL, thereby stimulating an avid, antigen specific, cytotoxic T cell response (Fig 1). This vaccine principle has been used successfully in murine models of cancer, in non-human primates for SIV vaccination and in clinical trials for the treatment of non-small cell lung cancer patients [1-9]. The generation of a powerful, cytotoxic PfCSP- and PfAMA1-specific CD8 CTL response by the vaccine is expected to provide prophylactic immunity against malaria liver-stage parasites.

2. Keywords

Malaria, vaccine, heat shock proteins, secreted gp96-Ig, CD8 T cells,

3. Accomplishments

What were the major goals of the project?



The overall goal of our project was to combine two Plasmodium falciparum (Pf) antigens circumsporozoite protein (CSP) and apical membrane antigen-1 (AMA1) with a novel method of immunization that is based on the heat shock protein (HSP) gp96-Ig vaccine platform to enable production of a strong, protective, and long lasting cell-mediated immune (CMI) response (interferon gamma [IFN-γ]-positive CD8+ cytotoxic T cells) (**Fig 1**).

Specific Aim 1: Construction of 293-gp96-IgPfAMA1-PfCSP and 293PfAMA1-PfCSP vaccine cell lines.
Task 1a: HEK-293 cells (purchased from ATCC) were transfected with DNA constructs encoding codon-optimized PfCSP and PfAMA1 antigens. The DNA constructs were provided by Dr. Villasante to Dr. Strbo for completion of this task. The transfection took place at UMSM and was performed by Dr. Strbo. Stable transfectants were selected by antibiotic resistance and surviving clones screened for expression of PfCSP and/or PfAMA1 antigens by Western blotting of whole-cell lysates and subsequent staining with commercially available antibodies to PfCSP and PfAMA1. Timeframe: Months 1-6. **Completed in this timeframe**
Task 1b: 293PfAMA1-PfCSP cells generated in Task 1a were additionally transfected with a DNA construct encoding gp96-Ig (either mouse or human), previously used by the Strbo laboratory for mouse, non-human primate and human clinical studies. These transfections were also performed by Dr. Strbo (with technical support) and stable transfectants selected by antibiotic resistance as described in Task 1a. Surviving clones were screened quantitatively for adequate secretion of gp96-Ig into cell culture supernatants by an anti- (mouse or human)-IgG ELISA test. Timeframe: Months 7-10. **Completed in this timeframe.**
Specific Aim 2: Immunogenicity of the 293-gp96-IgPfAMA1-PfCSP vaccine in mice

Task 2a: To determine the potential protective efficacy of the gp96-Ig vaccine, we will perform comparative immunogenicity studies in mice to a vaccine that was previously tested in mice and NHP and shown to be protective in humans. This vaccine, NMRC-M3V-D/Ad-PfCA, will be provided to the Strbo laboratory by Dr. Villasante. The first task in specific aim 2 is to determine whether the route of immunization of the gp96-Ig vaccine influences the degree of liver-infiltration by Pf-antigen specific CD8+ T cells following a primary immunization. Primary immunization with gp96-Ig vaccines is shown in mice to lead to CD8+ T cell proliferation which peaks at 5 days after immunization and can be detected in isolated tissues. These studies are currently undergoing at UMSM by Dr. Strbo (with technical support) and will require approximately 80 mice for completion. Immune responses will be measured by ELISpot and intracellular staining for Th1 cytokines (analyzed by flow cytometry) following re-stimulation of isolated cells with PfCSP and PfAMA1 overlapping peptide pools (provided to Dr. Strbo by Dr. Villasante). The objective for this task is to eliminate the immunization route which proves least effective at stimulating liver-infiltrating CD8+ T cells before advancing to task 2b. Timeframe: Months 11-14. **Completed in Month 15**.

Task 2b: The purpose of this task is to determine non-inferiority at the time of the vaccine 'memory' response by comparative vaccination with the 293-gp96-IgPfAMA1-PfCSP vaccine versus the NMRC-M3V-D/Ad-PfCA vaccine. To complete this task, Dr. Strbo (with technical support) will perform studies using the two optimal immunization routes identified in Task 2a for the gp96-Ig vaccine and treat mice with either the gp96-Ig vaccine or the NMRCM3V- D/Ad-PfCA vaccine on day 50 after the indicated 'priming' regimens for each treatment. The memory response will be measured on day 5 following the vaccine 'boost', and immune responses measured as described in task 2a from liver-infiltrating, splenic, mesenteric lymph node and peripheral blood T cells. An estimated 80 mice will be required to accomplish this aim in 2 different strains of mice. Timeframe: In progress, Months 15-20. **Completed in Month 24**.

Specific Aim 3: GMP Production, Safety and Immunogenicity analysis of 293-gp96-IgPfAMA1-PfCSP in Rhesus Macaques

Task 3a: The first objective of specific aim 3 is to manufacture GMP-grade vaccine material for use in non-human studies and potentially clinical studies during subsequent clinical trials. To complete this aim, Dr. Strbo will work with the GMP cell manufacturing facility in place within the Diabetes Research Institute at the UMMS, which is the GMP site where previous gp96-Ig vaccines were manufactured for human clinical studies for patient with advanced NSCLC. GMP production will take place by large-scale cell culture in approximately 120 T-175 flasks. Harvested cells will be irradiated and subsequently stored frozen in the vapor phase of liquid nitrogen until use. The release criteria for the manufactured product will include sterility testing (performed by WuXi Apptec), confirmation of adequate secretion of gp96-Ig (measured by anti-human IgG ELISA assays from cell culture supernatant) and expression of PfCSP and PfAMA1 (measured by Western blotting using monoclonal antibodies specific for PfCSP or PfAMA1). Timeframe: Months 15-20. **Completed in Timeframe**.

Task 3b: The objective of task 3b is to determine whether immunization with 293-gp96- IgPfAMA1-PfCSP is non-inferior to immunization with NMRC-M3V-D/Ad-PfCA in rhesus macaques. These studies will be performed at NMRC under the direction of Dr. Villasante and together with key personnel at NMRC including Dr. Martha Sedegah. In these studies two groups of 10 rhesus macaques will be immunized with either vaccine according to the schedule outlined in the project narrative and the CD8+ T cell mediated immune responses specific to PfCSP and PfAMA1 antigens evaluated at the 'memory' time points from the blood and various tissues including liver. Evaluation of the immune response will be performed by re-stimulation of isolated T cells with PfCSP and PfAMA1 overlapping peptides (available at NMRC) and then the production of inflammatory cytokines by those T cells will be evaluated both by ELISpot and FLUOROSpot assays. In addition to specifically evaluating the CD8+ T cell response, the PfCSP and PfAMA1 specific antibody responses will also be evaluated using serum samples collected at the 'memory' time points by ELISA assays. Timeframe: Months 21-36. **Completed in August 2019. Non-human portion of the study was delayed because of the administrative issues with renovation of nonhuman primate facility and transfer of funds for purchasing of nonhuman primates.**

What was accomplished under these goals?

1) Major activities: We generated secreted gp96-Ig vaccine that expresses PfCSP and PfAMA antigens, preformed all mouse vaccination experiments and nonhuman primate vaccination experiments including analysis of vaccine induced immune responses in mice and nonhuman primates. Presented results on the Annual Meeting of American Association of Immunologist (AAI) May 2016 and on upcoming meeting in May 2020. Manuscript will be submitted to Scientific Reports in January 2020.

2) Specific Objectives: We achieved all 3 specific Aims: Specific Aim 1: We generated 293-gp96-IgPfAMA1-PfCSP and 293PfAMA1-PfCSP vaccine cell lines; Specific Aim 2: We completed immunogenicity testing and analysis of the 293-gp96-IgPfAMA1-PfCSP vaccine in mice; Specific Aim 3: We finished GMP Production, Safety and Immunogenicity analysis of 293-gp96-IgPfAMA1-PfCSP in Rhesus Macaques. Significant results for each Aim are listed below.
3) Significant results or key outcomes, major findings:
We were successful in generating secreted cell vaccine, 293-gp96-IgPfCSP-PfAMA1 cells by co-transfecting 293 cells with gp96-Ig, PfCSP, and PfAMA1 (**Fig. 2**). Completion of the 293-gp96-IgPfCSP-PfAMA1 vaccine cell line served as the initial 'go', 'no-go' milestone for this application. We reached this milestone within the first 6 months of the grant period as stated in . We have also manufactured GMP-grade vaccine material for use in non-human primate studies and potentially clinical studies.

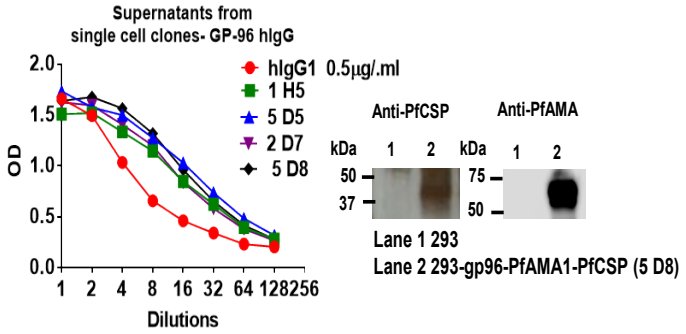


Figure 2. 293-gp96-Ig-PfCSP-PfAMA1 ELISA for gp96-Ig production and Western blotting for PfCSP and PfAMA1 expression. One million of 293-gp96-Ig-PfCSP-PfAMA1 single cell clones were plated in 1 ml for 24 h and gp96-Ig production in the supernatant was determined by ELISA using anti-human IgG antibody for detection with mouse IgG1 as a standard. Malaria antigens expression was confirmed by Western blotting

We also completed head-to-head immunogenicity comparison to the protective, T cell immunity-based NMRC-M3V-D/Ad-PfCA vaccine (DNA prime/adenovirus boost regimen). We demonstrated that the 293-gp96-Ig-PfAMA1-PfCSP cell-based vaccine is

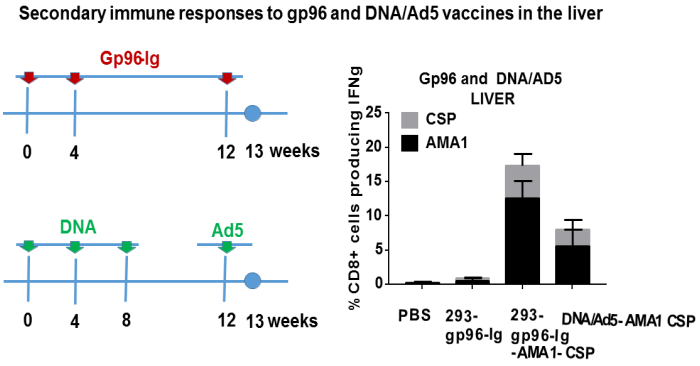


Figure 3. Comparison of frequencies of PfCSP and PfAMA1 specific CD8+ T cell responses in liver after immunization with secreted gp96-Ig and DNA/Ad5 vaccines. A) One million of 293-gp96-Ig and 293-gp96-Ig-PfCSP-PfAMA1 cells were injected in B6 mice by s.c. at week 0, 4 and 12. Plasmid VR1020 containing PfAMA1 gene (VR2571) and plasmid VR1020 containing PfCSP gene (VR2577) (100ug each) were injected by i.m.at week 0, 4 ,8 and on week 12 animals were boosted with adenovirus5-vectored vaccine encoding PfCSP and PfAMA1 (AdPfCSP and AdPfAMA1) (1x108 PU/administration) by i.m. Five days after the last boost, all mice were sacrificed and frequency of PfAMA1 and PfCSP specific CD8 T cell responses in the liver was measured by intracellular cytokine staining (ICS) assay and analyzed by flow cytometry. Total intrahepatic lymphocyte were cultured overnight in medium only or with pool of overlapping PfAMA1 and PfCSP peptides. B) Cells were stained for surface CD3, CD8 and intracellular cytokine IFNg. Cells were analyzed on flow cytometer and bar graph shows percentage of CD3+CD8+ cells that produce IFNg

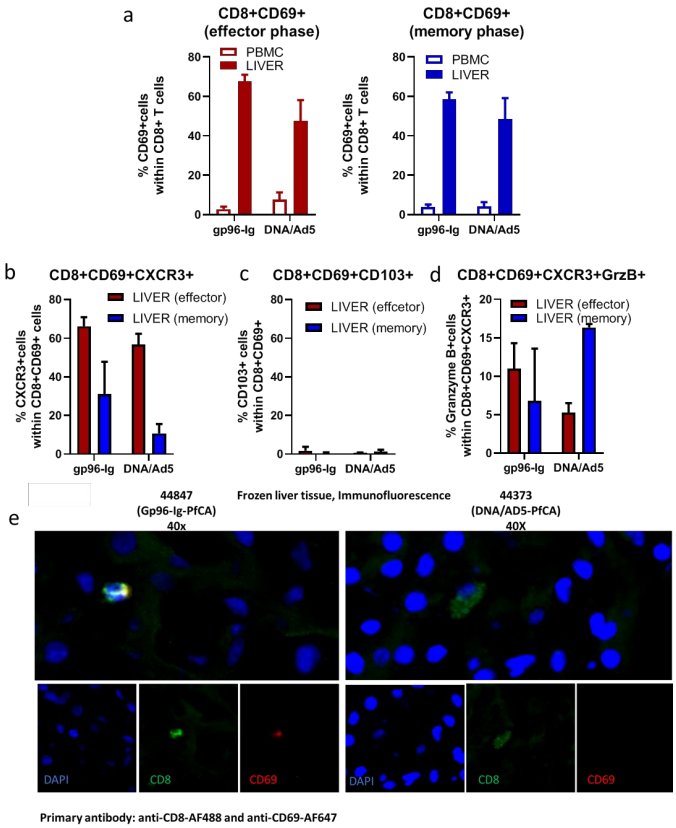


Figure 4. Gp96-Ig vaccine induces resident tissue memory responses in the liver. (a) Frequency of CD8+CD69+ T cells as a percentage of total CD8+ T cells in peripheral blood mononuclear cells (PBMC) and intrahepatic lymphocytes (Liver) at week 26 (for gp96-Ig) and 28 (for DNA/Ad5 (effector phase)) and week 36 (for both vaccines) (memory phase). (b) Frequency of CXCR3+ cells within CD8+CD69+cells (c) frequency of CD103+ cells within CD8+CD69+ cells and frequency of granzyme B+ cells within CD8+CD69+CXCR3+ cells (e) Frozen liver tissue sections of gp96-Ig (#44847) and DNA/Ad5 (#44373) vaccinated non-human primates. Immunofulorescence staining of CD8 (shown in green) and CD69 (shown in red) with co-localization of CD8 and CD69 (shown in yellow). Original magnification 40X with DAPI nuclear staining shown in blue.

immunogenic and can induce superior memory responses compared to NMRC-M3V-D/Ad-PfCA vaccine. Most importantly, we found that gp96-IgPfCSP-PfAMA1 subcutaneous route of vaccination induces dramatic increase in the liver-infiltrating PfCSP and PfAMA1-specific CD8+ T cells **(Fig 3**). The magnitude and quality of malaria antigen-specific CD8+ T cell responses are believed to play a critical role in eliminating intra-hepatic stages of the malaria parasite. Moreover, failure of RTS,S vaccine to induce CD8+ T cell responses was considered a main weakness of this vaccine approach. Thus our findings are strongly supportive of the novel gp96-Ig cell based vaccine as a unique liver-homing, CD8 + T cell pre-erythrocytic stage specific vaccine strategy. Following successful completion of second milestone, we completed all experiments under Specific Aim 1, 2, 3a and we met “go criteria” for moving forward to NHP studies (Specific Aim 3b). Our collaborators, Dr. Eileen F. Villasante and her team (Drs. Wijayalath and Sedegah) finished with the nonhuman primate studies in August 2019 and we finished analysis on vaccine induced immune responses by the end of September 2019. The key outcomes and major findings from the nonhuman primates studies were: We report, that DNA/Ad5 vaccine induced malaria-specific CD8+ T cell and antibody responses exclusively in the blood (**Fig 7**), while gp96-Ig predominantly induced malaria specific CD8+ T cells responses in the liver (**Fig 4-6**). The majority of the intrahepatic malaria specific CD8+ T cells expressed CD69 and CXCR3, hallmark of tissue resident memory T cells (TRM) (**Fig 4**). This is the first report describing vaccine induced intrahepatic CD8 T cells that produce IL-2 that is relevant for maintenance of effective memory responses (**Fig 6**).

What opportunities for training and professional development has the project provided?

Laura Romero and Dr. Natasa Strbo participated on conferences (Annual Meeting of AAI, May 12-17 2016 in Seattle, WA and May 8-12 2019 in Honolulu, HA) and seminars (June 8th 2018 in NMRC, Silver Spring, MD and September 30th 2019 University of Miami, FL).

How were the results disseminated to communities of interest?

Dr. Strbo was invited by Dr. Villasante to give a Seminar at Naval Medical Research Center on June 8th 2018 (Title” Bridging the gaps in vaccine development with secreted heat shock protein gp96-Ig”).

Dr. Strbo gave a Seminar at the Department of Microbiology and Immunology, University of Miami on September 30th 2019 (Title “Challenges and new strategies for development of efficacious and long-lasting malaria vaccine”). This seminar series are part of the Undergraduate Microbiology and Immunology Corse giving opportunity to undergraduate students to increase interest in learning about careers in science.
On March 24th through March 26th 2019, Dr Strbo participated in the annual Leadership Summit organized by the United Nations Foundation's Nothing But Nets campaign, a three-day event to train and mobilize the next generation of grassroots leaders in the fight against malaria. As a Summit participant and scientist, Dr. Strbo together with her colleagues went to Capitol Hill and urge policymakers to support U.S. funding for global malaria programs. In collaboration with UN Foundation Nothing but nets, Dr. Strbo will be one of the organizers of event that will explore the interconnective nature between health, climate, migration, and policy in the Western Hemisphere with a specific focus/case study on malaria. This event is planned for April 2020 at University of Miami Coral Gables Undergraduate Campus. Dr, Strbo will give a TED-talk about gp96-Ig malaria vaccine development.

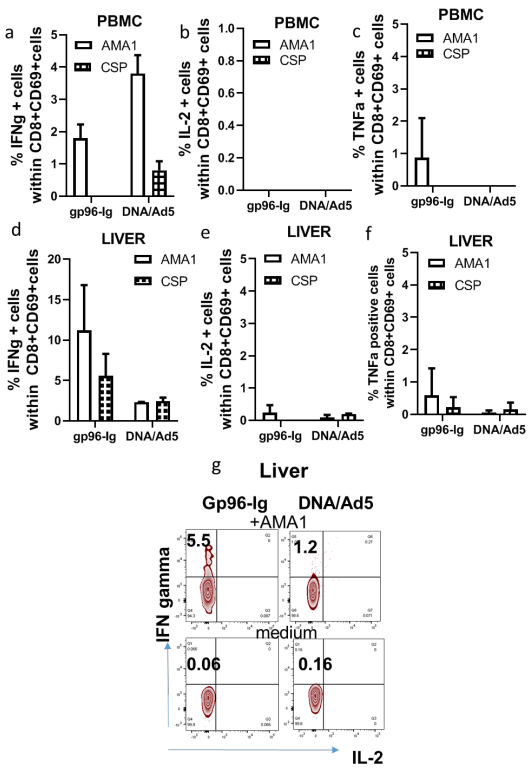


Figure 5. Gp96-Ig vaccine induces predominant IFN gamma-specific CD8+ T cell responses in the liver. Non-human primates were vaccinated with gp96-Ig, by s.c. route, at week 0, 5 and 25 or with DNA/Ad5 by i.m route at week 0, 4, 8 (DNA prime) and 25 (Ad5 boost). Two animals from each group were euthanized at week 26 (for gp96-Ig group) and at week 28 (for group DNA/Ad5). Peripheral blood mononuclear cells (PBMCs) and liver intrahepatic lymphocytes were stimulated with PfCSP and PfAMA1 overlapping peptides for 18h and intracellular expression of IFNgamma, IL-2 and TNFa in CD8+CD69+ cells were analyzed by flow cytometry. Frequency of cytokine expressing (IFNg, IL-2 and TNFa) as a percentage of CD8+CD69+ peripheral blood mononuclear cells (a-c,g) or intrahepatic cells (d-f,).

What do you plan to do during the next reporting period to accomplish the goals?

Nothing to report

4. Impact

a. **What was the impact on the development of the principal discipline(s) of the project?**

Malaria remains a significant disease threat to deployed military personnel with the risk varying year to year depending on the location of combat, humanitarian, and peacekeeping missions. Most military personnel from developed countries are non-immune to malaria and suffer high rates of morbidity and mortality when infected. Although, chemoprophylaxis to prevent malaria infection and personal protective measures to prevent mosquito bites are available, sub-optimal compliance with these measures along with spreading parasite resistance to anti-malarials, drive the need for a vaccine that can provide high level long-term protection. Unfortunately, malaria parasites are by far the most complicated organisms that anybody has ever tried to vaccinate against. The RTS,S (GSK) vaccine, farthest along in field testing, is based on a single malaria antigen, CSP. Another promising approach is the attenuated whole sporozoite vaccine, PfSPZ Vaccine (Sanaria). When immune responses to PfSPZ Vaccine are analyzed, no single target antigen has been identified that explains the full extent of host immunity. This suggests that the protective vaccines work by the summation of many immune responses against multiple antigens on the parasite. Whilst multiple immune mechanisms appear to contribute to protection against pre-erythrocytic stages, the generation of liver-resident parasite-specific memory CD8+ T cells is emerging as a key determinants of protective immunity. The design of strategies inducing these type of responses and the identification of

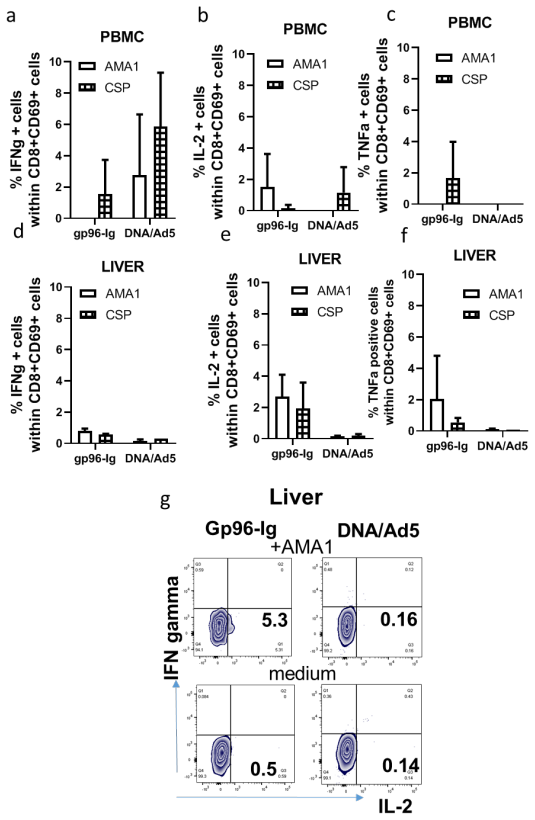


Figure 6. Gp96-Ig vaccine induced antigen specific memory CD8+ T cells in the liver produce IL-2. Non-human primates were vaccinated with gp96-Ig, by s.c. route, at week 0, 5 and 25 or with DNA/Ad5 by i.m route at week 0, 4, 8 (DNA prime) and 25 (Ad5 boost). Two animals from each group were euthanized 11 weeks after the last boost (week 36). Peripheral blood mononuclear cells (PBMCs) and intrahepatic lymphocytes were stimulated with PfCSP and PfAMA1 overlapping peptides for 18h and intracellular expression of IFNgamma, IL-2 and TNFa in CD8+CD69+ cells were analyzed by flow cytometry. Frequency of cytokine expressing (IFNg, IL-2 and TNFa) as a percentage of CD8+CD69+ peripheral blood mononuclear cells (a-c,g) or intrahepatic cells (d-f).

protective target antigens is instrumental for the development of an efficacious malaria vaccine. The main goal of our project was to developed novel heat shock protein (HSP)-based vaccine approach that induces multi-antigen specific CD8 T cell responses systemically and in the liver, a novel approach inducing long term immunity and memory responses that will ultimately prevent the parasite infection and/or development and clinical disease. These studies were designed to enable a phase I clinical trial in humans. The ultimate goal is to develop a malaria vaccine for the military that provides long-term protection and is highly effective and practical,

What was the impact on other disciplines?

We have developed and produced the first cell based-malaria vaccine platform that can be used as a novel vaccine platform for development of other emerging infectious diseases.

What was the impact on technology transfer?

In the next phase of this vaccine development, results from our study are very likely to make an impact, on commercial technology, including transfer of results to entities in government or industry (Heat Biologics Inc). Heat Biologics Inc. has license for gp96 cell-based vaccine derived from the non-small cell lung cancer (NSCLC) cell line AD100 for treatment of metastatic NSCLC and currently is running clinical studies. The gp96-Ig cancer vaccine has demonstrated good safety and tolerability (no reported SUSARs [Suspected Unexpected Serious

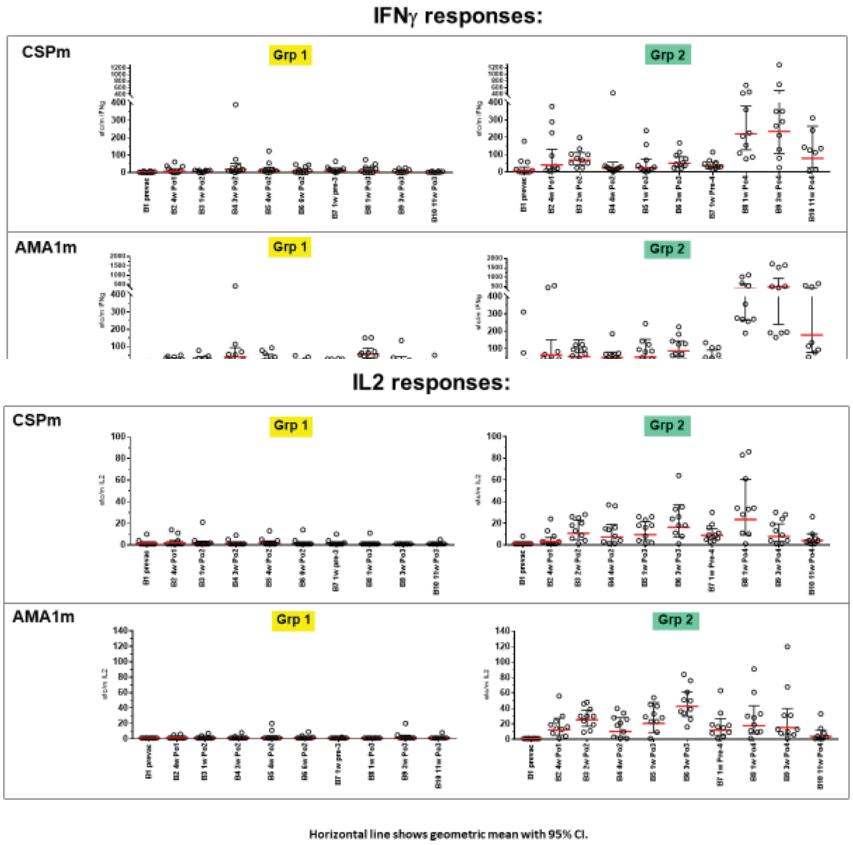


Figure 7. DNA/Ad5 vaccination induces higher magnitude of IFN gamma and IL-2 cellular responses in the blood compared to 293-gp96-Ig vaccination. Antigen-specific circulating peripheral blood mononuclear cells (PBMC) secreting single or multiple cytokines were evaluated using FluoroSpot kits. One million PBMCs were stimulated 40-42h with full-length PfCSP and PfAMA1 overlapping peptides at final concentration of 1.25 ug/mL. PHA was used as a positive control for cell viability. Negative control unstimulated PBMCs wells received medium only. The mean number of spots of the test sample was then calculated and expressed as sfc/million PBMCs (sfc/m). Group 1-293-gp96-Ig-PfCA Group 2: DNA/Ad5-PfCA.

Adverse Reactions]) and stimulates a potent immune response. These clinical studies and our preclinical studies provide important evidence that gp96-Ig-based cellular vaccines are safe and function similarly in humans, rodents and non-human primates.

What was the impact on society beyond science and technology?

Malaria-free countries have five times greater economic growth than those with malaria. Eradicating malaria could save around 11 million lives and $2 trillion in economic losses. That's why results from this project are likely to make an impact beyond the bounds of science and academic world on areas such as improving public health with overall impact on the improvement of social and economic conditions.

5. Changes/Problems

Actual or anticipated problems or delays and actions or plans to resolve them

During the period of the grant we had several changes in the Key personnel at the University of Miami as well as at NMRC: Dr. Strbo was appointed the PI role in May 2015, replacing Dr. Podack for medical reasons who unfortunately passed away in October 2015. Dr. Richie (NMRC partnering PI) was replaced with Dr. David Fryauff and subsequently with Dr. Kim Edgel and key personnel Dr. Wathsala Wijayalath, Dr. Martha Sedegah, and Noelle Patterson, M.S. In August 2017 Dr. Edgel received Permanent Change of Station orders and transferred from the Naval Medical Research Center (NMRC) to the US Naval Medical Research Unit No. 2 in Phnom Penh, Cambodia and Dr. Villasante became the PI. Changes in the Key personnel, in addition to administrative delays and NMRC animal facility renovations and transfer of the funds, were causes of the delayed nonhuman primate portion of this study. Despite the above personnel changes we established a very strong collaborative study with Dr. Villasante and her team, generated novel malaria vaccine, and accomplished all proposed objectives of the study.

6. Products

Publications, conference papers, and presentations

L. Romero, E.R. Podack E.F. Villasante, K.A. Edgel, N. Strbo. Secreted heat shock protein gp96-Ig vaccine for malaria prophylaxis. J. Immunol May 1, 2016, 196 (1 Supplement) 146.10. (Published Abstract). Acknowledgement of federal support (YES).

Laura Padula, Eva Fisher, Wathsala Wijayalath, Noelle B. Patterson, Jun Huang, Harini Ganeshan, Katelyn O'Neill, Denisse Garcia, Irina V. Etobayeva, Kimberly A. Edgel, Eckhard Podack, Martha Sedegah, Eileen F. Villasante and Natasa Strbo. Secreted heat shock protein gp96-Ig vaccine induces malaria specific intrahepatic CD8 T cell responses. J Immunol **May 8-12 2020** (Published Abstract). Acknowledgement of federal support (YES).

Laura Padula, Eva Fisher, Wathsala Wijayalath, Noelle Patterson, Martha Sedegah, Harini Ganeshan, Jun Huang, Francois Bates, Margaret A. Hanson, Monica L. Martin, Katelyn O'Neill, Denisse Garcia, Irina V. Etobayeva, Kim Edgel, Eckhard Podack, Thomas L. Richie, Eileen F. Villasante and Natasa Strbo. Secreted heat shock protein gp96-Ig malaria vaccine: A promising vaccine strategy for induction of intrahepatic CD8+ T cell responses. (In preparation for submission to Scientific Reports, **will be submitted in January 2020**). Acknowledgement of federal support (YES).

Presentations:

Dr. Strbo. " Bridging the gaps in vaccine development with secreted heat shock protein gp96-Ig", Naval Medical Research Center, June 8th 2018

Dr. Strbo "Challenges and new strategies for development of efficacious and long-lasting malaria vaccine", Department of Microbiology and Immunology, Miller School of Medicine University of Miami, September 30th 2019.

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.

We generated vaccine cell line that expresses PfCSP, PfAMA1 and gp96-Ig that will be distributed freely or deposited into a repository/stock center making them available to the broader research community, either before or immediately after publication. If we assume responsibility for distributing the newly generated vaccine cells, we will fill requests in a timely fashion. In addition, we will provide relevant protocols and published phenotypic data upon request. Back-up nonhuman primate specimens will also be made available to interested parties. The PI's will maintain unlimited and priority access to any back-up specimens in the repository for the primary purpose of repeating experiments or investigating alternate hypotheses. However, extra specimens from the repository that are not needed or used by any of the programs investigators will be made available as a resource for outside investigators for experiments outside the scope of work in this Program. These requests will be directed to Dr. Strbo who will inform Dr. Villasante of the request. If the requested specimen is no longer needed or anticipated to be needed by any of the investigators, then the specimen will be made available to the requestor. Material transfers will be made with no more restrictive terms than in the Simple Letter Agreement (SLA) or the Uniform Biological Materials Transfer Agreement (UBMTA) and without reach through requirements, as well as in accordance with institutional policy. Should any intellectual property arise, which requires a patent, we will ensure that the technology (materials and data) remains widely available to the research community in accordance with the NIH Principles and Guidelines document.

Inventions, patent applications, and/or licenses

Invention disclosure has been filed at University of Miami, Jul 23, 2018. (UM-D2019-0019 Title: Secreted gp96-Ig vaccine for Malaria Prophylaxis)

Other Products

We collected and stored nonhuman primate liver samples

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:	*Natasa Strbo*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	0000-0003-2152-2433
Nearest person month worked:	1
Contribution to Project:	*Dr. Strbo has been principal investigator and project leader, involved i management, coordination, study design, data analysis and she wrote manuscript.*
Funding Support:	
Name:	Laura Romero
Project Role:	Staff
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	2
Contribution to Project:	Ms. Romero generated vaccine cells, preformed mouse experiment, and analyzed nonhuman primate experiments.
Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

Collaborating PI, Dr. E. Villasante, NMRC.

8. Special Reporting Requirements

Independent report will be send from Collaborating PI, Dr. Eileen Villasante (NMRC).

Appendices