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MALARIA VACCINE INITIATIVE

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Acronyms and abbreviations

Ad	adenovirus
ADCI	antibody-dependent cellular inhibition assay
A. dirus	*Anopheles dirus*
AFRIMS	Armed Forces Research Institute of Medical Sciences
AFRO	Regional Office for Africa (WHO)
CBER	Center for Biologics Evaluation and Review (FDA)
CEF	cytomegalovirus, Epstein-Barr virus, and flu virus
CSP	circumsporozoite protein
DMF	Decision-Making Framework
FDA	US Food and Drug Administration
FY	Fiscal Year
GIA	growth inhibition assay
ICS	intracellular cytokine staining
IFA	immunofluorescent antibody
IFN-γ	interferon-gamma
IND	Investigational New Drug
MVI	Malaria Vaccine Initiative
LMIV	Laboratory of Malaria Immunology and Virology
NIBSC	National Institute for Biological Standards and Control
NIH	US National Institutes of Health
P. berghei	*Plasmodium berghei* (*Pb*)
P. falciparum	*Plasmodium falciparum* (*Pf*)
P. knowlesi	*Plasmodium knowlesi* (*Pk*)
PATH	Program for Appropriate Technology in Health
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PNG	Papua, New Guinea
rCSP	recombinant circumsporozoite protein
Seattle BioMed	Seattle Biomedical Research Institute
SOP	standard operating procedure
USAID	US Agency for International Development
WEHI	Walter and Eliza Hall Institute
WHO	World Health Organization
WRAIR	Walter Reed Army Institute of Research

Grant summary information

Date of report	October 31, 2010
Name of organization completing report	Program for Appropriate Technology in Health (PATH)
Address	2201 Westlake Avenue, Suite 200 Seattle, WA 98121 USA
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Any changes to nonprofit status?	No
Project name	PATH Malaria Vaccine Initiative
Brief description	US Agency for International Development funding supports science and technical activities related to the development of a malaria vaccine as well as policy activities in endemic countries
Location of project	Global, including the United States and sub-Saharan Africa
Grant amount	$23,000,000
Grant period	October 1, 2009–September 30, 2010
Type of report	Annual report

Executive summary

The PATH Malaria Vaccine Initiative (MVI) carried out the activities described in this report from October 1, 2009, through September 30, 2010. The cooperative agreement between MVI and the US Agency for International Development (USAID) was signed on October 8, 2004. On September 17, 2008, a cost extension was granted, which extended the term to September 16, 2013, and increased the ceiling from $8 million to $23 million.

During Fiscal Year (FY) 2010, USAID funds supported science and technical activities and policy and commercialization activities. The highlights of each are summarized below.

Science and technical highlights

- **Generation and characterization of *Pf*CSP (circumsporozoite protein) multivalent conjugates with enhanced immunogenicity and protection against *Plasmodium (P.) falciparum*:** Work since September 2009 focused on generating and testing both *Pf*s25- and CRM197-based candidates with more favorable immunogenicity profiles than those previously assessed. When formulated with saline, second-generation recombinant circumsporozoite protein (rCSP)-*Pf*s25 conjugates generated anti-CSP antibody titers up to 50 times greater than unconjugated rCSP alone. This benefit was not preserved following formulation with alum. In contrast, conjugation of CSP to CRM197 led to up to 300-fold greater anti-CSP antibody responses in outbred CD-1 mice either in the presence or absence of adjuvant, with best results achieved by CSP-CRM197 adjuvanted with GLA-SE. Unfortunately, no similar increase in CD4 T cell responses was observed by multicolor flow cytometry. This activity was terminated in February 2010, following release of the results of an independent comparative immunogenicity study, which compared the immunogenicity in mice of full-length rCS proteins generated by the Laboratory of Malaria Immunology and Virology (LMIV) and three other laboratories, with each protein formulated with CpG and poly I:C. The investigators concluded that the full-length rCSP generated by LMIV induced little to no antibody or T cell responses in comparison to two of the other proteins assessed, and that these responses were poorly protective.
- **Rodent model using transgenic *P. berghei* expressing *P. falciparum* on sporozoite surface:** The group at LMIV previously confirmed that the *P. berghei* transgenic parasite contains the *Pf*CSP transgene and not the *Pb*CSP gene and that the *Pf*CS protein was expressed on the transgenic sporozoite surface. The group has focused on repeated adaptation selections of the transgenic parasite in this mosquito-mouse combination. The rodent transgenic likely will not be viable due to the large numbers of mosquitoes needed to infect mice.
- **Rhesus model using transgenic *P. knowlesi* expressing *P. falciparum* on sporozoite surface:** LMIV performed a second transfection in which *P. knowlesi*-infected rhesus erythrocytes were transfected with the gene-replacement construct and infused to two recipient rhesus monkeys subjected to pyrimethamine selection. Transformants were detected and confirmed by PCR (polymerase chain reaction) testing. Presence of *Pf*CSP gene and expression of *Pf*CS protein, absence of *Pk*CSP gene and absence of *Pk*CS protein on the

surface of transgenic sporozoites were confirmed by PCR and immunofluorescent antibody testing. During passaging of the transformants between monkeys and mosquitoes, two monkeys died due to altered growth characteristics of the transformants. Proper treatment has since been implemented. The transformants were able to undergo gametogenesis, infect *Anopheles dirus* mosquitoes, and develop into oocysts in the mosquito midgut. Sporozoites were also detected in mosquito saliva, though they were lower in number compared to the wild type *P. knowlesi* parasites. LMIV consulted with several experts and adapted methodologies to improve feeding. A successful feeding was conducted, but the monkeys that received infectious bites were followed for four weeks and no blood-stage parasites were detected.

- **AMA1 polymorphism and optimization of AMA1 as a vaccine candidate/component:** These three projects are focused on the issue of polymorphism and optimization of AMA1. A team at the Walter and Eliza Hall Institute (WEHI) is developing a multivalent vaccine, while investigators at the Walter Reed Army Institute of Research and LaTrobe University are working on an optimized AMA1 immunogen that transcends heterogeneity. Highlights of FY2010 include the following:
  - ***Development of AMA1 transgenic parasite lines and recombinant proteins:*** Over the past year, the WEHI investigators adopted a new strategy to expand on the work of generating transgenic parasites by complete allelic replacement, and are focused on the C1L region of AMA1, since previous work has shown that this region is a target of invasion inhibitory antibodies and important in mediating immune escape. They have begun mutating specific residues and will determine which of those are important or how many are needed for escape of vaccine-induced antibodies or naturally acquired antibodies.
  - ***Progress toward developing a vaccine using a polyvalent approach:*** In the first year of the project, rabbits were immunized with polyvalent vaccine consisting of W2mef, HB3, 3D7, and FVO AMA1. Growth inhibition assay (GIA) results from this study showed that this vaccine elicited the best inhibition against both 3D7 and FVO parasites, plus six additional parasite isolates.
  - ***Summary of studies using monoclonal antibodies:*** Monoclonal antibodies generated during the first year were used in mixing experiments against 3D7 parasites in GIA, and interestingly, when two of the mAbs were mixed, the level of inhibition increased from 21 percent and 40 percent alone, respectively, to 94 percent mixed together. This is the first evidence of synergy using mAbs for inhibition of invasion.
  - ***Rabbit immunogenicity study—single antigen immunizations:*** The data, although incomplete, suggest that a single mutant form of AMA1 is unlikely to provide an effective immune response to a wide range of *P. falciparum* AMA1 genotypes. The LaTrobe group is focusing on combinations of mutant forms to determine whether this will provide an increased response to conserved epitopes over that achieved by combinations of wild type alleles.
- **Assay harmonization:**
  - ***Antibody-dependent cellular inhibition (ADCI) harmonization:*** In the past year, activities have focused on preparation for a first round of testing, in which the participating laboratories will test a panel of standard reagents with their own methods.

Based on the results of this first testing, a consensus standard operating procedure (SOP) for ADCI assay will be selected. Testing is expected to begin in Q4 2010.

- ***T cell assays:***
  - ✓ In the past year, the following standard reagents were identified: Peripheral blood mononuclear cells; tetanus toxoid peptide antigens; and cytomegalovirus, Epstein-Barr virus, and flu virus peptide antigens.
  - ✓ In preparation of the reports on the identification of key parameters and optimal approaches for evaluating T cell responses in pre-erythrocytic and blood-stage vaccinations and on the development and rationale of acceptance criteria for assay performance, key aspects of the ICS (intracellular cytokine staining) and ELISpot assays undertaken in the various laboratories were collected through a questionnaire.
  - ✓ SOPs for interferon-gamma ELISpot and ICS were obtained from Duke University. The National Institute for Biological Standards and Control is currently reviewing these SOPs and generating a guidance document on critical aspects of the assays, which will be distributed to participating laboratories. The first round of testing is scheduled to occur in Q1 2011.

- **GenVec's Ad28 (adenovirus type 28)-CSP:** One of the primary supporting arguments for developing Ad28 as a vector for malaria vaccines was the expectation of low neutralizing antibody prevalence in endemic regions. Work would continue beyond Q3 2010 only if prevalence of Ad28 neutralizing antibodies in adults in representative endemic regions of sub-Saharan Africa was demonstrated to be less than 30 percent. In Q2 2010, an independent assessment of Ad28 neutralizing antibody prevalence in a subset of sub-Saharan Africa was conducted by the US National Institutes of Health and demonstrated prevalence far greater than 30 percent. In light of these data, along with concerns regarding the long-term financial solvency of GenVec following the discontinuation of an unrelated Phase III clinical trial (TNFerade), MVI and GenVec agreed to terminate the study following completion of a limited scope of work, completed in August 2010.

- **Crucell's Ad26-CSP:**
  - Manufacturing, release, and testing of Ad26.CS.01 drug substance was completed in November 2009, and filling of the drug products (clinical trial materials) was performed in January 2010. Final release and testing of the clinical trial materials was completed in June 2010. The audited toxicology study report for Ad35.CS.01/Ad26.CS.01 immunizations showed no toxicity of the prime boost regimen. A decision was made to proceed to the clinical trial. Stability testing of all lots of Ad35.CS.01 and Ad26.CS.01 will continue through the completion of the clinical trial.
  - For the heterologous prime boost clinical study of Ad35.CS.01 and Ad26.CS.01, the clinical trial design consisted of one cohort of 20 subjects (with six controls) immunized two times with a dose of $5x10^{10}$ vp/ml Ad35.CS.01 and then immunized with a dose of $5x10^{10}$ vp/ml Ad26.CS.01 followed by infected mosquito challenge. The Seattle Biomedical Research Institute Malaria Clinical Trials Center was selected as the study site. The Investigational New Drug (IND) application was submitted to the US Food and Drug Administration Center for Biologics Evaluation and Review on July 20, 2010. The IND was placed on clinical hold on August 18, 2010.

Policy and commercialization highlights

- **Rollout of the malaria vaccine Decision-Making Framework (DMF) in non-RTS,S clinical trial countries:** Work has progressed on the malaria vaccine DMF, and several key milestones were achieved:
  - A proposal was developed and submitted to the steering committee, outlining an approach on the malaria vaccine DMF rollout in additional countries over the period 2010-2014.
  - Uganda and Nigeria initiated work around the malaria vaccine DMF. MVI worked with the Uganda Academy of Sciences to convene an experts meeting to discuss malaria vaccines and the use of the framework in Uganda. A subcommittee was set up to move the process forward. In Nigeria, MVI briefed the Director of the Public Health Department at the Nigeria Federal Ministry of Health about malaria vaccines development and the DMF.
  - World Health Organization (WHO) Regional Office for Africa (AFRO) staff worked with MVI to present updates on malaria vaccines and the DMF at three WHO AFRO subregional meetings on malaria and immunization.
  - Review of the guidance document, *Guide for Country Level Implementation of the Regional Malaria Vaccine Decision-Making Framework*, is in the final stages.

Science and technical

Generation and characterization of *Pf*CSP multivalent conjugates with enhanced immunogenicity and protection against *Plasmodium falciparum*

The goal of this study was to evaluate several chemical conjugation-based strategies aimed at enhancing the immunogenicity of recombinant *Plasmodium (P.) falciparum* circumsporozoite protein (rCSP). The rCSP utilized was produced in *Pichia pastoris* at the Laboratory of Malaria Immunology and Vaccinology (LMIV), and consisted of full-length CSP missing the N-terminal signal and C-terminal GPI anchor domains. Initial immunogenicity studies were to be conducted in mice, followed by a follow-on study in rhesus macaques, provided that any candidate(s) met the following Go/No-go criteria:

- The antibody levels induced by the conjugates have to be at least three times higher than the levels induced by unconjugated rCSP.
- Higher T cell responses and sustained T and B cell responses.

In the first year of the contract (executed in September 2008), LMIV attempted to increase the immunogenicity of rCSP through conjugation to the carrier protein recombinant exoprotein A from *Pseudomonas aeruginosa*. Preliminary rodent immunogenicity studies with this construct demonstrated a 1.8-fold increase in antibody responses compared to unconjugated rCSP, but this line of study was discontinued in favor of an alternative carrier protein based on diphtheria toxin, CRM197. LMIV scientists also conjugated rCSP to a transmission-stage malarial antigen, *Pf*s25, with the goal of producing a combination vaccine capable of generating immune responses against both proteins. Surprisingly, an initial rodent immunogenicity study comparing unconjugated rCSP to a conjugate of rCSP and *Pf*s25 showed a 1.5- to 7-fold decrease in antibody titers against CSP following conjugation to *Pf*s25.

Work after September 2009 focused on generating and testing both *Pf*s25- and CRM197-based candidates with more favorable immunogenicity profiles. When both were formulated with saline, second-generation rCSP-*Pf*s25 conjugates generated anti-CSP antibody titers up to 50 times greater than unconjugated rCSP alone. This benefit was not preserved following formulation with alum. In contrast, conjugation of CSP to CRM197 led enhanced anti-CSP antibody responses in outbred CD-1 mice either in the presence or absence of adjuvant (Table 1). This enhancement was most apparent in the presence of the TLR adjuvant GLA-SE (Groups 10–12). Unfortunately, no similar increase in CD4 T cell responses was observed by multicolor flow cytometry (Figure 1).

Table 1. Antibody responses in CD-1 mice following immunization with rCSP-CRM197 conjugates.

Group	Vaccine	Adjuvant	Dose (µg)	Anti-CSPM2 antibodies (GM* of ELISA units)
1	CSPM2	none	0.5	58
3	CSPM2/C-CRM197(2.5/1)	none	0.5	1,428
5	CSPM2/C-CRM197 (5.25/1)	none	0.5	198
2	CSPM2	none	2.5	106
4	CSPM2/C-CRM197 (2.5/1)	none	2.5	25,284**
6	CSPM2/C-CRM197 (5.25/1)	none	2.5	16,771**

Group	Vaccine	Adjuvant	Dose (µg)	Anti-CSPM2 antibodies (GM* of ELISA units)
7	CSPM2	alum	0.5	600
8	CSPM2/C-CRM197 (2.5/1)	alum	0.5	8,600**
9	CSPM2/C-CRM197 (5.25/1)	alum	0.5	16,315**
10	CSPM2	GLA-SE	0.5	434
11	CSPM2/C-CRM197 (2.5/1)	GLA-SE	0.5	142,099**
12	CSPM2/C-CRM197 (5.25/1)	GLA-SE	0.5	86,457**

** GM: geometric mean. ** 4 vs 2, 6 vs 2, 8 vs 7, 9 vs 7, 11 vs 10, 12 vs 10, P <0.05.*

CD-1 mice were immunized twice (Day 0 and Day 28) with 0.5µg or 2.5µg/mouse of unconjugated rCSP (CSPM2) or rCSP conjugated to CRM197 at a ratio of 2.5:1 (CSPM2/C-CRM197(2.5/1)) or 5.25:1 (CSPM2/C-CRM197(5.25/1)). Conjugates were formulated either in saline, alum, or GLA-SE. Serum was harvested 14 days after the second immunization (Day 42), and antibody responses were assessed by ELISA with homologous rCSP plate antigen.

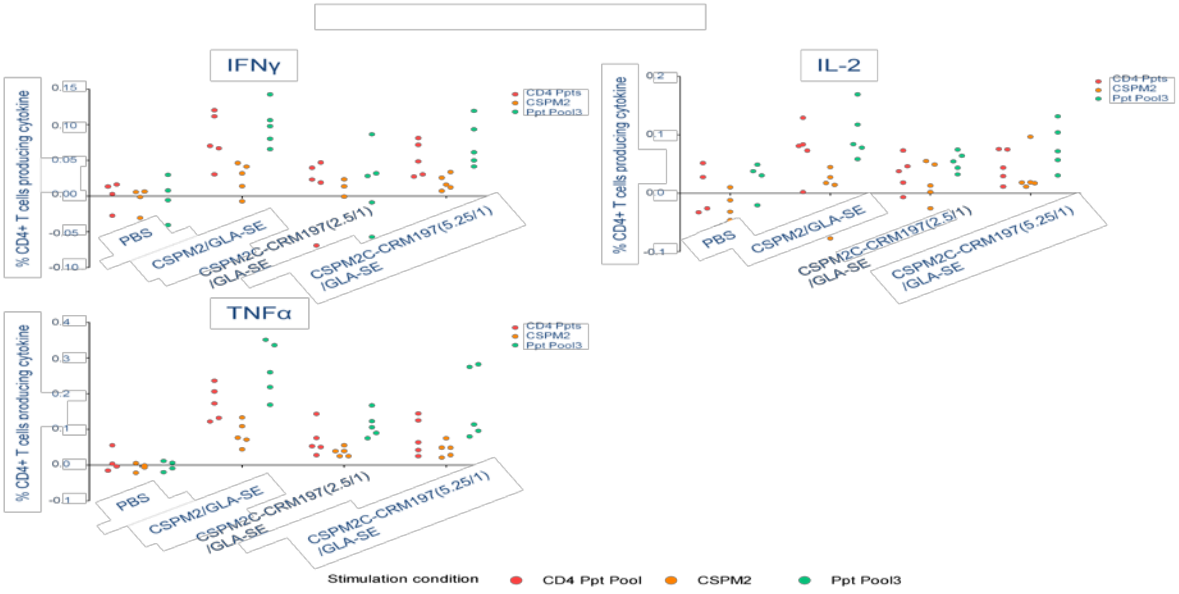

Figure 1. CD4 T cell responses in Balb/c mice following immunization with rCSP-CRM197 conjugates.

Balb/c mice received three doses (Days 0, 28, and 49) of PBS, 12.5µg unconjugated rCSP (CSPM2) or rCSP conjugated to CRM197 at a ratio of 2.5:1 (CSPM2/C-CRM197(2.5/1)) or 5.25:1 (CSPM2/C-CRM197(5.25/1)) formulated in GLA-SE. Spleens were harvested on Day 56 and splenocytes stimulated with a set of three immunodominant CSP peptides (CD4 Ppts), full-length homologous rCSP (CSPM2), or an overlapping peptide pool consisting of 20mers overlapping by 10 amino acids and spanning the C-terminal third of CSP, India strain (PptPool3). Stimulation was followed by intracellular cytokine staining (ICS) and flow cytometry analysis.

This activity was terminated in February 2010, following release of the results of an independent comparative immunogenicity study supported by the PATH Malaria Vaccine Initiative (MVI) through core funding from the Bill & Melinda Gates Foundation and conducted at the US National Institutes of Health (NIH) and Johns Hopkins University. This study compared the

immunogenicity in mice of full-length rCS proteins generated by LMIV and three other laboratories formulated with CpG and poly I:C. The investigators concluded that the full-length rCSP generated by LMIV induced little to no antibody or T cell responses in comparison to two of the other proteins assessed, and that these responses were poorly protective. In light of these results, and the lack of T cell responses generated by any of the LMIV conjugates assessed, the decision to terminate activities was relayed to LMIV on February 2, 2010. The status of all deliverables at the time of termination is detailed in Table 2.

Table 2. Status of deliverables for LMIV *Pf*CSP conjugation activities.

Activity	Deliverable	Status
Generation of conjugate vaccines	Production of conjugates	Completed
	Confirmation of stability and conjugation efficiency	Completed
Assessment of immunogenicity and protection in mice	Determination of antibody levels and longevity	Completed
	Selection of candidates for extensive immunogenicity study in mice: assessment of humoral and cellular immune responses	Terminated
	Mouse challenge study with transgenic *P. berghei*, if parasite is available	Terminated
	Selection of candidates to proceed to rhesus macaque and/or clinical testing	Terminated
	Preparation/formulation of candidates	Terminated
Assessment of immunogenicity and protection in rhesus macaques	Determination of antibody levels and longevity	Terminated
	Assessment of cellular immune responses	Terminated
	Assessment of protection against transgenic *P. knowlesi* challenge, if parasite is available	Terminated

Establishment of challenge models using transgenic *P. berghei* and *P. knowlesi* expressing *P. falciparum* CSP on sporozoite surface

Rodent model using transgenic *P. berghei* expressing *P. falciparum* on sporozoite surface

The group at LMIV previously confirmed that the *P. berghei* transgenic parasite contains the *Pf*CSP transgene and not the *Pb*CSP gene. The *Pf*CS protein was expressed on the transgenic sporozoite surface. They established a reproducible transgenic parasite stock and attempted to establish parameters for a rodent challenge model (mouse strain, quantity of sporozoites dissected from mosquito salivary glands, number of sporozoites needed to establish 100 percent infection, and pre-patent duration). Previous work indicated that the numbers of sporozoites found in infected mosquitoes was insufficient for 100 percent infection in mice. *Anopheles stephensi* mosquitoes and CD-1 mice were chosen for optimal infection rates and numbers of sporozoites. Mosquitoes fed sugar supplemented with para-aminobenzoic acid had slightly increased sporozoite counts. The group has focused on repeated adaptation selections of the transgenic parasite in this mosquito-mouse combination. The rodent transgenic most likely will not be viable due to large numbers of mosquitoes needed to infect mice.

Rhesus model using transgenic *P. knowlesi* expressing *P. falciparum* on sporozoite surface

After a first transfection study in which no transformants were detected, LMIV performed a second transfection in which *P. knowlesi*-infected rhesus erythrocytes were transfected with the gene-replacement construct and infused to two recipient rhesus monkeys subjected to pyrimethamine selection. In this second transfection study, the number of transfected parasites was increased, the pyrimethamine selection dose decreased, and the monkeys were splenoctomized. Transformants were detected and confirmed by PCR (polymerase chain reaction) testing. Presence of *Pf*CSP gene and expression of *Pf*CS protein, absence of *Pk*CSP gene and absence of *Pk*CS protein on the surface of transgenic sporozoites were confirmed by PCR and IFA (immunofluorescent antibody) testing using anti-*Pf*CSP repeat and anti-*Pk*CSP repeat antibodies. A frozen stock of the transformants was made.

LMIV began to test infectivity to mosquitoes by passaging the transformants between monkeys and mosquitoes. During this passaging, two monkeys died due to altered growth characteristics of the transformants. The study was temporarily placed on hold for animal safety review. After reviewing the literature and consulting with Dr. Montip Gettayacamin of the Armed Forces Research Institute of Medical Sciences (AFRIMS) and Dr. Walter Weiss of the Naval Medical Research Center, LMIV arranged procurement of Artesunate from Thailand (it is not licensed in the United States), and devised an appropriate treatment regimen. Working with NIH veterinarians, LMIV also modified the treatment endpoint for this rigorous transformant. The hold was lifted and the study resumed.

The transformants were able to undergo gametogenesis, infect *Anopheles (A.) dirus* mosquitoes, and develop into oocysts in the mosquito midgut. Sporozoites were also detected in mosquito saliva, though lower in number compared to the wild type *P. knowlesi* parasites. LMIV faced a new challenge when infecting naïve monkeys with infected mosquitoes: the infected *A. dirus* mosquitoes did not feed on naïve monkeys. After consulting with several entomologists, LMIV devised a new method to force the infected mosquitoes to lay eggs prior to feeding on naïve monkeys. This method significantly improved the feeding rate and has now been adopted by several groups, including AFRIMS and the Vaccine Research Center. LMIV also adopted suggestions by Dr. Weiss, such as allowing mosquitoes to feed longer (30 minutes instead of 15 minutes) and providing a more dimly lit setting for feeding. With these improvements in methodology, a successful feeding was conducted, wherein at least 17 to 36 infectious bites were confirmed. The two monkeys that received infectious bites were followed for four weeks and no blood-stage parasites were detected. Possible reasons for this failure are reduced salivary gland invasion ability, reduced hepatocyte invasion ability, or reduced development in the monkey liver.

Going forward, the group plans to:

- Prepare several constructs encoding chimeric *Pf*CSP and *Pk*CSP by changing N- C-termini and repeat segments. As the CSP termini are necessary for sporozoite invasion, retaining these regions of *Pk*CSP may improve liver cell invasion and thus ultimately enable parasitemia to develop.

- Intravenously administer the current transgenic sporozoites from hemocele to rhesus to reduce the stringency of development of parasitemia. If the monkeys can be infected this way, further adaptation will be performed.

Table 3 below outlines the status of the deliverables for these studies.

Table 3. Status of deliverables for LMIV rodent and rhesus challenge model transfection studies.

Activity	Deliverable	Status
Rodent challenge model		
PCR analysis	Presence of transgene confirmed Absence of *Pb*CSP gene confirmed	Completed
IFA	Expression of *Pf*CSP on sporozoite surface confirmed	Completed
Passage between mosquitoes and mice	Reproducible transgenic parasite stock established	Completed
Challenge naïve mice	Parameters for challenge model established	On hold
Two challenge studies in mice	Potential candidates to move to monkey studies selected	On hold
Rhesus challenge model		
Molecular design and construction	Gene-replacement vector for transfection	Completed
Infect monkeys, transfect, *in vivo* selection, cloning	*Pf-Pk*CSP transgenic parasite obtained	Completed
PCR, IFA, monkey/mosquito passage	Reproducible transgenic parasite stock established	In progress
Challenge naïve monkeys	Parameters for challenge model established	Expected Q2 2011
Two challenge studies in monkeys	Determination of whether conjugates provide superior protection	Expected Q4 2011–Q1 2012

AMA1 polymorphism and optimization of AMA1 as a vaccine candidate/ component

The US Agency for International Development (USAID) supports three projects aimed at overcoming the issue of polymorphism by either developing a multivalent vaccine or an optimized AMA1 immunogen that transcends heterogeneity. Investigators at the Walter and Eliza Hall Institute (WEHI) are pursuing the development of a multivalent vaccine under an agreement that initiated in July 2008. Different approaches are being taken by investigators at the Walter Reed Army Institute of Research (WRAIR) and LaTrobe University to develop a modified AMA1 vaccine under a collaborative agreement that was signed in October 2008. Descriptions of Fiscal Year (FY) 2010 major activities and results for each group appear below.

Studies toward the strategic development of a multivalent AMA1 vaccine (WEHI)

The WEHI multivalent AMA1 project entered its third year in May 2010, and thus far, the major project achievements include the development of an approach to transfect *P. falciparum* with different AMA1 alleles using complete allelic replacement or with mutant AMA1 genes. Using these transgenic parasites, they have established and validated an approach to measure AMA1-specific invasion inhibitory antibodies in human samples. They have also developed an approach to measure antibodies to functional epitopes of AMA1 by competition ELISA. Through these approaches, they have come to further understand AMA1 allele-specificity and cross-reactive

antibodies in naturally exposed persons. In the past year, progress toward major project milestones occurred in the following areas.

Development of AMA1 transgenic parasite lines and recombinant proteins

- The team successfully generated and established parasites transfected with W2mef, 7G8, *Pf*2004, and XIE. *Pf*2004 and XIE are recently adapted isolates from Africa and Papua, New Guinea (PNG), respectively. Work has continued toward expressing five additional AMA1 variants as recombinant proteins.
- Over the past year, the WEHI investigators adopted a new strategy to expand on the work of generating transgenic parasites by complete allelic replacement and are focused on the C1L region of AMA1, since previous work has shown that this region is a target of invasion inhibitory antibodies and important in mediating immune escape. They have begun mutating specific residues and will determine which of those are important or how many are needed for escape of vaccine-induced antibodies or naturally acquired antibodies.

Understanding antibodies to different AMA1 alleles in naturally exposed populations

- WEHI investigators have continued to use serum samples collected in the course of longitudinal cohort studies of naturally exposed children and adults in PNG. Using recombinant proteins, they measured the prevalence of antibodies to the AMA1 variants 3D7, W2mef, FVO, and HB3 by ELISA and their association with protection. In terms of allele-specificity of antibodies, they are examining the extent of cross-reactive antibodies among the different alleles, and their rate of acquisition with age. Antibodies from these exposed individuals have also been tested in growth inhibition assays (GIAs) using transgenic parasites.
- To understand how much antigenic difference there is between the variants, competitive ELISAs are performed in which the ELISA plate is coated with one antigen and then the serum antibodies are measured to that plate antigen in the presence or absence of a competing AMA1 variant. These assays allow for detecting how much of the response is strain-specific versus cross-reactive. WEHI investigators have done competition ELISAs using sera from 125 children and adults using five AMA1 proteins, for a total of 20 assay combinations. Data collected thus far suggest that 3D7 is antigenically an outlier; it is less related to the other three variants, which are more related to each other. A comparison of FVO and 3D7 suggests that the inclusion of 3D7 in a vaccine may not be valuable, since it does not provide significantly more antigenic coverage than FVO.
- WEHI investigators plan to use the competition ELISA for samples from the longitudinal cohort and determine the association between allele-specific antibodies, cross-reactive antibodies, and protection, and determine whether those who develop good cross-reactive antibodies are better protected than those who have highly strain-specific antibodies.

Testing AMA1 inhibitory antibodies among naturally exposed individuals using transgenic parasites

- Three transgenic parasite lines expressing different AMA1 alleles have been generated. They have been used in GIAs, and five additional variants have been generated. The parasites were

initially tested against rabbit antibodies, inhibitory mAbs, and then 100 sera from PNG to identify inhibitory samples.

- In summary, 20 percent of people had FVO-specific inhibitory antibodies and considerably fewer individuals had 3D7-specific antibodies. Very few individuals had HB3-specific antibodies, but final results are pending. With the additional transgenic parasites, WEHI investigators plan to measure inhibitory antibodies to the other variants.

Progress toward a multi-allele vaccine: preliminary recommendations

- Investigators will draw on the seroprevalence and competition ELISA data, the inhibition assays using transgenic parasites, and the mutation studies to identify residues for vaccine escape (no results yet) to identify which alleles to include in a vaccine.
- The preliminary recommendation is to include FVO, but it is debatable whether to include 3D7. Data also suggest that either W2mef or 7G8 should be included, but not both.

Development of a more effective malaria vaccine using a modified form of recombinant AMA1 (WRAIR)

The WRAIR team is pursuing both a monovalent and a polyvalent strategy to developing an AMA1 vaccine. The monovalent approach entails mapping the cross-reactive epitopes with chimeric proteins and mAbs. The polyvalent approach is using published data on the C1L loop to express AMA1 variants from specific phylogenetic groups and using them for immunization and cross-strain GIA testing to determine the minimum valency that will be adequate for clinical relevance.

Progress toward developing a monovalent vaccine based on mapping cross-reactive epitopes using chimeric proteins and mAbs

- Investigators tested *P. falciparum-P. berghei* chimeric proteins in GIA reversal assays and anti-3D7 rabbit polyclonal antibodies against 3D7 parasites (chimeras designed based on the crystal structure of AMA1). Overall, results indicate:
  - The single domains of AMA1 do not individually contribute significantly to the inhibitory activity of the antibodies, but when chimeras are mixed, the inhibitory activity increases significantly.
  - In various GIA reversal experiments, reversal of inhibition was greatest with the domain 3 crystal chimera, indicating that a domain 3-driven immune response is important; however, no one domain contains all of the inhibitory epitopes of AMA1.
  - The inhibitory activity of the polyclonal anti-3D7 sera was primarily distributed on the polymorphic face of AMA1.
- Investigators also tested antibodies raised in rabbits to the chimeric AMA1 proteins by GIA. These assay results confirmed the GIA reversal results and also showed that individual domain antibodies do not significantly inhibit invasion; however, antibodies to mixtures of domains yield significant levels of growth inhibition.

Progress toward developing a vaccine using a polyvalent approach

- In the first year of the project, rabbits were immunized with polyvalent vaccine consisting of W2mef, HB3, 3D7, and FVO AMA1. GIA results from this study showed that this vaccine elicited the best inhibition against both 3D7 and FVO parasites, plus six additional parasite isolates.
- The WRAIR team has also done GIA experiments to determine specifically how this mixed vaccine, referred to as "Quadvax," functions. Results indicate that per mg of the antibody, the Quadvax is significantly more inhibitory to the FVO parasite than the 3D7.
- Investigators have also performed cross-strain GIA reversal assays using chimeras to map where on AMA1 the response is coming from. Results indicate that almost all of the cross-strain inhibition seems to originate from domain 3 and the conserved face of AMA1.
- Phylogenetic analyses have been undertaken to augment the selection of strains for inclusion in testing of the polyvalent vaccine approach.
- To determine if the polyvalent approach can be improved by using chimeras, such that four strains can be incorporated into two proteins, WRAIR investigators have produced additional chimeric proteins that contain individual or two-domain portions of the HB3 and W2mef strains. Also, a rabbit study is underway using these chimeras in combination with the 3D7/FVO chimeras such that every rabbit will receive some part of each strain.
- WRAIR will soon start a rabbit study to address whether three strains perform as well as the Quadvax. They will look into combinations of two or if a three-strain combination can be identified that performs as well as the Quadvax.

Summary of studies using monoclonal antibodies

- Monoclonal antibodies generated during the first year were tested in GIAs against multiple parasite strains, and WRAIR investigators selected eight mAbs that had similarly high inhibitory activity against four strains. The mAbs that showed good cross-strain inhibition were then mapped to their specific location on AMA1 by testing them in inhibition assays using the *Pf-Pb* chimeric proteins that display specific domains of AMA1.
- The mAbs were then used in mixing experiments against 3D7 parasites in GIA; interestingly, when mAbs 5A6 and 1E10 were mixed, the level of inhibition increased from 21 percent and 40 percent alone, respectively, to 94 percent mixed together. This is the first evidence of synergy using mAbs for inhibition of invasion.

Development of a more effective malaria vaccine using a modified form of recombinant AMA1 *(*LaTrobe University)

During the first year of the project, the LaTrobe team generated mutant forms of AMA1 with five polymorphic residues in loop Id (similar to the C1L loop described by WRAIR investigators) mutated to alanine or serine. Recombinant proteins were produced for each mutant, as well as the wild type 3D7 and FVO, and rabbits were immunized with each mutant or wild type form. The rationale of this approach is that by introducing mutations in these highly polymorphic sites, the immune response may be diverted to more conserved epitopes. The LaTrobe investigators made important strides this year toward understanding whether this approach may be feasible for developing an immunogenic vaccine through the activities below.

Rabbit immunogenicity study: single antigen immunizations

- Rabbits were immunized intramuscularly with 50 µg each of AMA1 wild type or mutant antigen formulated in Montanide ISA720. The rabbits were given boosting doses with the same amount of antigen at four and ten weeks after the primary immunization.
- Serological analyses included a direct binding ELISA to measure the IgG antibody response to 3D7 and FVO in each sample. LaTrobe investigators observed a decrease in titer at bleed 3 for each rabbit, indicating that they may not have chosen the optimal immunization regimen. In rabbits immunized with the individual mutant and WT forms of 3D7 and FVO AMA1, the highest antibody responses were found to be in bleeds taken two weeks after the second and third immunizations. The responses measured with bleeds 1 and 2, however, showed that the mutations had very little effect on their ability to raise antibodies. The likely cause for the lower response at the third bleed was due to using a dosing schedule that was not appropriate for Montanide ISA720.
- Inhibition ELISAs were also completed to determine if the altered immunogenicity of loop Id is associated with an increased immune response to conserved epitopes in the rabbits immunized with the mutant forms of AMA1. Results of these assays indicated that the heterologous AMA1 antigens have a greater capacity to inhibit the binding of sera from rabbits immunized with the 3D7 AMA1 mutants to 3D7 AMA1 than sera from rabbits immunized with the wild type antigen. This suggests that by immunizing with the mutant forms of 3D7 AMA1, an antibody response occurs that is directed away from the strain-specific epitopes to the conserved epitopes. This is in contrast to the antibody response seen in rabbits immunized with the wild type 3D7 AMA1. This same effect was not observed in the rabbits immunized with the FVO mutants, and may be explained by the finding that FVO AMA1 induces less of a strain-specific antibody response than 3D7 AMA1, so the FVO mutants have little effect on redirecting the immune response to conserved epitopes.
- GIAs were performed by the WRAIR collaborators on IgG purified from sera from the third bleed from rabbits immunized with the individual forms of mutant or wild type AMA1. Results indicated that there is a large disparity between the level of heterologous inhibition between the WT 3D7 and WT FVO, reiterating that FVO is more cross-reactive than 3D7, and 3D7 did not induce a good heterologous response with any of the immunogens.
- Overall, the data, although incomplete, suggest that a single mutant form of AMA1 is unlikely to provide an effective immune response to a wide range of *P. falciparum* AMA1 genotypes. The LaTrobe group is focusing on combinations of mutant forms to determine whether this will provide an increased response to conserved epitopes over that achieved by combinations of wild type alleles.

Mouse immunogenicity study: single antigen immunizations

- The immunogenicity of each AMA1 antigen was also compared in two strains of mice, C57BL/6 and CBA, using a 5 µg dose and a prime + single boost regimen delivered subcutaneously with Montanide ISA720.

- Results led to the conclusion that immunizing with a single form of mutated AMA1 is unlikely to induce an antibody response that is more cross-reactive among different forms of AMA1 than a single form of the wild type antigen.

Rabbit immunogenicity study: combinations of mutant AMA1

- LaTrobe investigators then proceeded to address whether immunization with combinations of mutant forms of 3D7/FVO AMA1 offers an advantage over immunization with combinations of WT 3D7/FVO AMA1.
- Direct binding ELISAs were used to compare antibody responses for each of the three bleeds taken for each rabbit. The sera were titrated on each wild type form of AMA1, and the highest antibody responses were found to be in bleeds taken two weeks after the second and third immunizations. A decrease in response was again observed with the final bleed, when the binding of sera was measured to both wild type 3D7 and FVO, indicating that the vaccination schedule was not boosting the response, so this study is being repeated.
- The team completed inhibition ELISAs using various strains of recombinant AMA1, wherein the ELISA was set up to compete the binding of the sera with the various recombinant proteins. The same trend was evident for binding to WT 3D7 and FVO in that the homologous strain competed the best among the strains tested. This indicates that either the immunization schedule used was not optimal or the approach is not sufficient for eliciting cross-reactive antibodies.
- Inhibition ELISAs were also used to determine the IC50 values, in which rabbit sera raised against combinations of WT or mutant versions of 3D7 and FVO AMA1 were allowed to bind to immobilized WT 3D7 AMA1 in the presence of increasing concentrations of 3D7, FVO, W2mef, HB3, or 7G8 AMA1. The IC50 values represent the concentration of antigen required to inhibit 50 percent of the binding of each rabbit serum to WT 3D7 AMA1. Preliminary results suggest that immunization with combinations of mutant forms of 3D7 and FVO AMA1 does not appear to increase the immune response to conserved epitopes when compared with combinations of the wild type antigens. However, these results are from sera collected at the first bleed; investigators plan to complete this analysis on the later bleeds.

The status as of September 30, 2010, of all deliverables for each of the three projects focused on AMA1 optimization is detailed in Table 4 below.

Table 4. Key activities for the coming year, deliverables, and status of each of the three AMA1 optimization projects.

Activity	Deliverable	Status
WEHI		
Conduct AMA1 allele-specific competition ELISAs using additional AMA1 variants expressed	Further understanding of polymorphic versus conserved targets of antibodies to AMA1 by ELISA	In progress (75 percent complete)
Generate additional AMA1 transgenic parasites	Characterized transgenic parasite lines to be used in invasion inhibition assays	In progress
Conduct GIAs with transgenic parasites	Further results on allele-specific inhibitory antibodies in naturally exposed individuals	In progress
Generate and test mAbs and rabbit antibodies in GIAs	Further data on allele-specificity of antibodies	In progress
WRAIR		
Conduct prime/boost immunization using the full-length AMA1 as the prime and boosting with individual domains of AMA1	Immunogenicity results from prime/boost immunization	In progress
Express properly folded fragments of AMA1	Fragments to be used in immunization studies	In progress
Conduct immunization studies to determine if three strains would be sufficient	Conclusion about whether broad enough coverage would be possible with a trivalent vaccine	In progress
Conduct immunization studies to determine if chimeras can improve immunogenicity	Immunogenicity results to indicate whether two strains can be represented in a single protein	In progress
Map cross-reactive and synergistic polyclonal and mAbs for rational design of an engineered vaccine	Visit LaTrobe and work with them on epitope mapping	Q4 2010
LaTrobe University		
Perform GIA from pools of other bleeds from rabbit study sent to WRAIR	GIA results	In progress
Repeat combination immunization study	Final immunogenicity results of combination immunization study	Not yet started
Map epitopes using mAbs and sera collected in Quadvax immunization at WRAIR	Phage library displaying AMA1 fragments will define epitopes of cross-reactive sera	Not yet started
Conduct crystallographic studies of AMA1	Complete structural analysis if crystals are obtained	In progress
Conduct mouse immunogenicity study using 3D7 AMA1 complexed with hydrophobic pocket binders	Determination of whether the antibody response can be diverted to conserved regions of AMA1	Not yet started
Generate a more highly strain-specific reagent by depleting sera of antibodies that recognize other forms of AMA1 (in addition to 3D7/FVO)	Strain-specific reagents to be used in immunogenicity assays	Not yet started
Produce recombinant 3D7/FVO AMA1 with four loop Id residues replaced with heterologous residues, and initiate immunogenicity study using 3D7 with four FVO loop Id residues	Results of testing various sera on these proteins to further understand the immune response to this region	Not yet started

Assay harmonization

USAID has been supporting assay harmonization efforts through the consultancy of Patrice Dubois. His efforts have focused largely on harmonization of the antibody-dependent cellular inhibition assay (ADCI) and the T cell assay (interferon-gamma [IFN-γ] ELISpot and ICS) efforts within the Optimalvac project.

Antibody-dependent cellular inhibition assay harmonization

In the past year, activities have focused on preparation for a first round of testing, in which the participating laboratories will test a panel of standard reagents with their own methods. Based on the results of this first testing, a consensus standard operating procedure (SOP) for ADCI will be selected. Testing is expected to begin in Q4 2010.

The standard reagents to be used were identified as follows:

- Sera from endemic and non-endemic areas: one from Institut Pasteur (collected in Côte d'Ivoire) and one from Radboud University Nijmegen Medical Centre (collected in Gabon). Work progress was slowed due to issues connected with ethical considerations regarding the use of human samples as comparators in the harmonization process. These issues were resolved and the National Institute for Biological Standards and Control (NIBSC) has given ethical approval for the use of serum samples as reference reagents in ADCI testing.
- Monocytes: All laboratories will use their own source of cells.
- Parasites: All laboratories will use their own source of parasites.
- A monoclonal antibody, RAM 1 (human monoclonal antibody anti-*P. falciparum* MSP3), developed by Pierre Druilhe and known to display specific activity in ADCI has been produced and will be used as a standard throughout the project. The production of this antibody was delayed because of administrative issues. The antibody is completing quality control testing. If the vialing process at NIBSC does not alter RAM 1 activity, it will be distributed to the participating laboratories in Q4 2010.

T cell assays

The objective of this project is to harmonize T cell-mediated immune assays within the context of malaria vaccine development, involving the following tasks:

- Identification of key parameters and optimal approaches in ELISpot and ICS, which are relevant to malaria vaccine evaluation.
- Identification of sources of activation and detection standards for the selected assays.
- Harmonization and optimization SOPs for selected assays (including ELISpot and/or flow cytometry-based assays) by liaising with the current ongoing standardization exercises in the HIV and tuberculosis vaccine communities, which could contribute to the malaria community's efforts to harmonize T cell assays.
- Ensuring the training of personnel.
- Liaising with the HIV and tuberculosis T cell assay harmonization effort.

- Distribution of reference cell preparations and reagents to participants.
- Performance of intra- and inter-laboratory assay variability tests.

In the past year, the following standard reagents were identified:

- Peripheral blood mononuclear cells (PBMCs): Dr. Giuseppe Pantaleo's laboratory at University of Lausanne Hospital will act as supplier of PBMCs for inclusion in ICS and ELISpot panels. The laboratory has extensive experience in assay standardization (they have participated in multiple HIV panel testing) and works under Good Laboratory Practices. Following the identification and characterization of donors, the pools will be sent to each participant in one single shipment in dry cryoshippers. The PBMCs will be distributed to participating laboratories in Q1 2011 for testing.
- Tetanus toxoid peptide antigens: A free supply of tetanus toxoid (provided by Serum Institute of India) for use as an antigen has been secured and distributed to all participants.
- Cytomegalovirus, Epstein-Barr virus, and flu virus (CEF) peptide antigens: The CEF peptides will be purchased by NIBSC from JPT Peptide Technologies GmbH in Berlin and distributed to all participants at no cost.

In preparation of the reports on the identification of key parameters and optimal approaches for evaluating T cell responses in pre-erythrocytic and blood-stage vaccinations and on the development and rationale of acceptance criteria for assay performance, key aspects of the ICS and ELISpot assays undertaken in the various laboratories were collected through a questionnaire.

On the advice of the Optimalvac Technical Advisory Committee, SOPs for IFN-γ ELISpot and ICS were obtained from Duke University. NIBSC is currently reviewing these SOPs and generating a guidance document on critical aspects of the assays, to be distributed to participating laboratories. In the first round of testing, the laboratories will follow their own procedures altered to incorporate the guidance document. The first round of testing is scheduled to occur in Q1 2011.

Adenovirus-based vaccine candidates

In 2010, MVI continued work to develop alternatives to adenovirus type 5 (Ad5) as vaccine candidates for malaria. Collaborations with GenVec (Ad28) and Crucell (Ad26), supported in part by funds from USAID, were instrumental in this work.

GenVec's Ad28-CSPΔGPI program

In 2009, GenVec generated a research vector stock of Ad28 expressing CSP from the E1 region (Ad28-CSPΔGPI). This work was conducted as part of a larger study also supported by core funding from the Bill & Melinda Gates Foundation, ultimately aiming to develop a multivalent, pre-erythrocytic stage Ad28 vaccine vector for *P. falciparum* malaria. In 2010, characterization of Ad28-CSPΔGPI continued. Growth of the vector was confirmed to be equivalent to E1 deleted Ad28. Preliminary rodent immunogenicity studies comparing Ad28-CSPΔGPI to equivalent Ad5 and Ad35 constructs demonstrated that CD8 IFN-γ T cell responses to Ad28

were lower than that of Ad5 but higher than Ad35 at the same dose, as expected. Anti-CSP antibody responses were difficult to interpret, due to small group sizes.

Additional work outside the scope of USAID funding demonstrated the feasibility of generating E1/E3/E4 multiply-deleted Ad28 backbones for insertion of multiple antigens. GenVec also attempted to generate an Ad28-CSP prototype vector with enhanced immunogenicity by replacing all or some of the Ad28 fiber shaft with that of Ad5. This work revealed a fundamental incompatibility between Ad28 and Ad5 fiber shafts; only two very small insertions, KKTK and RGD, were viable. Neither of these modifications resulted in enhanced immunogenicity in initial mouse studies.

One of the primary supporting arguments for developing Ad28 as a vector for malaria vaccines was the expectation of low neutralizing antibody prevalence in endemic regions. A Go/No-go criterion for the project was incorporated to reflect this. Work would continue beyond Q3 2010 only if prevalence of Ad28 neutralizing antibodies in adults in representative endemic regions of sub-Saharan Africa was demonstrated to be less than 30 percent. In Q2 2010, an independent assessment of Ad28 neutralizing antibody prevalence in a subset of sub-Saharan Africa was conducted by the NIH, and demonstrated prevalence far greater than 30 percent. In light of these data, along with concerns regarding the long-term financial solvency of GenVec following the discontinuation of an unrelated Phase III clinical trial (TNFerade), MVI and GenVec agreed to terminate the study following completion of a limited scope of work, completed in August 2010. The status of all deliverables at the time of termination is detailed in Table 5.

Table 5. Status of deliverables for GenVec's Ad28-CSPΔGPI program.

Activity	Deliverable	Status
Construct generation	Report	Completed
Immunogenicity studies (mice) Fiber shaft modified Ad28	Study report to include: • Interim data from mouse immunogenicity studies • Generation of fiber shaft modified Ad28	Completed
Project decision point	Report on: • Final mouse immunogenicity studies • Construct generation • Multiple deletion prototype • Ad28 seroprevalence	Decision: No-go (seroprevalence), May 2010
Generation of multivalent pre-erythrocytic Ad28 vector	Interim study report	Terminated
Statement of Work completed	Final study report	Terminated

Crucell's Ad35.CS.01/Ad26.CS.01 program

Manufacturing, release, and testing of Ad26.CS.01 drug substance was completed in November 2009 and filling of the drug products (clinical trial materials) was performed in January 2010. Final release and testing of the clinical trial materials was completed in June 2010 as evidenced by receipt of the certificate of analyses for two concentrations of Ad26.CS.01 ($1x10^{10}$ vp/ml and $1x10^{11}$ vp/ml). We received the audited toxicology study report for Ad35.CS.01/Ad26.CS.01 immunizations in June 2010, which showed no toxicity of the prime boost regimen. A Go decision was made to proceed to the clinical trial. Stability testing of all lots of Ad35.CS.01 and Ad26.CS.01 will continue through the completion of the clinical trial.

For the heterologous prime boost clinical study of Ad35.CS.01 and Ad26.CS.01, the clinical trial design consisted of one cohort of 20 subjects (with six controls) immunized two times with a dose of $5x10^{10}$ vp/ml Ad35.CS.01 and then immunized with a dose of $5x10^{10}$ vp/ml Ad26.CS.01 followed by infected mosquito challenge. The design is based on immunology data acquired from the Ad35.CS.01 clinical trial sponsored by the NIH/National Institute of Allergy and Infectious Diseases. The Seattle Biomedical Research Institute (Seattle BioMed) Malaria Clinical Trials Center was selected as the study site and the Clinical Trial Agreement was signed on August 13, 2010. The Investigational New Drug (IND) application was submitted to the US Food and Drug Administration (FDA) Center for Biologics Evaluation and Review (CBER) on July 20, 2010. The IND was placed on clinical hold on August 18, 2010. FDA CBER identified four major safety concerns and made corresponding recommendations:

- They did not agree that the current clinical data for previous Ad35 and Ad26 human studies support the proposed dose of $5x10^{10}$ vp/ml for either vector. They recommend a dose escalation study design.
- They recommend that we increase the time interval that follows the Ad26 pioneer group from 12 hours to 72 hours.
- They recommend that we re-evaluate the timing for safety blood draws (i.e., essentially add more time points).
- They recommend that we include a neurological assessment, as the Ad35 study identified a potential for neurological adverse events.
- They recommend that we assess hematuria based on the significant rate of hematuria in female subjects in the Ad35 study.

Next steps for resolution of the clinical hold are being discussed with Crucell and Seattle BioMed. Study initiation is anticipated in December 2010.

USAID funding is being used to fund Crucell's activities under this project. MVI will utilize non-USAID funding sources to cover the clinical trial. The status of this project is outlined in Table 6 below.

Table 6. Status of deliverables for Crucell's Ad35.CS.01/Ad26.CS.01 program.

Activity	Deliverable	Status
IND for Ad35.CS.01/Ad26.CS.01 clinical trial	• Finalized IND application for review and comment by PATH • Report on status of submission to FDA • Copy of FDA response, if any, and documented evidence that vaccine has been cleared by the FDA to begin clinical trial • Go/No-go decision on clearance of IND by FDA	Q4 2010
Stability and clinical sample analyses	Stability data reports on Ad35.CS.01 and Ad26.CS.01 drug products	Q4 2010

Policy and commercialization projects

Framing the malaria vaccine introduction decision: the Decision-Making Framework

It is well-recognized that many new lifesaving interventions are often accessible to public health programs in developing countries only decades after they have been licensed. These delays are due to a lack of evidence for rational policy decision-making and the lack of necessary mechanisms to support the decisions once made.

To avoid such delays when a malaria vaccine becomes available, the MVI Policy and Access Unit began working in 2006 with decision-makers from malaria-endemic countries and partners on a process that would ensure that, once a malaria vaccine does become available, policymakers will have all the necessary information to decide on its use within one to three years after the vaccine is licensed.

Since 2006, USAID has been supporting MVI on this process, through the development of the Decision-Making Framework (DMF) for malaria vaccine, to help policymakers identify data and processes needed to make a decision about the adoption of a malaria vaccine to complement existing malaria control interventions.

After the development period of the framework (2006-2008), MVI started actively engaging and supporting national malaria and EPI programs to initiate a process that will pave the way for a timely decision on a malaria vaccine.

To continue this in 2010, MVI had the following objectives:

1. Roll out the malaria vaccine DMF in non-RTS,S clinical trial countries.
2. Support DMF technical advisory groups for malaria vaccine (mechanisms of consultation between researchers and ministries of health) to coordinate data collection and establishment of processes for policy and decisions.
3. Continue to update national and regional stakeholders on malaria vaccine development progress each year.
4. Continue to work on information requested in the DMF from global partners.

Activities related to objectives 2, 3, and 4 were supported through other MVI resources. MVI received funds from USAID to implement activities related to objective 1.

In the section below, we report on the activities that were carried out during the reporting period.

Develop and agree with the steering committee on a strategy for the rollout of the DMF for malaria vaccine

As described in the section above, the process of engaging countries in the malaria vaccine DMF started in 2006, when results of proof-of-concept trials showed that a malaria vaccine was

feasible and could enter the last phase before licensure. At that time, the clinical development plan anticipated that a vaccine would be licensed within the next five years. Also, five years was estimated to be the adequate time for good policy preparation.

As it now stands, possible licensure of the most advanced malaria vaccine candidate is anticipated for 2015 or so; therefore, it is important to pace country-level preparation with the vaccine development time frame in order to keep the momentum going, and to avoid "fatigue" in these countries while ensuring that countries stay engaged in the process of preparing for a malaria vaccine. To this end:

- MVI developed a proposal to the steering committee outlining an approach for the rollout of the DMF for malaria vaccine in additional countries over the period 2010-2014. The document is available on the DMF website: www.malvac.decision.net.
- Additional countries have initiated work around the DMF for malaria vaccine: MVI worked with the Uganda Academy of Sciences to convene an expert meeting to discuss malaria vaccines and the use of the framework in Uganda. A subcommittee was set up to move the process forward. MVI also briefed the Director of the Public Health Department in the Nigeria Federal Ministry of Health about malaria vaccine development and the DMF for malaria vaccine. Plans are underway to hold a stakeholders meeting in Abuja before the end of 2010.

Convene a meeting with the World Health Organization (WHO) to discuss collaboration around the rollout of the DMF in Africa, including finalization of the DMF guide

Since the inception of the malaria vaccine DMF, MVI has worked closely with partners—in particular with WHO, as the leading countries' technical partner—to develop the framework. This year, MVI's objective was to meet with WHO to develop joint activities to continue support for countries that have started working on the framework and for rolling out the framework in other countries.

- MVI's Christian Loucq and Alan Brooks met in November in Geneva with the Director of the WHO Regional Office for Africa (AFRO) and the Regional Focal Points for WHO's malaria and immunization programs. The regional office held off on a formal agreement with MVI for joint activities at country level, but did agree to participate in subregional briefing meetings with the national program managers.
- WHO AFRO staff worked with MVI to present updates on malaria vaccines and the DMF at three WHO AFRO subregional meetings on malaria and immunization.

Finalize and publish the DMF guide

MVI is in the final stages of reviewing the guidance document, *Guide for Country Level Implementation of the Regional Malaria Vaccine Decision-Making Framework*. MVI sought inputs from external experts with competencies in malaria and immunization and with research or program management experience. A group of five external reviewers provided input into the document.

Jointly convene with WHO three subregional workshops

MVI will continue discussions with WHO to reach an agreement on the roles of each organization in providing technical support to countries in implementing the DMF. Once this has been established, the three subregional meetings that were originally planned for this year will be scheduled. See Table 7 below for a status update.

Table 7. Status of deliverables for DMF activities.

Activity	Deliverable	Status
Develop and agree with the steering committee on a strategy for DMF rollout	Document outlining steps to roll out the DMF in non-clinical trials	Completed
Convene a meeting with WHO to discuss collaboration to roll out the DMF in Africa, including finalization of the DMF guide	Agreement with WHO on technical support to countries for the DMF and on publication of the DMF guide	Meeting held with WHO AFRO senior management
Finalize and publish the DMF guide	Finalized and published malaria vaccine DMF implementation guide	In progress
Jointly convene with WHO three subregional workshops on the implementation of the malaria vaccine DMF	Plans to implement the DMF according to the rollout strategy and in line with lessons from other countries	Put on hold until further discussion with WHO AFRO