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TITLE: GMP Production and Clinical Trial of a Self-Assembling Protein Nanoparticle and Toll-Like Receptor Liposomal MPL Adjuvanted Malaria Vaccine

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14. ABSTRACT

There are two main efforts in this project: First, the manufacture of a protein nanoparticle, (FMP014) as the protein base for a malaria vaccine. Second, the development and manufacture of an adjuvant system (Army Liposome Formulations) that was designed to increase the immune response to the protein nanoparticle FMP014. The first year of this three year project was focused on the GMP manufacture of these two key components and was reported last year. The results of second year of this project, reported here, are focused on the evaluation of the two components, both chemically and immunologically. To evaluate the components chemically we focused on identification of the bio-physical characteristics (identification by sequence analysis, size assembled nanoparticle, identification by monoclonal and stability over time) of each component; for evaluation of the immunological characteristics we focused on the immune responses (titer to NANP repeat and C-terminal epitopes, demonstration of induction of protective antibodies; and induction of cellular cytokines in mice and non-human primates) when the FMP014 and adjuvant were combined and injected into the animals. In addition we began an evaluation of the potential toxicity of the components ether individually or combined. These efforts were accomplished by a standard multi-dose toxicology study in rabbits. This investigation is still in progress. The results of these evaluations are reported here.

15. SUBJECT TERMS

None listed

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1. INTRODUCTION

The JW14011 project consisted of two main cGMP product development efforts with the goal of translation into first-in-human, Phase I clinical trials to assess the safety, reactogenicity, immunogenicity and efficacy of a novel malaria vaccine candidate. Initially, the Malaria Vaccine Branch (MVB) focused on the development and manufacture of the malaria target, a self-assembling protein nanoparticle (SAPN) based on displaying polypeptide epitopes from the *P. falciparum* Circumsporozoite protein (CSP) on the surface of SAPN. The final molecule was designated, FMP014, for Falciparum Malaria Protein #14. Concomitantly, the Military HIV Research Program (MHRP) focused their efforts on the development and manufacture of an adjuvant system, Army Liposome Formulations, (ALF) designed to increase the immune response to the protein nanoparticle FMP014 and other militarily relevant targets of interest (i.e. HIV, Campylobacter, Dengue, etc.). The initial years of this multi-year project focused on the GMP manufacture of the two key components (antigen and adjuvant). Results from these cGMP manufactures and the evaluation of the two vaccine components, chemically and immunologically, were reported previously and will only be briefly summarized here.

Briefly, for the evaluation of the biophysical characteristics of the FMP014 SAPN molecule, various tests were performed to assess the identity, purity and homogeneity of the molecule. These tests included full plasmid nucleotide sequence of the clone, N-terminal sequencing of the protein monomer, mass spectrometry to assess the size of the monomer, dynamic light scattering to analyze the size of the final-assembled SAPN, and SDS-PAGE/Western blotting to verify the homogeneity and stability of the monomer protein over time. For the evaluation of the immunological characteristics induced by the FMP014/adjuvant vaccine candidate, we focused on measuring immune responses such as the antibody titer to NANP repeat and C-terminal epitopes, induction of protective antibodies, and induction of cellular cytokines in mice and non-human primates (NHP). To assess the safety of FMP014 and the various ALF adjuvants including ALFQ, ALFQA and ALFA, several repeat-dose toxicity studies in New Zealand white rabbits were performed. Overall, there was no antigen or adjuvant related safety signal triggered causing concerns for advancing FMP014 and ALFQ or the other adjuvants evaluated into first in human, Phase I clinical trials.

Evaluation of ALF adjuvants, ALFQ and ALFA, for product release and stability used Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) as the analytical method for lipids

quantification. LC/MS/MS measurements were performed by Avanti Polar Lipids (Alabaster, AL).

Extensive delays were experienced due to unexpected outcomes during standard stability testing of the ALF adjuvants. Briefly, the regulatory timelines for implementing a Phase 1 clinical trial of the FMP014/ALFQ were delayed due to an Out of Specification (OOS) OOS-279 investigation initiated by Avanti Polar Lipids (contractor that performed the analytical methods to quantitate the lipids and adjuvant components) pursuant to failures in stability testing of ALFQ lot #1974 and ALF43 lots #1962 and #1967 for 2-8°C stability samples at the 12 month stability time point. While initial investigations found no obvious reasons for the testing failures, subsequent investigations identified several inconsistencies in the performance of the analytical methods by Avanti Polar Lipids. Most notably, there was an uncontrolled change to the instrumentation, which could have had an effect on the qualification status of the methods and instrument. Secondly, it was determined that possible laboratory errors related to the routine preparation and use of the working calibration standards may have impacted their stability (and concentration). Pursuant to Avanti's internal QC investigation, Avanti's Quality Unit deemed the methods performance unreliable and the system suitability parameters an insufficient measure of assay performance. These failures were associated with the performance of the analytical methods and not to the final adjuvant products, ALF43 (ALFA) and ALFQ. Avanti Polar Lipids initiated methods development under a Corrective and Preventative Action proposal, (CAPA-039), September 2017, to better understand the capabilities of all five adjuvant component test methods and to improve their system suitability parameters to better monitor test method performance. Approximately one year later (Sept 2018), following extensive methods development, all cGMP lots of adjuvants were retested using the requalified methods and calibrated standards. All adjuvant lots passed identity and composition testing within the established reset specifications. Based on these positive results, MVB/MHRP initiated translational efforts of the FMP014 (PfCSP- SAPN) formulated in ALFQ to a Phase 1 trial with Controlled Malaria Infection Challenge (CHMI). The focus of the work in the next period shifted to the development of clinical protocol and completion of the CMC sections of the IND; substantially undertaken from Oct 2018 through the end of the previous reporting period (ending about 31 July 2019).

Following extensive consideration on a study design and documents preparation for filing an application to the FDA on the clinical product, FMP014/ALFQ [IND# 19100 & DMF# 18760 – ALF55 containing QS-21(ALFQ) adjuvant for vaccines], the safety portion of the study (Part

A) initiated in April 2020 at the WRAIR CTC in the midst of a global COVID-19 pandemic. See Table 1 for details on key review and approval dates.

2. KEYWORDS:

Plasmodium falciparum
Malaria
Vaccine
SAPN
Nanoparticle
Adjuvant

3. ACCOMPLISHMENTS:

What was accomplished under these goals in Year 4?

a) Successful requalification of the analytical methods used to assess adjuvant components, lipids, 3D-PHAD and QS-21.

b) Retesting of cGMP adjuvant materials for product release and stability testing.

c) The final clinical protocol study design identified.

d) CMC sections of the FMP014 IND completed.

e) FMP014 IND was submitted to the FDA August 2019

f) Subject screening and recruiting for the trial

g) WRAIR CTC initiates FMP014/ALFQ Phase 1 Part A portion of the trial in April 2020

h) Safety data on low dose and high doses of antigen and adjuvant collected

Key Reviews and Approval

Table 1:

List ofkey events and approvals for FMP014/ALFQ clinical study	Date
Protocol approval by WRAIR Scientific Review Committee (SRC)	14-Jun-19
Protocol approval by Sponsors Protocol Review Board (PRB)	21-Jun-19
Protocol approval by WRAIR Investigation Review Board (IRB) with stipulation	10-Jul-19
Protocol approval by WRAIR Investigation Review Board (IRB) final approval	3-Sep-20
Protocol Update #1 approval by WRAIR SRC	24-Oct-19
Protocol Update #1 approval by WRAIR IRB	12-Dec-19
Protocol approval by Human Research Protection Office (HRPO)	6-Jan-20
US FDA authorization to move forward with Part A (via email message)	15-Jan-20
Protocol Update #2 approval by WRAIR IRB	31-Jan-20
Sponsor Implementation Memo	20-Feb-20
WRAIR Commander Approval Authorization	29-Feb-20
WRAIR IRB Correction to September Protocol Approval	17-Mar-20
Protocol IRB Approval Correction to Update 1	17-Mar-20
Amendment 1 IRB approval by WRAIR IRB	23-Mar-20
Amendment 1 IRB approval by WRAIR Command	25-Mar-20
Amendment 2 approval by Sponsor PRB	27-Feb-20
Amendment 2 IRB approval by WRAIR IRB	9-Apr-20
Amendment 2 approval by WRAIR Command	17-Apr-20
Amendment 3 IRB approval by WRAIR IRB	10-Apr-20
Amendment 3 approval by WRAIR Command	10-Apr-20
Amendment 4 IRB approval by WRAIR IRB	6-May-20
Amendment 4 approval by WRAIR Command	11-May-20
Amendment 5 IRB approval by WRAIR IRB	19-May-20
Amendment 5 approval by WRAIR Command	29-May-20
Amendment 6 approval by Sponsor PRB	23-Jun-20
Amendment 6 IRB approval by WRAIR IRB	25-Jun-20
Amendment 6 approval by WRAIR Command	26-Jun-20

3.1 Clinical Study Design

The study is designed as a single center, open-label immunization with Controlled Human Malaria Infection (CHMI). The trial design is described and illustrated in Figure 1. Healthy, malaria-naïve adults (males and non-pregnant, non-lactating females), aged 18 to 55 years old (inclusive) will be recruited from the surrounding area to participate in this immunization with CHMI study. Up to 40 subjects will be enrolled (defined as receiving experimental product) into one of 5 experimental cohorts in two parts. In part A two experimental cohorts of 5 subjects each will receive a series of three vaccinations at 0, 1, and 2 months at a two doses (the “low dose” arm and the “high dose arm”). In part B three experimental cohorts of 10 subjects will receive a series of 3 vaccinations at 0, 1, 6 months (called the “delayed dose” arm), the “delayed fractional dose” arm is vaccinated at 0, 1, and 6 months with the 6 month dose being 1/5 the other doses, and the “standard” arm” at the 4th, 5th, and 6th month (after the first vaccination of the other two arms in part B). As both the adjuvant and the antigens will be first-in-human inter- and intra-

cohort safety staggers will be used. In part A, low and high dose groups will be staggered by 14 days, with the “low dose” arm vaccinated prior to “high dose” arm. In addition, both arms will utilize an internal safety stagger, with one subject vaccinated on Day 1, followed by the remainder of each cohort 24-72 hours later (if no stopping criteria are met). After the third vaccination of cohorts from part A are complete, a SMC meeting will occur. Part B will start only after the Safety Monitoring Committee (SMC) evaluates safety data from Part A and part B will start if the study is determined to be safe to proceed and at either the high or low dose used in part A.

Up to 6 subjects will be enrolled (defined as receiving malaria challenge) later in the trial to serve as challenge controls. Additional subjects may be recruited as alternates to ensure that 6 control subjects undergo each challenge. Any alternates not challenged will be released from the study at day of challenge.

All subjects in part B will undergo CHMI 3-4 weeks after the final vaccine is administered. Challenge will consist of exposure to Plasmodium falciparum 3D7 sporozoites through the bites of infected mosquitoes. Between post-challenge day 5 and post-challenge day 20, subjects will be evaluated daily for the development of malaria infection utilizing validated qPCR. Subjects will be monitored post-challenge in a clinic setting; a hotel phase will not be utilized. Subjects who do not become parasitemic by Day 20 will be seen every other day in the clinic until diagnosis or Day 28. All subjects without parasitemia through Day 28 after challenge will be treated empirically.

All subjects diagnosed with malaria infection will be prescribed a standard treatment regimen consisting of chloroquine (CQ), atovaquone-proguanil (AP; Malarone®), or artemether/lumefantrine (AL; Coartem®), to begin on the day that parasitemia is detected. For purposes of confirming treatment effectiveness, daily qPCR will be continued after diagnosis until two consecutive negative qPCR results has been recorded. If a treated subject has not met the effectiveness threshold by Day 20, they will be followed daily in the CTC for blood draws and continued testing until they meet criteria.

All part B experimental subjects will be followed for an additional 56 days following completion of the original challenge phase for safety and immunogenicity, and then released from the trial.

Part A subjects will not undergo CHMI. Instead, they will be followed for an additional 112 days (4 months post-third immunization) for safety and immunogenicity, and then released from the trial.

3.2 Criteria for Evaluation

Safety:

1. Occurrence of solicited adverse events (AE) during the study period (enrollment to final follow-up visit).
2. Occurrence of unsolicited AEs at any time during the study period (enrollment to final follow-up visit).
3. Occurrence of serious adverse events (SAE) at any time during the study period (enrollment to final follow-up visit).

Efficacy:

1. Vaccine efficacy defined as the proportion of subjects who do not have a positive qPCR following CHMI.
2. Identification of parasitemia by positive qPCR after the *P. falciparum* challenge.
3. Time to parasitemia defined as quantification of time to positive qPCR in subjects that develop a positive qPCR.

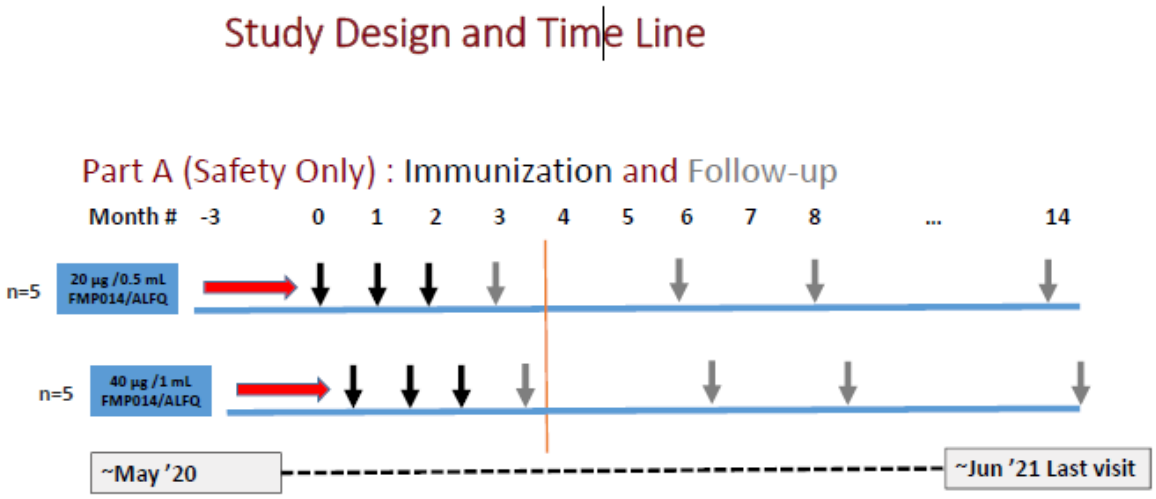
Immunogenicity:

1. Measure antibody, innate, and cellular responses to PfCSP present in serum/blood at specified time-points: prior to vaccination (or challenge for control subjects) and on study days specific for each group.

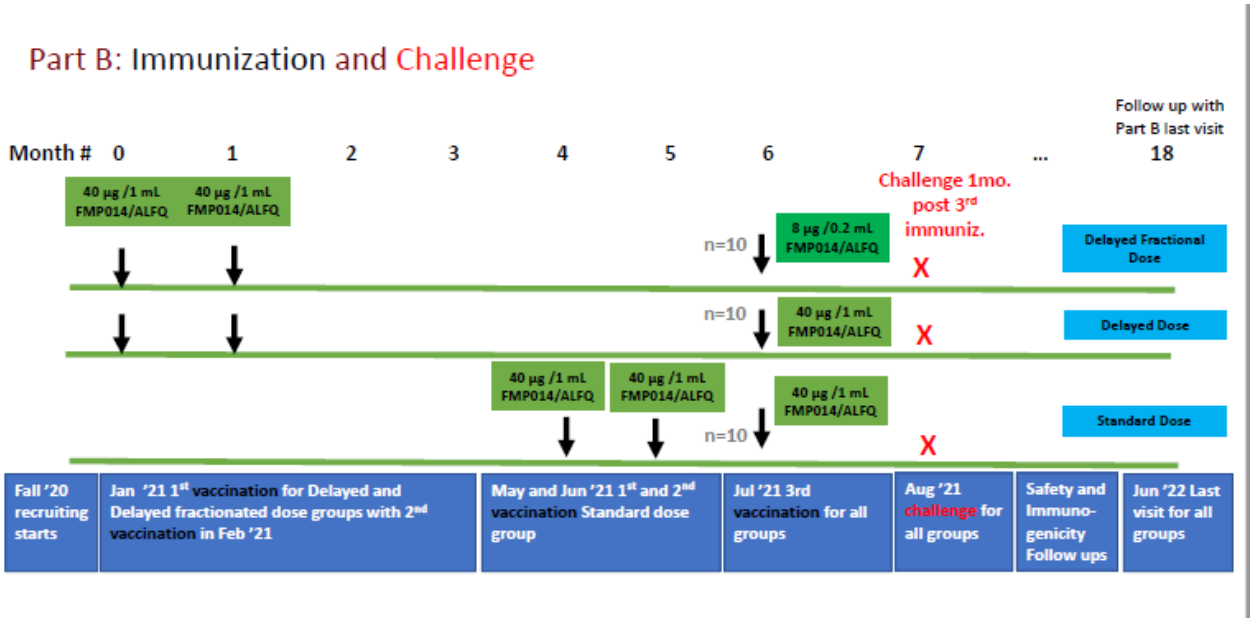
3.3 Clinical Schema

Figure 1: Clinical Study Design

Part A (Safety)



Part B (Immunogenicity and Efficacy)



Note: For Part B, SMC may choose to proceed with lower dose (20 µg of FMP014 and 0.5 mL ALFQ), depending on safety evauation from part A of the study. In this case the fractioal dose would be 4 µg of FMP014 and 0.1 mL ALFQ.

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

In the context of COVID-19, we will participate in virtual conferences and meetings to present the study data.

How were the results disseminated to communities of interest?

Currently we are engaging with partners and attending regular weekly and monthly working group meetings to discuss results and information related to the trial.

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

1. Complete immunogenicity tests on subjects from Part A to assess the vaccine response.
2. Convene a Safety Monitoring Committee to assess the safety and adverse events from Part A, as a determination for progressing to Part B (expanded immunogenicity and efficacy in CHMI).
3. Initiate recruiting and screening for subjects to participate in the Part B phase of the clinical trial of the FMP014/ALFQ (Winter 2020/2021)
4. Complete all immunizations and sample collections during Fiscal year 2021
5. Submit information on the safety and any adverse events through reporting to the Sponsor
6. Perform Controlled Human Malaria Infections (CHMI), summer 2021.

4. IMPACT

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

The development of the self-assembling nanoparticles approach used for FMP014 is an innovative approach for vaccine antigen design and display, one that has increasingly gained attention. The nanoparticle design for FMP014 produces a highly repetitive, symmetrical display of CSP-derived antigens. Results from animal models yielded encouraging results for safety, tolerability and protective efficacy. In addition, the FMP014 design is highly stable relative to long-term storage, and thus is economical for production and distribution. The use of WRAIR's ALFQ adjuvant is also groundbreaking; its composition is similar to the highly potent and protective AS01 adjuvant developed by GSK. While the AS01 adjuvant has been essential to the

success of the malaria vaccine candidate, RTS,S, it is not available to the US Army for developing clinically relevant vaccines. Application of ALF-like adjuvants in humans, has proven that the immunostimulatory molecules (QS-21 and 3D-PHAD) are safe and suitable for enhancing immune responses, and support the Phase I studies of FMP014/ALFQ. Results from safety testing of FMP014/ALFQ in mice, rabbits and rhesus macaques support the further testing of this vaccine candidate in human subjects. The objective of the current study is to develop a novel malaria vaccine based on the CSP antigen that is both safe and effective in healthy, naïve adults. Importantly, because of improvements in the antigen and adjuvant, the vaccine has the potential for eliciting potent and long-lasting protection against infection.

What was the impact on other disciplines?

The ALF adjuvants are being applied to evaluate other vaccine approaches being developed by WRAIR research community as well as with external collaborations.

In MVB Dr. Evelina Angov and Dr. Sheetij Dutta are evaluating the ALFQ adjuvant in preclinical studies using PfCelTOS, PfCel-PfCel SAPN and the soluble *P. falciparum* CSP (FMP013), PfCSP-repeat specific tobacco mosaic virus nanoparticles, respectively.

In collaboration with Dr. Evelina Angov, Dr. Martha Sedegah and Dr. Peter Burkhard have received CDMRP funding to evaluate the next generation of SAPN antigen display as a multi-epitope/multi-antigen delivery system for malaria. This project will initiate in OCT FY20 and is funded for three years.

What was the impact on technology transfer?

1. Scalable, cGMP processes for manufacture of both the antigen (FMP014) and adjuvants (ALF43 and ALFQ) were developed.

2. Methods for the qualification of the adjuvant components were developed by Avanti Polar Lipids. APL plans to validate these methods in the future.

3. A US Patent for ALFQ adjuvant has issued.

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

The company, Avanti Polar Lipids, who manufactures the liposomes and phospholipids used to manufacture the base component, ALF55, of the ALF adjuvants experienced significant delays in the quantitative analysis of the GMP vialed products during the normal stability-testing plan. During the second-third quarter of FY17, adjuvant stability testing yielded an out of specifications finding for ALF adjuvants. Due to an Out of Specifications (OOS) investigation initiated by Avanti Polar Lipids, Inc. on March 27, 2017 and the pursuant investigations into the matter, it was determined that additional methods development is required to establish well qualified and validated methods for quantifying and identifying the ALFQ adjuvant components and degradants of the GMP batch lots for product release and stability testing. The impact of this action is the invalidation of the lot release for the GMP adjuvant ALFQ Lot #1974 and #2010.

COVID-19 pandemic in the context of a clinical trial is challenging. Given its central mission for the development and evaluation of medical countermeasures, the WRAIR has continued to conduct mission-critical clinical trials in the setting of the COVID pandemic. Early in this experience, a working group of DoD Infectious Diseases and clinical trials experts outlined consensus guidance for the safe conduct of trials activities. These guidance's were based upon expert opinion as well as federal, DoD, and local treatment center policy. Encompassing pre-visit symptom screening, active screening on arrival, infection control procedures, personal protective equipment posture, quarantine criteria, as-needed COVID testing on-site, and even procedures for off-site or home visits, these practices have allowed the WRAIR to safely conduct

trials activities which directly support the COVID countermeasures development effort, and more recently, other mission-relevant investigations. In addition, the WRAIR has instituted a rapid review committee to evaluate and advice on practices for new and resuming trials in the setting of COVID-19.

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

GMP ALFQ product re-testing and release occurred in Sept 2018 using the re-qualified methods. All adjuvants passed composition testing and were released in October 2018. Ongoing annual stability testing indicates that the adjuvant is stable.

Changes that had a significant impact on expenditures

1. Due to unforeseen delays related to ALFQ stability and requalification of assays, the clinical trial was delayed until further remediation; funds were returned to CDMRP in July 2017. Once trial activities resumed, CDMRP provided 'new' 2 year, FY18 funds on about March 2019, for the clinical trial execution.

2. In July 2020, WRAIR MBB requested exchange of FY19 DHP RDT&E $350K remaining funds with FY20 DHP RDT&E to allow for appropriate obligation of clinical activities in Fiscal year 2021. WRAIR received the exchanged funds in July 2020.

3. WRAIR, Pilot BioProduction Facility closure had a significant impact on the ability to formulate new ALFQ adjuvant lots.

4. The PBF has initiated process development activities in response to COVID-19 and is currently scaling up activities to produce the WRAIR Spike Ferritin Nanoparticle (SpFN) Vaccine in September. The PBF has completed all operational and performance qualifications for each of the four cGMP production suites and is finalizing the documentation to meet full cGMP operational capability. The Viral suite use for SpFN will open for full GMP production next week with the fill/finish suite opening in early August.

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

Nothing to report

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals.

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. **PRODUCTS:**

Publications, conference papers, and presentations:

Journal publications

All subject matter discussed in the reports and presentations relates to either the malaria antigen FMP014, or the ALF adjuvants, ALFQ and ALFA (ALF43).

Publications (All used ALF or ALFQ)

1. Ramakrishnan., A., Schumack, N.M., Gariepy, C.L, Eggleston, H., Nunez, G., Espinoza, N., Nieto, M., Castillo, R., Rojas, J., McCoy, A.J., Beck, Z., **Matyas, G.R.**, Alving, C.R., Guerry, P., Poly, F., Laird, R.M.. Enhanced Immunogenicity and Protective Efficacy of a Campylobacter jejuni Conjugate Vaccine Coadministered with Liposomes Containing Monophosphoryl Lipid A and QS-21. mSphere (2019) 4. pii: e00101-19.

2. Cawlfield, A., Genito, C.J., Beck, Z., Bergmann-Leitner, E.S., Bitzer, A.A., Soto, K., Zou, X., Hadiwidjojo, S.H., Gerbasi, R.V., Mullins, A.B., Noe, A., Waters, N.C., Alving, C.R., **Matyas, G.R.**, Dutta, S. Safety, toxicity and immunogenicity of a malaria vaccine based on the circumsporozoite protein (FMP013) with the adjuvant army liposome formulation containing QS21 (ALFQ). Vaccine (2019) 37:3793-3803.

3. Singh, P., Bodycomb, J., Travers, B., Tatarkiewicz, K., Travers, S., **Matyas, G.R.**, Beck, Z. Particle size analyses of polydisperse liposome formulations with a novel multispectral advanced nanoparticle tracking technology. Int J Pharm. (2019) 566:680-686.

4. Karch, C.P., Burkhard, P., Matyas, G.R., Beck, Z. Production of E. coli-expressed Self-Assembling Protein Nanoparticles for Vaccines Requiring Trimeric Epitope Presentation. J Vis Exp. (2019) 150.

5. Martin, M.L., Bitzer, A.A., Schrader, A., Bergmann-Leitner, E.S., Soto, K., Zou, X., Beck, Z., Matyas, G.R., Dutta, S. Comparison of immunogenicity and safety outcomes of a

malaria vaccine FMP013/ALFQ in rhesus macaques (Macaca mulatta) of Indian and Chinese origin. Malar J. (2019) 18:377.

6. Verma, A., Schmidt, B.A., Elizaldi, S.R., Nguyen, N.K., Walter, K.A., Beck, Z., Trinh, H.V., Dinsarapu, A.R., Lakshmanappa, Y.S., Rane, N.N., Matyas, G.R., Rao, M., Shen, X., Tomaras, G.D., LaBranche, C.C., Reimann, K.A., Foehl, D.H., Gach, J.S., Forthal, D.N., Kozlowski, P.A., Amara, R.R., Iyer, S.S. Impact of T(h)1 CD4 T(FH) skewing on antibody responses to an HIV-1 vaccine in Rhesus macaques. J Virol. (2019) 94:e01737-19.

7. Langowski, M.D., Khan, F.A., Bitzer, A.A., Genito, C.J., Schrader, A.J., Martin, M.L., Soto, K., Zou, X., Hadiwidjojo, S., Beck, Z., Matyas, G.R., Livingstone, M.C., Batchelor, A.H., Dutta, S., Langowski, M.D. Optimization of a Plasmodium falciparum circumsporozoite protein repeat vaccine using the tobacco mosaic virus platform. Proc Natl Acad Sci U S A. (2020) 117:3114-3122.

8. Alving, C.R., Peachman, K.K., Matyas, G.R., Rao, M., Beck, Z., Alving, C.R. Army Liposome Formulation (ALF) family of vaccine adjuvants Expert Rev Vaccines. (2020) 19:279-292.

9. Karch C.P., Paquin-Proulx D., Eller M.A., Matyas G.R., Burkhard P., Beck Z. Impact of the expression system on the immune responses to self-assembling protein nanoparticles (SAPNs) displaying HIV-1 V1V2 loop. Nanomedicine. (2020) 29:102255.

10. Singh P., Matyas G.R., Beck Z. Biophysical characterization of polydisperse liposomal adjuvant formulations. Biochem. Biophys. Res. Comm. (2020) 529:362-365.

Note: All the heroin/fentanyl work uses ALF43.

Published or Web Available Abstracts

Matyas, G. R., Torres, O.B., Sulima, A., Jacobson, A.E., **Beck, Z.**, Rice, K.C., Heroin Hapten Vaccine Formulation to be Used for a Phase 1 Clinical Trial Induces Long Duration Antibody Titers that Block the Antinociceptive Effects of Heroin and other Opioids in Animals, International Society for Vaccines Annual Congress, Ghent, Belgium, October 26-29, 2019 (Poster).

Matyas, G. R., Torres, O.B., Sulima, A., Jacobson, A.E., Beck, Z., Rice, K.C., Heroin Hapten Vaccine Formulation to be Used for a Phase 1 Clinical Trial Induces Long Duration Antibody Titers that Block the Antinociceptive Effects of Heroin and other Opioids in Animals International Narcotics Research Conference, New York City, July 7-11, 2019 (poster).

Renee M. Laird[1,2], Christina L. Gariepy[1,2], Heather Eggleston[1,2], Nina M. Shoemaker[1,2], Mario A. Monteiro[3], Zoltan Beck[1,4], Gary R. Matyas[4], Frédéric Poly Application of a *Campylobacter jejuni* mouse infection model to test efficacy of a *C. jejuni* capsule conjugate vaccine delivered with a potent liposome adjuvant containing monophosphoryl lipid A and QS-21 Immunology 2020; meeting canceled.

Chen, B, Barnafo, E, Alani, N, Butler, B, Lambert, L, Anderson, C, Zaidi, I, Narum, D, Beck, Z, Matyas, G, Nahas, D, Lucas, B, Duffy, P, Scaria, P, Rowe, C. Evaluation of Pfs230D1M-OMPC conjugate in Rhesus as transmission-blocking vaccine for malaria. ASTMH National Harbor 20-24 Nov 2019 (poster).

Sirinan Madnote[1], Surawach Rittiroongrad[1], Somsak Chantakulkij[1], Jiraporn Puangkaew[1], Zoltan Beck[2,3], Mangala Rao[2], Gary Matyas[2], Eugene Kroon[4], Donn J. Colby[2,3,4], Carlo Sacdalan[4], Robert J. O'Connell[5], Mark M. Fukuda[1], Thomas Musich granulocyte response to Army Liposome adjuvant Formulations CROI Conference Retroviruses and Optimistic Infections, Boston MA, 8-11 Mar 2020 (poster).

Beck, Z, Matyas, GR, Lanar, DE, Ramakrishnan, A, Poly, F, Angov, E, Laird, RM, Dutta, S. Immunogenicity and Safety of the Army Liposome Formulations containing QS-21 (ALFQ) in Non-human Primates, Liposome Research Days, Sapporo, Japan, 15-18 Sep 2020 (talk)

Mangala Rao[1], Kristina K. Peachman[2], Mary E. Akpan[2], Alexander Anderson[2], Zoltan Beck[2], Gary R. Matyas[1], Sanjay Phogat[3] and Carl R. Alving[1] Army Liposome Formulations Induce Durable Binding and Functional Antibody Responses to HIV-1 Envelope gp120 Protein ISV 2019

Pushpendra Singh, [1,2] Zoltan Beck,[1,2] Gary Matyas, [1] Carl Alving, Saturated Phospholipids Are Required for QS21-Induced Nano- to Micron-Size Transformation of Cholesterol-Containing Small Unilamellar Liposomes. Liposome Researh Days Japan

Matyas, GR, Torres, O, Rice, KC, Thomas, S. A Phase I/IIa Clinical Trial Testing the Safety and Immunogenicity of a Heroin Vaccine Heal Investigators Meeting, Bethesda, MD 16-17 Jan 2020 (poster).

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers, and presentations.

Invited Scientific Lectures

Matyas, GR Immunotherapeutic approaches to counteract the fentanyl overdose epidemic minisymposia College of Problems of Drug Dependence Zoom meeting, 22-24 Jun 2020.

Matyas, Gary R. Heroin Vaccine Overview, Ethical and Regulatory Issues Involved in the Clinical Development of Substance Abuse Countermeasures Symposia, SUNY Upstate Medical University, Institute for Global Health and Translational Science, October 7, 2019

Matyas, Gary. R. A Phase I/IIa Clinical Trial Testing the Safety and Immunogenicity of a Heroin Vaccine, Opiant Pharmaceuticals, Inc. Scientific Advisors Meeting, New York, NY 27 Aug 2019

Matyas, Gary R., Update of A Phase I/IIa Clinical Trial Testing the Safety and Immunogenicity of a Heroin Vaccine and its Efficacy Against Morphine Challenge, Virtual meeting with Indiana Bioscience Research Institute, 30 July 2019.

Army Presentation

Nothing to report

Website(s) or other Internet site(s)

Nothing to report

Technologies or techniques

Nothing to report

Inventions, patent applications, and/or licenses

Alving, C.R Beck, Z. "Non-toxic Adjuvant Formulation Comprising a Monophosphoryl Lipid A (MPLA)-Containing Liposome Composition and a Saponin. Compositions and Methods for Vaccine Delivery". This patent was issued on 8 October 2019 (U.S. Patent No. 10,434,167).

Matyas, GR, Torres, O, Rice, KC, Jacobson, AE, Sulima, A, Bow, E. Fentanyl Haptens for the Preparation of a Fentanyl Vaccine. United States Provisional Patent Application Serial No.: 62/960,187 13 January 2020 (HJF).

Other Products

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Dr. Evelina Angov
Project Role:	Principle Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0003-2814-3057
Nearest person month worked:	12
Contribution to Project:	Dr. Angov is the Project PI.
Funding Support:	N/A

Name:	Dr. Gary Matyas
Project Role:	Consultant
Researcher Identifier (e.g. ORCID	

ID):	
Nearest person month worked:	12
Contribution to Project:	ALFQ expertise
Funding Support:	N/A

Name:	Dr. Peter Burkhard
Project Role:	Consultant
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	invented protein nanoparticles as a platform for vaccine design
Funding Support:	N/A

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

No change during this period.

What other organizations were involved as partners?

Organization name:	Avanti Polar Lipids
Location of the organization	Alabaster, AL, USA
Partner's contribution to the project:	
In kind support	Partner manufactures the ALF (with 43% or 55% cholesterol) portion of the adjuvants used with FMP014; performed the ongoing stability testing on the ALFQ composition

Organization name:	Desert King International
Location of the organization	San Diego, CA, USA
Partner's contribution to the project:	
In kind support	Partner manufactures the QS21 that is part of the adjuvant used with FMP014

Organization name:	Alpha-O peptides
Location of the organization	Riehen , Switzerland
Partner's contribution to the project:	
Collaboration	Dr. Burkhard designed the SAPN FMP014

Organization name:	WRAIR Pilot Bioproduction Facility (PBF)
Location of the organization	Silver Spring, MD, USA
Partner's contribution to the project:	
Collaboration	PBF staff performed the scale-up and release testing of the drug product and substance
Facilities	The project staff uses the partners facilities for stability sample storage

Organization name:	WRAIR Veterinary Medicine Support
Location of the organization	Silver Spring, MD, USA
Partner's contribution to the project:	
In kind support	Partner makes equipment such as cages etc. available to the project staff when needed
Facilities	Project staff uses the partner's facilities to perform all animal work
Collaboration	Partner's staff works with the project staff to ensure and maintain the safety of the animals used in the studies
Personnel exchanges	Project staff uses the partners facilities to immunize and bleed the mice for the studies

Organization name:	WRAIR Entomology Branch
Location of the organization	Silver Spring, MD, USA
Partner's contribution to the project:	
In kind support	Partner helped train staff in mosquito dissection
Facilities	Project staff uses entomology facilities to retrieve mosquitoes for dissection

Collaboration	Partners staff provides the mosquitoes for mouse challenge studies and works with project staff in the event help is required with dissections
Personnel exchanges	Project staff uses the entomology facilities while training and collecting mosquitoes

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Nothing to report

QUAD CHARTS:

Quad Chart July 2020 – Attachment 1

9. APPENDICES:

Attachment 1 – Quad Chart July 2020 – Annual Report updated.