Blood stage vaccines for *Plasmodium falciparum*

Current status and the way forward

Ruth D. Ellis,^{1,*} Issaka Sagara,² Ogobara Doumbo² and Yimin Wu¹

¹Laboratory of Malaria Immunology and Vaccinology; National Institute of Allergy and Infectious Diseases; National Institutes of Health (NIAID/NIH); Bethesda, MD USA

²Malaria Research and Training Center; Faculty of Medicine Pharmacy and Dentistry; University of Bamako; Bamako, Mali

Key words: malaria, vaccine, blood stage, falciparum, merozoite

Abbreviations: AdCh63, adenovirus strain 63; AMA1, apical membrane antigen 1; AMANET, African Malaria Network Trust; AS (01, 02), adjuvant system; BSAM, blood stage antigen mixture; CSP, circumsporozoite protein; DNA, deoxyribonucleic acid; DMID, Division of Microbiology and Infectious Diseases; EMVI, European Malaria Vaccine Initiative; FMP (1, 2.1), falciparum malaria protein; FP9, fowlpox 9; GLURP, glutamate rich protein; GMZ, GLURP + MSP 3; LMIV, Laboratory of Malaria Immunology and Vaccinology (formerly Malaria Vaccine Development Branch); LSP, long synthetic peptide; MSP (1, 2, 3, 4), merozoite surface protein; MVA, modified vaccinia ankara; NIAID, National Institute of Allergy and Infectious Diseases; NYVAC-PF7, highly attenuated vaccinia virus with 7 *P. falciparum* genes inserted into its genome; PEV (3A, 301, 302), pre-erythrocytic vaccines; PfCP-2.9, *Plasmodium falciparum* chimeric protein; PfEMP1, *Plasmodium falciparum* erythrocyte membrane protein 1; QIMR, Queensland Institute of Medical Research; RESA, ring-infected erythrocyte surface antigen; SERA, serine repeat antigen; TLR-4, toll-like receptors-4; TRAP, thrombospondin-related anonymous protein; WEHI, Walter and Eliza Hall Institute; WHO, World Health Organization; WRAIR, Walter Reed Army Institute of Research

Since the recent call for a shift from malaria control to eradication, the role of asexual blood stage vaccines for falciparum malaria, which are not expected to prevent infection, has become less clear. However, blood stage antigens remain likely to be a critical component of a highly effective malaria vaccine. The inclusion of a blood stage component in a multistage malaria vaccine would not only prevent disease caused by “leaky” pre-erythrocytic immunity, but would also protect against epidemics in newly vulnerable populations. Recent clinical results of blood stage vaccine candidates have shown strain specific and partial efficacy, although no protection against clinical outcomes has been demonstrated in experimental infection or field trials to date. The current status of *Plasmodium falciparum* blood stage vaccine development is summarized and the potential role of these vaccines in the changed malaria landscape is discussed. Alternative preclinical and clinical development paths will speed iterative development.

Introduction

Recent declines in malaria transmission have prompted the announcement of a shift in long term strategy from malaria control to eradication or elimination.¹ However, while significant reductions in transmission and disease have been achieved in

widely varying settings,² the tools used to make these advances (artemisinin combination drug therapy, intermittent preventive therapy, indoor residual spraying and insecticide treated bednets) are vulnerable to the development of resistance to drugs by parasites and resistance to insecticides by mosquitoes. Furthermore, current efforts rely on intensive interventions that may not be sustainable over the long term, particularly in resource constrained settings or areas suffering from political instability. Even when comprehensive integrated control strategies have been implemented in well circumscribed areas local elimination has proved to be an elusive goal, despite impressive reductions in transmission and all cause mortality.^{3–5} Indeed the only examples of disease eradication or near eradication (smallpox and polio) provide evidence that a highly effective vaccine is needed to make elimination of malaria from high transmission areas a real possibility, as opposed to a distant dream.

Naturally occurring protection against malaria disease occurs in adults living in malaria endemic areas and is thought to be antibody mediated, based on experiments conducted using passive transfer of immunoglobulin.^{6,7} However, natural immunity is imperfect, does not completely develop until the immune system matures, and appears to wane if exposure is interrupted.⁸ Evidence that blood stage immunity can be induced experimentally has been demonstrated by the repeated administration of low doses of infected red cells,⁹ with subsequent protection correlated with T-cell responses, and was also recently demonstrated when volunteers were infected with sporozoites under chloroquine “cover” and were subsequently found to be protected from homologous challenge, with protection again associated with T-cell responses.¹⁰ Furthermore, the mechanism of action of the malaria vaccine which is furthest in clinical development (RTS,S) may be partially due to “leaky” pre-erythrocytic protection

*Correspondence to: Ruth D. Ellis; Email: ellisru@niaid.nih.gov

Submitted: 01/06/10; Accepted: 02/07/10

Previously published online:

www.landesbioscience.com/journals/vaccines/article/11446

DOI: 10.4161/hv.6.8.11446

resulting in continued exposure to blood stage parasites and the resulting acquisition of blood-stage immunity.¹¹ Thus protection against blood stages appears to be a critical element of an effective immune response to malaria, and also likely to be a critical component of a highly effective malaria vaccine. Including a blood stage component in a multi-stage vaccine would also protect against epidemic malaria, which becomes an increased risk once natural immunity wanes in a newly protected population.

Current Clinical Development

Many candidate blood stage vaccines have progressed to early phase clinical development, with several completing Phase 2b studies in the target population, young malaria exposed children. **Table 1** summarizes current clinical development. Phase 1a trials are typically conducted first, in malaria naïve adults, followed by Phase 1b in malaria exposed adults, and Phase 1b in malaria exposed children. Phase 2 studies can be either experimental challenge studies (2a) or field efficacy trials (2b). While at first glance the development pipeline appears robust, the number of antigens evaluated in the clinic to date is limited to 8: apical membrane antigen 1 (AMA1), merozoite surface protein (MSP) 1, MSP2, MSP3, glutamate rich protein (GLURP), ring-infected erythrocyte surface antigen (RESA), serine repeat antigen (SERA5), and erythrocyte-binding antigen 175 (EBA 175). Varying formulations of AMA1 and MSP1₄₂ account for the majority of the vaccines which have been taken forward to the clinic, particularly those which have gone to Phase 2 studies. Most candidate vaccines are recombinant proteins, but peptides, virosomes, DNA and viral vectored approaches are also being used, as reviewed by Richards and Beeson.⁴⁸ The adjuvants in use include alum, Montanide ISA 720 (Seppic), adjuvant systems including AS01 and AS02 from Glaxo-Smith Kline (GSK), and CPG 7909 (Coley Pharmaceuticals, a Pfizer Company). Antigens are also being used in combination, both with other blood stage antigens and with pre-erythrocytic antigens such as circumsporozoite protein (CSP) and thrombospondin-related anonymous protein (TRAP).

Five blood stage vaccines (four recombinant proteins and one synthetic peptide) have been or are being evaluated for impact on clinical or parasitologic endpoints in malaria exposed children, with results known for four of these. Combination B (the proteins RESA, MSP1 and MSP2, formulated with ISA 720) was shown to reduce parasite density by 62% in children not pretreated with sulfadoxine-pyrimethamine, and to reduce the prevalence of parasites carrying the allelic form of MSP2 contained in the vaccine.³⁹ However, there was no overall effect on clinical malaria. Falciparum malaria protein (FMP) 1, MSP1₄₂ (3D7 allele) adjuvanted with AS02A, had no impact on first episodes of clinical malaria (fever with parasitemia of $\geq 50,000/\mu\text{L}$), or on the overall incidence of clinical malaria or malaria infection, or parasite density.³³ AMA1-C1, a combination of FVO and 3D7 allelic proteins, adjuvanted with Alhydrogel, also showed no impact on frequency of parasitemia $>3,000/\mu\text{L}$ or on clinical malaria, and had a negative impact on hemoglobin, with a higher frequency of anemia (hemoglobin $<8.5 \text{ g/dL}$) in the

vaccinees.¹⁵ Preliminary results from the fourth trial, of FMP2 (the 3D7 allelic protein of AMA1) adjuvanted with AS02A, were presented at the 5th Multilateral Initiative on Malaria meeting in November, 2009 and also at the 2009 annual meeting of the American Society of Tropical Medicine and Hygiene. While the impact on the primary outcome (time to first clinical malaria defined as fever and parasite density $>2,500/\mu\text{L}$) was non-significant, there was a consistent reduction of $\sim 20\%$ on not only the primary outcome, but multiple secondary outcomes relating to parasite density and clinical malaria using other cutoffs. There was no adverse impact on the frequency of anemia, and allele specific protection appears to have been induced. The fifth trial is of MSP3 long synthetic peptide adjuvanted with aluminum hydroxide, and is expected to be unblinded in mid-late 2010 (ClinicalTrials.gov Identifier: NCT00652275). It is interesting to note that of these five trials, three have been conducted in Mali, an area of intense seasonal transmission. A multi-site Phase 2b trial of a chimeric protein vaccine, GMZ2 (MSP3 and GLURP, adjuvanted with aluminum hydroxide) is planned to begin in African children in 2010 (5th MIM Pan-African Malaria Conference, 2009, Symposium 14).

Two vaccines containing AMA1 have also been evaluated for efficacy in the sporozoite challenge model. Since AMA1 is also thought to be expressed on sporozoites,⁴⁹ an AMA1 vaccine could also have some pre-erythrocytic effects. A virosomal formulation containing AMA1 and CSP peptides, PEV3A, was shown to decrease in vivo parasite growth rates and to induce morphologically abnormal parasites, with one volunteer not becoming smear positive until 20 days after a challenge with heterologous parasites.²⁸ In a challenge study of AMA1 adjuvanted with AS01B or AS02A no delay in patency was seen, but estimation of parasite growth rates suggested a partial biologic effect of the vaccine against parasites homologous for the vaccine strain.²⁵ Thus while no overall clinical impact of vaccination with AMA1 has been demonstrated either in field studies or in a challenge model to date, partial efficacy and strain specific effects appear to have been demonstrated, particularly with the highly immunogenic formulations using GSK adjuvant systems. Another highly immunogenic formulation, AMA1-C1/Alhydrogel + CPG 7909, is planned to be evaluated in a homologous blood stage challenge trial (ClinicalTrials.gov Identifier: NCT00984763).

Immunogenicity has been variable for the many vaccines evaluated in Phase 1 trials. Aluminum based adjuvants have an extensive safety record and have been widely used. However, immunogenicity has been suboptimal, and stronger adjuvants such as GSK's adjuvant systems, Montanide ISA 720, and CPG 7909 have also been used extensively. Local reactogenicity is typically increased with these adjuvants, particularly for the water-in-oil emulsions, and for ISA 720 is likely to limit further development. Toll like receptor agonists such as CPG 7909 may be uniquely useful against parasitic infections, but have a limited safety record, particularly in healthy populations and children. Immunogenicity varies with the population vaccinated; responses in malaria naïve adults (typically the first to receive a new vaccine) are not necessarily predictive of responses in malaria exposed adults and children. Reactogenicity also may

Table 1. Vaccines targeting blood stages of *P. falciparum*

Antigen(s)	Vaccine(s)	Developer or sponsor	Phase	Location	Reference
AMA1	AMA1-C1/Alhydrogel	LMIV (formerly MVDB)	1a	USA	12
AMA1	AMA1-C1/Alhydrogel	LMIV	1b (children)	Mali	13
AMA1	AMA1-C1/Alhydrogel	LMIV	1b (adults)	Mali	14
AMA1	AMA1-C1/Alhydrogel	LMIV	2b (children)	Mali	15
AMA1	AMA1-C1/Alhydrogel +/- CPG 7909	LMIV	1a (adults)	USA	16
AMA1	AMA1-C1/Alhydrogel with CPG 7909	LMIV	1a (adults)	USA	17
AMA1	AMA1-C1/Alhydrogel +/- CPG 7909	LMIV	1b (adults)	Mali	18
AMA1	AMA1-C1/Alhydrogel with CPG 7909	LMIV	2a (adults)	UK	ClinicalTrials.gov Identifier: NCT00984763
AMA1	FMP2.1/AS02A	WRAIR	1a (adults)	USA	19
AMA1	FMP2.1/AS02A	WRAIR	1b (adults)	Mali	20
AMA1	FMP2.1/AS02A	WRAIR	1b (children)	Mali	21
AMA1	FMP2.1/AS02A	WRAIR	2b (children)	Mali	ClinicalTrials.gov Identifier: NCT00460525
AMA1	AMA1-C1/ISA 720	LMIV	1a (adults)	Australia	22
AMA1	AMA-1/ISA 720	QIMR, Biotech Australia Pty Ltd., Cooperative Research Centre for Vaccine Technology	1a (adults)	Australia	23
AMA1	AMA1/Alhydrogel, AMA1/ISA 720, AMA1/AS02	AMANET	1b (adults)	Mali	ClinicalTrials.gov Identifier: NCT00431808
AMA1	AMA1/Alhydrogel, AMA1/ISA 720, AMA1/AS02	EMVI	1a (adults)	Netherlands	24
AMA1	AMA1/AS01B and AMA1/AS02A	WRAIR	1/2a (adults)	USA	25
AMA1, MSP1	PfCP-2.9 chimeric protein/ISA 720	WHO	1a (adults)	China	26
AMA1, MSP1	BSAM2/Alhydrogel + CPG 7909	LMIV	1a, 1b (adults)	US, Mali	ClinicalTrials.gov Identifier: NCT00889616
AMA1 and CSP	PEV301 and PEV302 virosomes	Swiss Tropical Institute	1a (adults)	Switzerland	27
AMA1 and CSP	DNA prime, Adenovirus 5 boost	WRAIR	1, 2a (adults)	US	ClinicalTrials.gov Identifier: NCT00870987
AMA1, CSP, TRAP	PEV3A virosome, FP9/MVA ME-TRAP	Swiss Tropical Institute, Peven, University of Oxford	1, 2a (adults)	Oxford	28
MSP1 ₄₂	MSP1 ₄₂ -FVO/Alhydrogel and MSP1 ₄₂ -3D7/Alhydrogel	LMIV	1a (adults)	USA	29
MSP1 ₄₂	MSP1 ₄₂ -C1/Alhydrogel +/- CPG 7909	LMIV	1a (adults)	USA	30
MSP1 ₄₂	FMP1/AS02A	WRAIR	1a (adults)	USA	31
MSP1 ₄₂	FMP1/AS02A	WRAIR	1b (adults)	Kenya	32
MSP1 ₄₂	FMP1/AS02	WRAIR	2b (children)	Kenya	33
MSP1 ₄₂	FMP1/AS02A	WRAIR	1b (children)	Kenya	34
MSP1	AdCh63-MSP1/MVA-MSP1	University of Oxford	1, 2a	UK	ClinicalTrials.gov Identifier: NCT01003314
MSP2	MSP2/ISA 720	QIMR	1a (adults)	Australia	ACTR Number: ACTRN12607000552482
MSP1, MSP2 and RESA	Combination B (MSP1, MSP2, RESA/ISA 720)	WEHI, QIMR, Biotech Australia, CSL, F. Hoffmann—La Roche and Roche Products Australia	1a (adults)	Australia	35

Table 1. Vaccines targeting blood stages of *P. falciparum*

MSP1, MSP2 and RESA	Combination B (MSP1, MSP2, RESA/ISA 720)	WEHI, QIMR, Biotech Australia, CSL, F. Hoffmann—La Roche and Roche Products Australia	2a (adults)	Australia	36
MSP1, MSP2 and RESA	Combination B (MSP1, MSP2, RESA/ISA 720)	WEHI, QIMR, Biotech Australia, CSL, F. Hoffmann—La Roche and Roche Products Australia	1b (adults)	Papua New Guinea	37
MSP1, MSP2 and RESA	Combination B (MSP1, MSP2, RESA/ISA 720)	WEHI, QIMR, Biotech Australia, CSL, F. Hoffmann—La Roche and Roche Products Australia	1b (children)	Papua New Guinea	38
MSP1, MSP2 and RESA	Combination B (MSP1, MSP2, RESA/ISA 720)	WEHI, QIMR, Biotech Australia, CSL, F. Hoffmann—La Roche and Roche Products Australia	1/2b (children)	Papua New Guinea	39
CSP, PfSSP2, LSA1, MSP1, SERA, AMA1, Pfs25	NYVAC-Pf7	WRAIR	1, 2a	US	40
MSP3	MSP3 with Montanide ISA 720 or aluminum hydroxide	EMVI	1a (adults)	Switzerland	41
MSP3	MSP3-LSP/aluminum hydroxide	AMANET	1b (adults)	Burkina Faso	42
MSP3	MSP3-LSP/Aluminum hydroxide	AMANET	1b (children)	Tanzania	43
MSP3	MSP3-LSP/Aluminum hydroxide	AMANET	1b (children)	Burkina Faso	44
MSP3	MSP3-LSP/Aluminum hydroxide	AMANET	2b (children)	Mali	ClinicalTrials.gov Identifier: NCT00652275
GLURP	GLURP with Aluminum Hydroxide or ISA 720	EMVI	1a (adults)	Netherlands	45
GLURP + MSP3	GMZ2 (chimeric protein)/aluminum hydroxide	AMANET	1b (adults)	Gabon	ClinicalTrials.gov Identifier: NCT00424944
GLURP + MSP3	GMZ2 (chimeric protein)/aluminum hydroxide	EMVI	1a (adults)	Germany	46
GLURP + MSP3	GMZ2 (chimeric protein)/aluminum hydroxide	AMANET	1b (children)	Gabon	ClinicalTrials.gov Identifier: NCT00703066
EBA175	EBA175/aluminum phosphate	NIAID/DMID	1a (adults)	US	ClinicalTrials.gov Identifier: NCT00347555
SERA	SERA5/aluminum hydroxide	Osaka University	1a (adults)	Japan	47

vary: Combination B (adjuvanted with ISA 720) was highly reactogenic in Australian adults but was very well tolerated in adults and children in Papua New Guinea.³⁵⁻³⁹ AMA1/Alhydrogel + CPG 7909 also demonstrated more reactogenicity in US adults than in Malian adults.^{16,18} Formulations such as virus like particles or conjugates may offer higher immunogenicity with less need for strong adjuvants, although this remains to be demonstrated in the clinic. Prime boost strategies, such as a DNA vaccine followed by a vectored vaccine, may enhance antibody and cellular responses, and are also in the clinic (ClinicalTrials.gov Identifier: NCT00870987).

Since protection against blood stage malaria is thought to be antibody mediated the primary immunologic endpoint for most studies has been the number of seroconverters or the level of induced antibody, with biologic function demonstrated by various methods such as in vitro growth inhibition, immunofluorescent

antibody, or antibody-dependent cellular inhibition of parasite growth. In vitro growth inhibition is the most widely used and best standardized of these functional assays.^{12,16,50} However, none of these assays have been validated as a surrogate of protection, and the level or type of protective antibody needed is unknown. Cellular responses may also be required to induce high affinity antibody of the correct subtypes, and to maintain antibody levels over time, and are being increasingly examined as endpoints in clinical trials and as correlates of protection in longitudinal cohort studies. The lack of validated correlates of protection remains a critical issue in the development of blood stage vaccines, with criteria for down selection unclear. To date vaccines found to be safe and sufficiently immunogenic (as judged by sponsors) have been taken forward.

Antigenic variation is a major concern with blood stage vaccines, as this is thought to be one of the principal mechanisms

by which *Plasmodium falciparum* evades the immune response. AMA1 in particular is extremely diverse, with hundreds of haplotypes identified.⁵¹ In vitro growth inhibition results suggest that antibody responses to AMA1 are strain specific,⁵² a finding which is supported by recent clinical results as discussed above. Approaches to broadening responses include the addition of diverse allelic proteins,^{52,53} and attempting to dampen responses to diverse epitopes while heightening responses to conserved regions.⁵⁴ The other lead blood stage antigen, MSP1₄₂, is dimorphic in the MSP1₃₃ region and largely conserved in the MSP1₁₉ domain, and a bi-allelic vaccine should cover most strain diversity for this protein.²⁹ MSP2 is similarly dimorphic, with evidence of allele specific protection demonstrated in a field trial.³⁹ MSP3 and GLURP, the other antigens furthest along in clinical development, are largely conserved.

Multi-antigen vaccines currently in the clinic include combinations of AMA1 and MSP1, AMA1 with pre-erythrocytic antigens, and MSP3 and GLURP, as discussed. The rationale for which combination of blood stage antigens is likely to be protective is not clear, with conflicting epidemiologic evidence.⁵⁵⁻⁵⁸ The choice of what antigens to combine has to some extent been driven by the availability of clinical grade materials. It seems likely that for an organism as complex as *Plasmodium* more than one antigen is likely to be needed, both to cover antigenic diversity and diversity in individual responses to vaccination. A vaccine which combined multiple targets from the parasite life cycle, such as a pre-erythrocytic antigen like CSP combined with one or more blood stage antigens and one or more transmission blocking antigens, would be ideal. One such vaccine has been evaluated in the clinic, although immunogenicity to the various components was variable and no protection was seen.⁴⁰ Such an ideal vaccine would provide protection from infection, protection from disease in case of breakthrough, and also reduce transmission.

Preclinical Development

Besides the 8 blood stage malaria antigens that have been tested in human trials, a handful of additional antigens have been produced by scalable processes and are being tested in preclinical phases. Among these are MSP4, a merozoite surface protein that induces naturally acquired antibodies,⁵⁹ and VAR2CSA, a variant of PfEMP1 (a highly variable virulence factor expressed on the surface of red blood cells) that mediates parasite-infected erythrocytes binding to the placenta during pregnancy.^{60,61} To assess the feasibility of developing a blood stage vaccine combining available antigens and to understand correlates of protection, if any, in a non-human primate model, a vaccine comprised of MSP1, MSP2, MSP3, MSP4 and AMA1 is being tested in an Aotus challenge model (Wu Y et al., unpublished). One caveat to this combination approach is potential immune interference among antigens, as demonstrated by Pichyangkul S et al.⁶² In this study, immunogenicity of a combination of AMA1, MSP1 and RTS,S was evaluated in rhesus monkeys. MSP1 was found to suppress antibody and T-cell responses to AMA1, whereas RTS,S, responses seemed to not be affected by being combined with AMA1 and MSP1. Thus the design of a

multi-component vaccine requires careful assessment to avoid immune interference.

A vaccine based on killed whole *P. falciparum* blood stage parasites is also in preclinical evaluation.^{9,63} Significant challenges exist in the production, safety and delivery of a killed whole parasite vaccine. In addition to the potential use of killed whole parasites as a vaccine, a live parasite inoculum produced under cGMP conditions would be a useful tool to study correlates of immune protection and would facilitate discovery of new vaccine targets in experimental infections.⁶⁴

The RTS,S development pathway demonstrates the critical role of vaccine formulation and adjuvants in enhancing immune responses against a subunit vaccine. Three adjuvant systems were evaluated for their potential use with blood stage vaccine candidates in an Aotus challenge model.⁶⁵ In this study, monkeys were immunized with the 3D7 allele of AMA1 formulated with AS02A or Montanide ISA720 and were challenged with FCH/4, a *P. falciparum* strain with an AMA1 sequence close to the 3D7 strain. Limited protection, shown as the ability to control or self-limit parasitemia without drug treatment, was observed in the AS02A formulation group, but the ISA720 formulation induced higher antibody titers and better strain-specific protection. No statistical significance was reported. A similar Aotus challenge study evaluated AMA1 formulated with a synthetic TLR-4 agonist. The monkeys receiving AMA1 formulated with this novel adjuvant were able to control or self-limit parasitemia after challenge with a homologous parasite strain, though the protection was not statistically significant due to the small number of animals per group (Wu Y, Rausch K, Lambert L, Miura K, Vedvick T, Howard R, et al. Protection Against Malaria Challenge by Vaccination of Aotus Monkeys with Adjuvanted Recombinant AMA1. Abstract Presented at the Modern Vaccine Adjuvant and Delivery Systems 2009). Combining subunit vaccines with viral vaccines in a prime-boost delivery strategy is also being tested for optimal immune responses against the target antigen(s).

Discussion

The RTS,S vaccine, which contains the pre-erythrocytic antigen CSP and targets the liver stages of malaria, has begun Phase 3 clinical trials, and is likely to be licensed if results in the Phase 3 trials are consistent with earlier studies.⁶⁶ However, this vaccine has only shown 30–65% efficacy.⁶⁶ A vaccine based on attenuated sporozoites is entering clinical development and has the potential to be highly effective both in preventing infection and thereby also preventing transmission.⁶⁷ However, immunogenicity of a highly purified preparation of attenuated sporozoites has yet to be demonstrated, and even if long lasting high level protection is achieved with whole parasite vaccines, significant issues with production, purification and distribution may limit actual usefulness in the field. Transmission blocking vaccines also may be useful tools in elimination or eradication, although clinical development of the only formulation shown to induce high transmission blocking activity was halted due to safety concerns.⁶⁸ Thus while the current status of malaria vaccine development is

encouraging, much remains to be done, and blood stage vaccines remain a critical element of this effort.

The overarching lesson of the experience with CSP and RTS,S is that development is both lengthy and iterative. For pre-erythrocytic vaccines this iterative process was greatly facilitated by the existence of a human sporozoite challenge model which was felt likely to predict protection in the field, and which has subsequently been shown to do so. Results from challenge studies were also used to provide down selection criteria for immunogenicity in non-human primates. Blood stage vaccine development has been significantly hampered by the lack of such a model and the resulting lack of rational criteria for advancement, or perhaps more importantly, for elimination of antigens and formulations. For example, AMA1-C1/Alhydrogel entered clinical development in 2003 with a Phase 1 study in US adults, and was not definitively shown to be non-protective in a Phase 2b study until 2007, after going through Phase 1b in Malian adults and children. This was relatively fast, with a combined Phase 1, 2b study conducted in 336 Malian children in 2006; AMA1/AS02A entered clinical trials in 2003 and results from the Phase 2b study in Malian children were not known until late 2009. In the meantime other groups also devoted resources to developing AMA1 vaccines which, given the results of these two field trials, are not likely to go forward as formulated, either due to insufficient immunogenicity or likely lack of breadth of protection. Reduced transmission intensity in many settings, while a very welcome development, also means that small field trials may no longer be sufficient to demonstrate protection or lack thereof. Meanwhile the regulatory burden continues to increase, with lengthy delays in trial initiation and unblinding not uncommon, and costs continually escalating.

But what are rational criteria for down selection for clinical development, and for further advancement to field studies? Small animal models are useful for early demonstration of immunogenicity and biologic function of induced antibody, but immunogenicity in non-human primates is much more likely to be predictive of responses in humans. Such studies should be conducted with formulations and adjuvants which are suitable for use in humans. While clear cutoffs are still not known, comparative studies can be conducted and the candidates with higher antibody and cellular responses in primates can be chosen for clinical development. Aotus challenge studies using clinical formulations may also be useful in down selection, although this model has not yet been validated. For vaccine candidates already in clinical

development, in addition to safety, induction of antibody levels in malaria naïve adults similar to those seen in malaria exposed adults (where antibody is caused by exposure) may be reasonable criteria for advancement. Experimental challenge studies in humans may also allow for down selection prior to large field trials. Sporozoite challenge studies have been conducted with blood stage vaccines, but may be too stringent a model to demonstrate partial protection, given the relatively short time during which protective antibody (or other mechanisms of protection) would be interacting with the parasites before treatment is required (a median of 4 days from when merozoites emerge from the liver until symptomatic or smear positive).⁶⁹ Detection of partial protection would allow for iterative improvement, as occurred with CSP and subsequent formulations of RTS,S. A human blood stage challenge using parasite-infected erythrocytes is under development and may prove more useful in detection of partial efficacy.^{69,70} Challenge studies suffer from the same potential limitation as Aotus studies, in that treatment may be required sooner than protective mechanisms have time to act. However, in the absence of better models these may be the best options for screening vaccine candidates.

Previously, vaccines which were judged to be safe and adequately immunogenic have gone forward to Phase 2b trials in African children, but given the lack of clinical protection demonstrated to date this approach no longer appears to be feasible, particularly for formulations with a limited safety profile or perceived safety risks. The current malaria paradigm, in which disease modifying vaccines are no longer considered to be adequate (even though RTS,S is a disease modifying vaccine), also requires rethinking preclinical and clinical development strategies. Many new antigens are likely to be identified through the recent publication of the genome for *Plasmodium falciparum*,⁷¹ and the range of formulations and adjuvants available to vaccine developers is broader than ever before. Thus the burden is on malaria vaccinologists to focus their efforts, with elimination of vaccines not likely to be protective from the pipeline, and the speedy advancement of those which show promise in preclinical and early clinical development.

Acknowledgements

This work was supported by the Intramural Program of NIAID/NIH. Thanks to Louis Miller for his thoughtful review and comments on the manuscript and to Alemush Imeru for her help with manuscript preparation.

References

1. Roberts L, Enserink M. Did they really say... eradication? Science 2007; 318:1544-5.
2. WHO publication. World Malaria report 2009; http://whqlibdoc.who.int/publications/2009/9789241563901_eng.pdf.
3. Kleinschmidt I, Schwabe C, Benavente L, Torrez M, Ridl FC, Segura JL, et al. Marked increase in child survival after four years of intensive malaria control. AJTMH 2009; 80:882-8.
4. Bhattarai A, Ali AS, Kachur SP, Mårtensson A, Abbas AK, Khatib R, et al. Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. PLoS Med 2007; 4:309.
5. Barnes KI, Chanda P, Ab Barnabas G. Impact of the large-scale deployment of artemether/lumefantrine on the malaria disease burden in Africa: case studies of South Africa, Zambia and Ethiopia. Malar J 2009; 8:8.
6. Cohen S, McGregor IA, Carrington (1961) Gamma-globulin and acquired immunity to human malaria. Nature 192:733-7.
7. Sabchareon A, Burnouf T, Ouattara D, Attanath P, Bouharoun-Tayoun H, et al. Parasitologic and clinical human response to immunoglobulin administration in falciparum malaria. Am J Trop Med Hyg 1991; 45:297-308.
8. Doolan DS, Cobano C, Baird JK. Acquired Immunity to Malaria. Clin Microbiol Rev 2009; 22:13-36.
9. Pombo DJ, Lawrence G, Hirunpetcharat C, Rzepczyk C, Bryden M, Cloonan N, Anderson K, et al. Immunity to malaria after administration of ultra-low doses of red cells infected with *Plasmodium falciparum*. Lancet 2002; 360:610-7.
10. Roestenberg M, McCall M, Hopman J, Wiersma J, Luty AJ, van Gemert GJ, et al. Protection against a malaria challenge by sporozoite inoculation. N Engl J Med 2009; 361:468-77.
11. Guinovart C, Aponte JJ, Sacarlal J, Aide P, Leach A, Bassat Q, et al. Insights into long-lasting protection induced by RTS,S/AS02A malaria vaccine: further results from a Phase IIb trial in Mozambican children. PLoS One 2009; 4:5165. doi:10.1371/journal.pone.0005165.

12. Malkin EM, Diemert DJ, McArthur JH, Perreault JR, Miles AP, Giersing BK, et al. Phase 1 clinical trial of apical membrane antigen 1: an asexual blood-stage vaccine for *Plasmodium falciparum* malaria. *Infect Immun* 2005; 73:3677-85.
13. Dicko A, Sagara I, Ellis RD, Miura K, Guindo O, Kamate B, et al. Phase 1 study of a combination AMA1 blood stage malaria vaccine in Malian children. *PLoS One* 2008; 3:1563.
14. Dicko A, Diemert DJ, Sagara I, Sogoba M, Niambele MB, Assadou MH, et al. Impact of a *Plasmodium falciparum* AMA1 vaccine on antibody responses in adult Malians. *PLoS ONE* 2007; 2:1045.
15. Sagara I, Dicko A, Ellis RD, Fay MP, Diawara Sory, Assadou MH, et al. A randomized controlled phase 2 trial of the blood stage AMA1-C1/Alhydrogel malaria vaccine in children in Mali. *Vaccine* 2009; 27:3090-8.
16. Mullen GED, Ellis RD, Miura K, Malkin E, Nolan C, Hay M, et al. Phase 1 trial of AMA1-C1/alhydrogel plus CPG 7909: An asexual blood-stage vaccine for *Plasmodium falciparum* malaria. *PLoS ONE* 2008; 3:2940.
17. Ellis RD, Mullen GED, Pierce M, Martin LB, Miura K, Fay MP, et al. A Phase 1 study of the blood-stage malaria vaccine candidate AMA1-C1/Alhydrogel® with CPG 7909, using two different formulations and dosing intervals. *Vaccine* 2009; 27:4104-9.
18. Sagara I, Ellis RD, Dicko A, Niambele MB, Kamate B, Guindo O, et al. A randomized and controlled Phase 1 study of the safety and immunogenicity of the AMA1-C1/Alhydrogel + CPG 7909 vaccine for *Plasmodium falciparum* malaria in semi-immune Malian adults. *Vaccine* 2009; 27:7292-8.
19. Polhemus ME, Magill AJ, Cummings JF, Kester KE, Ockenhouse CF, Lanar DE, et al. Phase I dose escalation safety and immunogenicity trial of *Plasmodium falciparum* apical membrane protein (AMA-1) FMP2.1, adjuvanted with AS02A, in malaria-naïve adults at the Walter Reed Army Institute of Research. *Vaccine* 2007; 25:4203-12.
20. Thera MA, Doumbo OK, Coulibaly D, Diallo DA, Kone AK, Guindo AB, et al. Safety and immunogenicity of an AMA-1 malaria vaccine in Malian adults: Results of a phase 1 randomized controlled trial. *PLoS ONE* 2008; 3:1465.
21. Thera M, Doumbo OK, Coulibaly D, Laurens MB, Kone AK, Guindo AB, et al. Safety and immunogenicity of an AMA-1 malaria vaccine in Malian children: Results of a phase 1 randomized controlled trial. *PLoS One* 2010; 5:9041.
22. Pierce MP, Ellis RD, Martin LB, Malkin E, Tierney E, Miura K, et al. Phase 1 safety and immunogenicity trial of the *Plasmodium falciparum* blood-stage malaria vaccine AMA1-C1/ISA 720 in Australian adults. *Vaccine* 2010; 28:2236-42.
23. Saul A, Lawrence G, Allworth A, Elliott S, Anderson K, Rzepczyk C, et al. A human phase 1 vaccine clinical trial of the *Plasmodium falciparum* malaria vaccine candidate apical membrane antigen 1 in Montanide ISA720 adjuvant. *Vaccine* 2005; 23:3076-83.
24. Roestenberg M, Remarque E, de Jonge E, Hermesen R, Blythman H, Leroy O, et al. Safety and immunogenicity of a recombinant *Plasmodium falciparum* AMA1 malaria vaccine adjuvanted with Alhydrogel, Montanide ISA 720 or AS02. *PLoS ONE* 2008; 3:3960.
25. Spring MD, Cummings JF, Ockenhouse CF, Dutta S, Reidler R, Angov E, et al. Phase 1/2a study of the malaria vaccine candidate apical membrane antigen-1 (AMA-1) administered in adjuvant system AS01B or AS02A. *PLoS ONE* 2009; 4:5254.
26. Hu J, Chen Z, Gu J, Wan M, Shen Q, Kieny MP, et al. Safety and immunogenicity of a malaria vaccine, *Plasmodium falciparum* AMA-1/MSP-1 chimeric protein formulated in montanide ISA 720 in healthy adults. *PLoS One* 2008; 3:1952.
27. Genton B, Pluschke G, Degen L, Kammer AR, Westerfeld N, Okitsu SL, et al. A randomized placebo-controlled phase Ia malaria vaccine trial of two viro-some-formulated synthetic peptides in healthy adult volunteers. *PLoS ONE* 2007; 2:1018.
28. Thompson FM, Porter DW, Okitsu SL, Westerfeld N, Vogel D, Todryk S, et al. Evidence of blood stage efficacy with a viro-some malaria vaccine in a phase IIa clinical trial. *PLoS ONE* 2008; 3:1493.
29. Malkin E, Long CA, Stowers AW, Zou L, Singh S, MacDonald NJ, et al. Phase 1 study of two merozoite surface protein 1 (MSP1₁₂) vaccines for *Plasmodium falciparum* malaria. *PLOS Clin Trial* 2007; 2:12.
30. Ellis RD, Martin LB, Shaffer D, Long CA, Miura K, Fay MP, et al. Phase 1 trial of the *Plasmodium falciparum* blood stage vaccine MSP1₁₂-C1/Alhydrogel with and without CPG 7909 in malaria naïve adults. *PLoS One* 2010; 5:8787.
31. Ockenhouse CF, Angov E, Kester KE, Diggs C, Soisson L, Cummings JF, et al. Phase I safety and immunogenicity trial of FMP1/AS02A, a *Plasmodium falciparum* MSP-1 asexual blood stage vaccine. *Vaccine* 2006; 24:3009-17.
32. Stoute JA, Gombé J, Withers MR, Siangla J, McKinney D, Onyango M, et al. Phase 1 randomized double-blind safety and immunogenicity trial of *Plasmodium falciparum* malaria merozoite surface protein FMP1 vaccine, adjuvanted with AS02A, in adults in western Kenya. *Vaccine* 2007; 25:176-84.
33. Ogutu BR, Apollo OJ, McKinney D, Okoth W, Siangla J, Dubovsky F, et al. Blood stage malaria vaccine eliciting high antigen-specific antibody concentrations confers no protection to young children in western Kenya. *PLoS ONE* 2009; 4:4708.
34. Withers MR, McKinney D, Ogutu BR, Waitumbi JN, Milman JB, Apollo OJ, et al. Safety and reactivity of an MSP-1 malaria vaccine candidate: A randomized phase Ib dose-escalation trial in Kenyan. *PLOS Clin Trial* 2006; 1:32.
35. Saul A, Lawrence G, Smillie A, et al. Human phase I vaccine trials of 3 recombinant asexual stage malaria antigens with Montanide ISA720 adjuvant. *Vaccine* 1999; 17:3145-59.
36. Lawrence G, Cheng QQ, Reed C, Taylor D, Stowers A, Cloonan N, et al. Effect of vaccination with 3 recombinant asexual-stage malaria antigens on initial growth rates of *Plasmodium falciparum* in non-immune volunteers. *Vaccine* 2000; 18:1925-31.
37. Genton B, Al-Yaman F, Anders R, Saul A, Brown G, Pye D, et al. Safety and immunogenicity of a three-component blood-stage malaria vaccine in adults living in an endemic area of Papua New Guinea. *Vaccine* 2000; 18:2504-11.
38. Genton B, Al-Yaman F, Betuela I, Anders RF, Saul A, Baea K, et al. Safety and immunogenicity of a three-component blood-stage malaria vaccine (MSP1, MSP2, RESA) against *Plasmodium falciparum* in Papua New Guinean children. *Vaccine* 2003; 22:30-41.
39. Genton B, Betuela I, Felger I, Al-Yaman F, Anders RF, Saul A, et al. A recombinant blood-stage malaria vaccine reduces *Plasmodium falciparum* density and exerts selective pressure on parasite populations in a phase 1-2b trial in Papua New Guinea. *Am J Infect Dis* 2002; 185:820-7.
40. Ockenhouse CF, Sun PF, Lanar DE, Welde BT, Hall BT, Kester K, et al. Phase I/IIa safety, immunogenicity, and efficacy trial of NYVAC-Pf7, a pox-vectored, multiantigen, multistage vaccine candidate for *Plasmodium falciparum* malaria. *J Infect Dis* 1998; 177:1664-73.
41. Audran R, Cachat M, Lurati F, Soe S, Leroy O, Corradin G, et al. Phase I malaria vaccine trial with a long synthetic peptide derived from the merozoite surface protein 3 antigen. *Infect Immun* 2005; 73:8017-26.
42. Sirima SB, Nébédé I, Ouédraogo A, Tiono AB, Konaté AT, Gansané A, et al. Safety and immunogenicity of the *Plasmodium falciparum* merozoite surface protein-3 long synthetic peptide (MSP3-LSP) malaria vaccine in healthy, semi-immune adult males in Burkina Faso, West Africa. *Vaccine* 2007; 25:2723-32.
43. Lusingu JP, Gesase S, Msham S, Francis F, Lemnge M, Seth M, et al. Satisfactory safety and immunogenicity of MSP3 malaria vaccine candidate in Tanzanian children aged 12–24 months. *Malar J* 2009; 8:163.
44. Sirima SB, Tiono AB, Ouédraogo A, Diarra A, Ouédraogo AL, Yaro JB, et al. Safety and immunogenicity of the malaria vaccine candidate MSP3 long synthetic peptide in 12–24 months-old Burkinabe children. *PLoS One* 2009; 4:7549.
45. Hermesen CC, Verhage DF, Telgt DS, Teelen K, Bousema JT, Roestenberg M, et al. Glutamate-rich protein (GLURP) induces antibodies that inhibit in vitro growth of *Plasmodium falciparum* in a phase 1 malaria vaccine trial. *Vaccine* 2007; 25:2930-40.
46. Esen M, Kremsner PG, Schleucher R, Gessler M, Imoukhuede EB, Imbault N, et al. Safety and immunogenicity of GMZ2—a MSP3-GLURP fusion protein malaria vaccine candidate. *Vaccine* 2009; 27:6862-8.
47. Horii T. Malaria Vaccine. *Nippon Rinsho* 2008; 66:1990-8.
48. Richards JS, Beeson JG. The future for blood-stage vaccines against malaria. *Immunol Cell Biol* 2009; 87:377-90.
49. Silvie O, Franetich JF, Charrin S, et al. A role for apical membrane antigen 1 during invasion of hepatocytes by *Plasmodium falciparum* sporozoites. *J Biol Chem* 2004; 279:9490-6.
50. Bergmann-Leitner ES, Duncan EH, Mullen GE, Burge JR, Khan F, Long CA, et al. Critical evaluation of different methods for measuring the functional activity of antibodies against malaria blood stage antigens. *Am J Trop Med Hyg* 2006; 75:437-42.
51. Takala SL, Coulibaly D, Thera MA, Batchelor AH, Cummings MP, Escalante AA, et al. Extreme polymorphism in a vaccine antigen and risk of clinical malaria: implications for vaccine development. *Sci Transl Med* 2009; 1:2-5. DOI:10.1126/scitranslmed.3000257.
52. Duan J, Mu J, Thera MA, Joy D, Kosakovsky Pond SL, Diemert D, et al. Population structure of the genes encoding the polymorphic *Plasmodium falciparum* apical membrane antigen 1: implications for vaccine design. *Proc Natl Acad Sci USA* 2008; 105:7857-62.
53. Remarque EJ, Faber BW, Kocken CH, Thomas AW. A diversity-covering approach to immunization with *Plasmodium falciparum* apical membrane antigen 1 induces broader allelic recognition and growth inhibition responses in rabbits. *Infect Immun* 2008; 76:2660-70.
54. Dutta S, Dlugosz LS, Clayton JW, Pool CD, Haynes JD, Gasser RA, 3rd, et al. Alanine mutagenesis of the primary antigenic escape residue cluster (C1) of Apical Membrane Antigen-1. *Infect Immun* 2009; 78:661-71.
55. Dodoo D, Aikins A, Kwadmo AK, Lamptey H, Remarque E, et al. Cohort study of the association of antibody levels to AMA1, MSP1₁₉, MSP3 and GLURP with protection from clinical malaria in Ghanaian children. *Malaria J* 2008; 7:142; DOI:10.1186/1475-2875-7-142.
56. Roussillon C, Ouevray C, Muller-Graf C, Tall A, Rogier C, et al. Long-term clinical protection from falciparum malaria is strongly associated with IgG3 antibodies to Merozoite Surface Protein 3. *PLoS Med* 2007; 4:320.
57. Osier FA, Fegan G, Polley SD, Murungi L, Verra F, et al. Breadth and magnitude of antibody responses to multiple plasmodium falciparum merozoite antigens are associated with protection from clinical malaria. *Infect Immun* 2008; 76:2240-8.

58. Iriemenam NC, Khirelsied AH, Nasr A, ElGhazali G, Giha HA, et al. Antibody responses to a panel of *Plasmodium falciparum* malaria blood-stage antigens in relation to clinical disease outcome in Sudan. *Vaccine* 2009; 27:62-71.
59. Wang L, Richie TL, Stowers A, Nhan DH, Coppel RL. Naturally acquired antibody responses to *Plasmodium falciparum* merozoite surface protein 4 in a population living in an area of endemicity in Vietnam. *Infect Immun* 2001; 69:4390-7.
60. Oleinikov AV, Francis SE, Dorfman JR, Rossnagle E, Balcaitis S, Getz T, et al. VAR2CSA domains expressed in *Escherichia coli* induce cross-reactive antibodies to native protein. *J Infect Dis* 2008; 197:1119-23.
61. Avril M, Hathaway MJ, Cartwright MM, Gose SO, Narum DL, et al. Optimizing expression of the pregnancy malaria vaccine candidate, VAR2CSA in *Pichia pastoris*. *Malar J* 2009; 8:143.
62. Pichyangkul S, Tongtawe P, Kum-Arb U, Yongvanitchit K, Gettayacamin M, Hollingdale MR, et al. Evaluation of the safety and immunogenicity of *Plasmodium falciparum* apical membrane antigen 1, merozoite surface protein 1 or RTS,S vaccines with adjuvant system AS02A administered alone or concurrently in rhesus monkeys. *Vaccine* 2009; 28:452-62.
63. McCarthy JS, Good MF. Whole parasite blood stage malaria vaccines: A convergence of evidence. *Hum Vaccin* 2010; 6:114-23.
64. Doolan DL, Mu Y, Unal B, Sundaresh S, Hirst S, Valdez C, et al. Profiling humoral immune responses to *P. falciparum* infection with protein microarrays. *Proteomics* 2008; 8:4680-94.
65. Dutta S, Sullivan JS, Grady KK, Haynes JD, Komisar J, Batchelor AH, et al. High antibody titer against apical membrane antigen-1 is required to protect against malaria in the aotus model. *PLoS One* 2009; 4:8138.
66. Ballou WR. The development of the RTS,S malaria vaccine candidate: challenges and lessons. *Parasite Immunol* 2009; 31:492-500.
67. Luke TC, Hoffman SL. Rationale and plans for developing a non-replicating, metabolically active, radiation-attenuated *Plasmodium falciparum* sporozoite vaccine. *J Exp Biol* 2003; 206:3803-8.
68. Wu Y, Ellis RD, Shaffer D, Fontes E, Malkin EM, Mahanty S, et al. Phase 1 trial of malaria transmission blocking vaccine candidates Pf525 and Pvs25 formulated with montanide ISA 51. *PLoS One* 2008; 3:2636.
69. Sanderson F, Andrews L, Douglas AD, Hunt-Cooke A, Bejon P, et al. Blood-stage challenge for malaria vaccine efficacy trials: a pilot study with discussion of safety and potential value. *Am J Trop Med Hyg* 2008; 78:878-83.
70. Moorthy VS, Diggs C, Ferro S, Good MF, Herrera S, et al. Report of a consultation on the optimization of clinical challenge trials for evaluation of candidate blood stage malaria vaccines, 18–19 March 2009, Bethesda MD, USA. *Vaccine* 2009; 27:5719-25.
71. Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW, et al. Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* 2002; 419:498-511.