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EXPERT REVIEWS

The RTS,S vaccine candidate for malaria

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Malaria continues to be a worldwide leading cause of morbidity and mortality, and the development of an effective malaria vaccine remains a research imperative. Of the multiple approaches that have been pursued, the RTS,S/AS01 vaccine candidate represents the most developed and clinically validated malaria vaccine formulation. Throughout its development, increasingly more effective adjuvants have been key in improving the potency of the vaccine. RTS,S-based vaccine formulations have been demonstrated to be safe, well tolerated, immunogenic, and to confer partial efficacy in both malaria-naive and -experienced adults as well as children. Further research to optimize and improve vaccine efficacy is ongoing.

Keywords: adjuvant • falciparum • malaria • malaria vaccine • prime–boost • RTS,S

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The development of a highly protective malaria vaccine is an imperative that has eluded researchers for over 40 years. Despite the implementation of effective antimalarial drugs, personal protective measures and vector control efforts, it is estimated that over 3 billion people are at risk of infection, with as many as 300 million cases of malaria occurring annually, and nearly a million attributable deaths worldwide [1,101]. *Plasmodium falciparum* is the most deadly of the malarial species, ranking as one of the most common causes of morbidity and mortality within endemic regions, accounting for the vast majority of malaria-associated deaths [2]. A disproportionate number of these deaths occur in young children and pregnant women in developing countries. Worldwide, malaria exacts an extraordinary cost in terms of human morbidity, mortality and economic burden [3].

The malaria parasite is a complex organism with a complex lifecycle spanning both humans and *Anopheles* spp. mosquitoes (Figure 1). This complexity contributes to the difficulty in engineering an effective vaccine. Three major stages of its lifecycle have been targeted for vaccine development. The first is the pre-erythrocytic stage, from sporozoite inoculation, to hepatocyte infection and then hepatic merozoite release. The second is the asexual erythrocytic stage (blood stage), which propagates itself through the continuous infection of red blood cells. Finally, there is the sporogonic cycle (sexual stage), beginning with the development of gametocytes in the human host through to the development of sporozoites in the mosquito. Blood-stage vaccines are typically envisioned as decreasing the severity of illness and preventing death via controlling, but not preventing, parasitemia. A sexual-stage vaccine would impact the pathogen on a population or altruistic level, not preventing disease in the recipient, but preventing malaria development in mosquitoes, thus ending the propagation of parasites from person to mosquito to person. A pre-erythrocytic vaccine has the most appeal because it has potential for complete sterilizing immunity, arresting parasite development early (at the sporozoite or liver stage), preventing both clinical disease in the human host and infection of mosquitoes. It is postulated that even a partially effective pre-erythrocytic vaccine would have some impact in reducing the severity of clinical disease, perhaps via a reduction in early blood-stage parasite production. Furthermore, the pre-erythrocytic stage is the only stage for which high-level, sterilizing immunity against heterologous strains of *P. falciparum* has been demonstrated. Building upon successful preclinical animal work, Clyde *et al.* demonstrated during the early 1970s that human volunteers could be protected from experimental sporozoite challenge via the administration of a large number of radiation-attenuated sporozoites [4–6]. Although the large-scale production and delivery of radiation-attenuated sporozoites, at the present time, remains infeasible for implementation as a malaria vaccine, this pivotal demonstration has fueled over three decades of investment into

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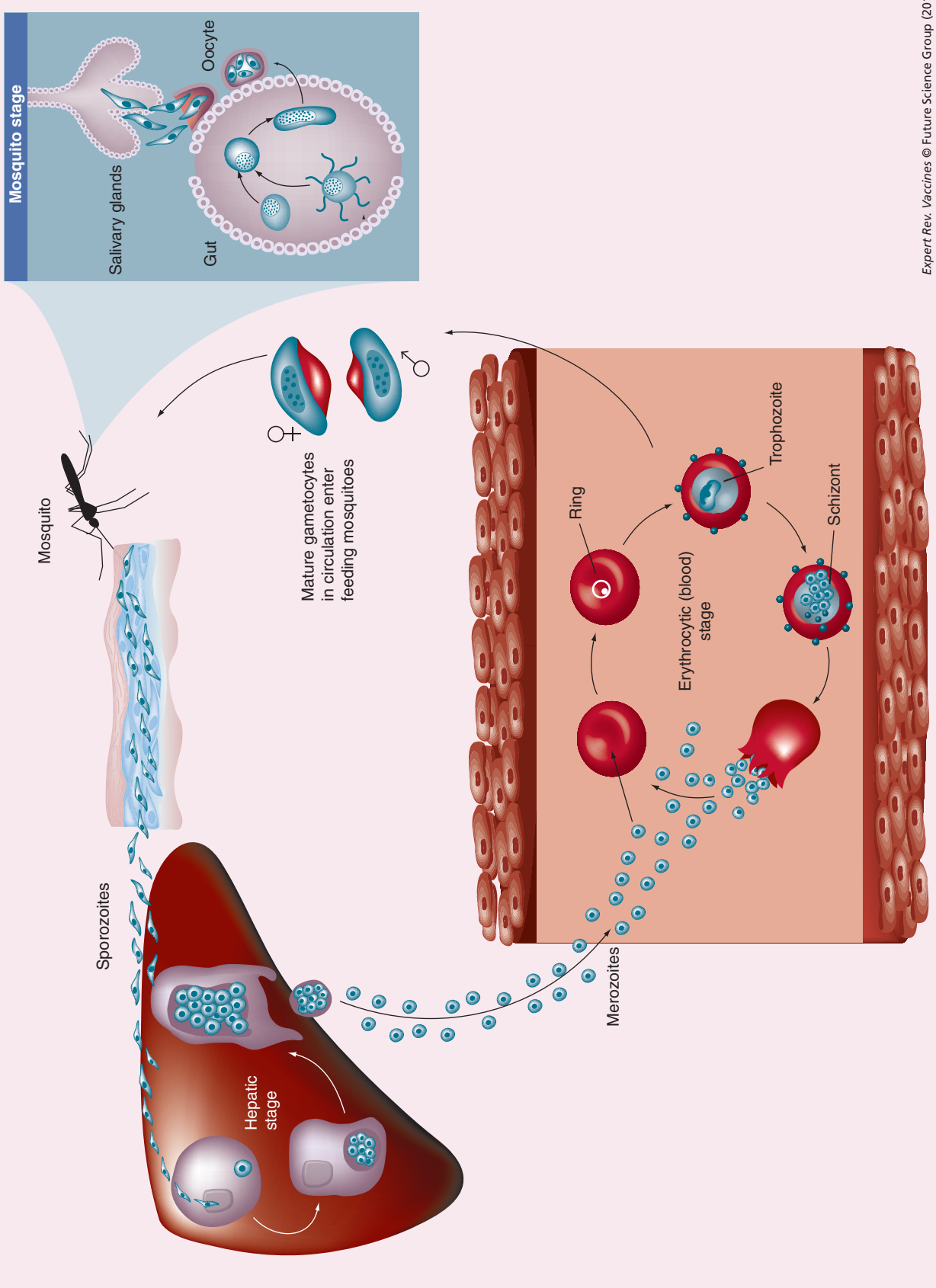

Figure 1. *Plasmodium falciparum* lifecycle. Parasite lifecycle, spanning both humans and mosquitoes.

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a viable pre-erythrocytic protein-based vaccine. Among the many candidates that have been explored, the hybrid RTS,S particle vaccine, in its various adjuvant formulations, has been the most developed and most promising.

Development of the RTS,S antigen

The immunodominant surface antigen that covers the surface of the sporozoite, the circumsporozoite (CS) protein, was first described in the rodent malarial parasite, *Plasmodium berghei* [7]. It was demonstrated that antibodies to this protein completely protected mice from malaria challenge [8]. In a relatively short span of time, CS proteins for primate-infecting malarial species were also identified, and the gene structure for the *P. falciparum* parasite CS protein was elucidated [9,10]. Although immunogenic, initial vaccines based upon the genetically conserved, central repeat region of the CS protein were felt to have a poor representation of T-cell epitopes, and it was suggested that perhaps the fusion of an independent T-cell epitope would address this shortfall. The solution, a hybrid vaccine particle, consisting of the *P. falciparum* CS protein and the hepatitis B surface antigen (HBsAg), was the product of a collaboration between GlaxoSmithKline (GSK) and the Walter Reed Army Institute of Research (WRAIR) during the mid-1980s [10]. The initial product, designated R16HBsAg, consisted of 16 tandem repeats of the immune-dominant epitope of the *P. falciparum* CS protein fused to the pre-S2 region of the hepatitis B virus (HBV) surface antigen (Asn–Ala–Asn–Pro, or NANP). The hybrid protein, expressed in transformed *Saccharomyces cerevisiae* yeast cells, self-assembled into virus-like particles similar to native HBsAg, with exposure of the CS epitope upon their exteriors. It was demonstrated that, adjuvanted with alum, R16HBsAg induced a high-titer antibody response towards the CS epitope in both mice and rabbits and could also prevent parasite invasion of hepatoma cells [10].

In 1988, a Phase I trial of R16HBsAg was able to demonstrate both immunogenicity and safety. A total of 20 adult males, with antibody-negative serologies for hepatitis B and CS protein, were administered three 20-g intramuscular doses of vaccine at monthly intervals. All 20 volunteers demonstrated an anti-CS antibody response, with 17 developing an antibody titer (measured with ELISA) ≥1:200 after the first vaccination, and 13 subjects with anti-CS antibody detection up to 10 months after the final immunization. The vaccine demonstrated a threefold or greater boosting effect of antibody titers in six volunteers, however, this was not observed in the remaining 14 subjects [11].

Given a desire to include what were felt to be important T- and B-cell epitopes, R16HBsAg was redesigned to include nonrepeat sequences from the C-terminal region of the CS protein (Figure 2). Amino acid sequences were initially derived from the 7G8 parasite strain, but later from the NF54 strain [12–14]. This novel particle was named RTS: 'R' for the CS repeats, 'T' for T-cell epitopes and 'S' for HBsAg. The genetically transformed yeast strain used to produce these antigens already contained multiple integrated copies of an 'S' expression cassette, and thus expressed two polypeptides, RTS and S, with a resulting 1:4 ratio [12].

RTS,S adjuvants

Although the mechanisms of action are not entirely understood, adjuvants (from Latin *adjuvare*, 'to help') have long been utilized to enhance the immune response to antigens [15]. The French veterinarian and biologist, Gaston Ramon, originally described adjuvants as, 'substances used in combination with a specific antigen that produce more immunity than the antigen alone' [16]. While increased immunological potency is an emphasis of adjuvant development, clinically feasible products must achieve this while maintaining tolerable reactogenicity. The development of the RTS,S vaccine has been inextricably linked to the evolution of its various adjuvants. Most of these adjuvants have been proprietary GSK products, consisting of varied component formulations, and utilized in a number of different vaccine candidates. The GSK adjuvant formulations, originally labeled with the prefix 'SBAS', were later labeled 'AS' for 'Adjuvant System', followed by a specific formulation number. A comparison of the different RTS,S adjuvants can be found in Table 1. Although the components are similar, AS02 and AS01 also each have pediatric formulations (AS02D and AS01E, respectively) containing a 25-µg dose of RTS,S rather than the standard 50-µg adult dose.

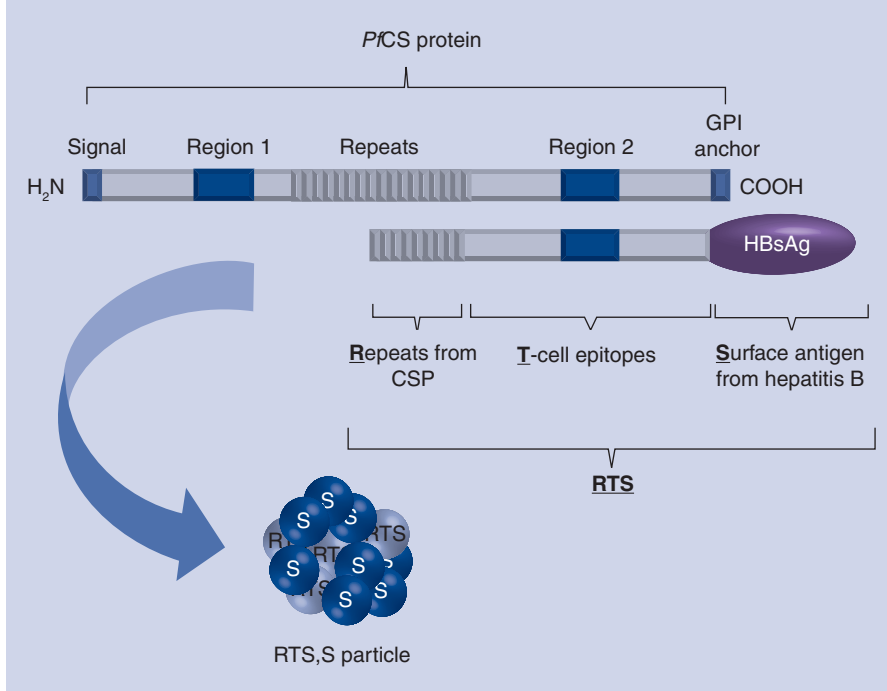

Figure 2. RTS,S particle. RTS derived from *Pf*CS protein and HBsAg.
HBsAg: Hepatitis B surface antigen; *Pf*CS: *Plasmodium falciparum* circumsporozoite.

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Table 1. Comparison of RTS,S vaccine formulations.

Adjuvant	Formulation	Components				
		Alum	*MPL*	*Oil-in-water emulsion*	*QS21*	*Liposome*
Alum	Adult	+	-	-	-	-
AS04	Adult	+	+	-	-	-
AS03	Adult	-	-	+	-	-
AS02A	Adult	-	+	+	+	-
AS02D	Pediatric	-	+	+	+	-
AS01B	Adult	-	+	-	+	+
AS01E	Pediatric	-	+	-	+	+

+: Present; -: Not present; MPL: Monophosphoryl lipid A.

The first two RTS,S vaccines examined in humans contained aluminum salts. Aluminum salt-based adjuvants, referred to as 'alum', have a long history of safe use, and are currently among the most widely used adjuvants in clinical practice. Proposed mechanisms of action include prolonged immune stimulation via antigen deposition, recruitment and activation of antigen-presenting cells (APCs) via production of inflammation and danger signals such as uric acid, and conversion of antigen to a multivalent particulate form that is more efficiently internalized by APCs. Although adept at boosting humoral immunity, alum is a poor stimulator for cell-mediated immune responses [15,17,18].

In addition to other components, AS04, AS02 and AS01 also contain 3-deacylated monophosphoryl lipid A (MPL). First developed in 1979, MPL is a detoxified product of the Re595 strain *Salmonella minnesota* lipopolysaccharide [19,20]. In its natural form, lipopolysaccharide is a highly immunogenic endotoxin found on the membrane of Gram-negative bacteria and, during infection, contributes to the pathophysiology of septic shock. MPL acts upon TLR-4, promoting the maturation of APCs. These APCs migrate to the T-cell areas of draining lymph nodes, where they participate in the priming of naive T cells [18,20]. Ultimately, activation of TLR-4 leads not only to the activation of innate immunity, but also potentiates both humoral and cellular immune responses [21–23].

The AS03 and AS02 adjuvants utilize a proprietary GSK oil (squalene)-in-water-based emulsion. The oil phase contains DL-α-tocopherol and squalene, and the aqueous phase includes the nonionic detergent polysorbate 80. The inclusion of a liposoluble, plant-derived, vitamin E analog, namely, DL-α-tocopherol, makes the GSK oil-in-water formulation unique among the commercially available oil-in-water adjuvants. It has been shown that DL-α-tocopherol can enhance the magnitude of the antigen-specific adaptive response, early eosinophil and neutrophil migration to draining lymph nodes, antigen loading in monocytes, and affect cytokine production [24]. Unlike water-in-oil adjuvants, squalene-in-water adjuvants, in general, do not appear to work via antigen deposition at the site of injection [18]. Rather, these adjuvants work via a methodology not dissimilar to MPL, triggering chemoattraction and maturation of APCs at the site of vaccination, then encouraging migration of APCs to draining lymph nodes for effective presentation of antigen to T cells and priming of an effective adaptive immunity [25].

The AS02 and AS01 vaccine adjuvants contain the saponin QS21, derived from the bark of the South American *Quillaja saponaria* tree. QS21 and its antecedent, Quil A, have been utilized as adjuvants for a broad array of candidate vaccines, including those against HIV, malignancy, viruses and other parasites [16]. This adjuvant has been shown to stimulate antibody, cell-mediated Th1, as well as cytotoxic T lymphocyte (CTL) responses to subunit antigens [26]. QS21 is intrinsically lytic, a property that accounts for some degree of increased reactogenicity. It is postulated that its mechanism of action may be related to its lytic nature, perhaps causing the release of intrinsic 'danger' signals, with subsequent immune activation [15].

The AS01 adjuvant replaces the oil-in-water component with a liposome formulation. Liposomes have been tested for decades as potential adjuvants and are, essentially, artificial vesicles with an aqueous center enclosed by one or more phospholipid layers. Having been demonstrated as safe and efficacious, liposomes have been utilized for commercial vaccines against hepatitis A and influenza [27].

RTS,S clinical trials with experimental challenge

Based upon promising murine and nonhuman primate data, the first RTS,S clinical trial in malaria-naive adults was conducted by WRAIR and SmithKline Beecham Biologicals (later GSK) during the early 1990s. Utilizing RTS,S antigen adsorbed to alum alone, or alum with MPL, vaccine was administered via intramuscular injection at 0, 2 and 6 months with live sporozoite challenge 10–14 days after the final vaccination. Investigators were able to demonstrate that both vaccine formulations were immunogenic and well tolerated [12]. However, upon sporozoite challenge, all six vaccinated volunteers in the RTS,S/alum group, and six out of eight volunteers in the RTS,S/AS04 group developed patent malaria. The two protected volunteers in the RTS,S/AS04 group demonstrated higher anti-CS antibodies than nonprotected volunteers, and one of the protected volunteers demonstrated increased CTL activity against CS, leading the investigators to speculate that CTL activity may also have contributed to protection.

Encouraged by these initial results, and hypothesizing that more potent adjuvants would improve the efficacy of the vaccine, WRAIR and GSK Biologicals went on to compare RTS,S adjuvanted with either AS04, AS03 or AS02 [13]. In this trial, volunteers were randomly assigned to receive three doses of one of these vaccine formulations at 0, 1 and 7 months. A total of 27 subjects completed their full vaccination series and 22 underwent sporozoite challenge approximately 3 weeks after final vaccination. Although

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all three formulations were found to be safe, because of increased constitutional symptoms and pain with the second dose of the AS03 and AS02 formulations, the third doses of these formulations were reduced from 0.5 to 0.1 ml, which was well tolerated. CS-repeat antibody responses to the AS03 and AS02 formulations were much greater than seen with the AS04 formulation. Vaccine efficacy was greatest in the RTS,S/AS02 group, with six out of seven (86%) sporozoite-challenged volunteers being protected against patent malaria infection, as opposed to only one out of eight, and two out of seven for the RTS,S/AS04 and RTS,S/AS03 groups, respectively. Protected subjects tended to have higher antibody titers against CS tandem repeat epitopes as compared with subjects who developed patent malaria. However, after comparing the RTS,S/AS03 and RTS,S/AS02 groups, the investigators concluded that antibody responses to the tandem repeats alone were not sufficient to confer protection, and postulated that the AS02 formulation may also confer significant cellular immunity, as had been demonstrated in preclinical murine and nonhuman primate studies. Of note, seven out of the protected volunteers underwent a 6-month rechallenge, where only two volunteers were protected – one with the RTS,S/AS04 vaccine and one with the RTS,S/AS02 vaccine – punctuating that further enhancement of long-term immunity would be needed to produce a feasible vaccine. The results of this small trial could not demonstrate any statistically significant correlation between humoral immunity, cellular immunity and protection [28]. In subsequent trials utilizing RTS,S/AS02, a protective efficacy of 86% would never be reproduced, and without further testing of the unique dosing used in this trial (namely reduction of the third dose to one-fifth of the components contained in the previous doses), it remains unanswered as to whether or not this dose reduction accounts for the observed increased efficacy.

Pursuing the promising results for RTS,S/AS02, further work was done to optimize vaccine dosing and administration. In an evaluation of the protection offered by varied regimens and doses of RTS,S/AS02, it was demonstrated that the 25-µg dose administered at 0, 1 and 9 months and the 50-µg dose at either 0 and 1 months or 0, 1 and 9 months were both safe and comparably efficacious, providing protection against challenge 1 month after vaccination in the range of 50–57% [29]. Given concern about long-term stability of the liquid formulation of the RTS,S antigen when mixed with adjuvant, a lyophilized formulation in a two-vial presentation (one antigen, one adjuvant) was formulated, and was found to be as well tolerated and immunogenic as the conventional liquid formulation in a Phase I/IIa trial [30]. The lyophilized vaccine, given on a 0, 1 month schedule protected eight out of 19 volunteers (42%) upon sporozoite challenge 2 weeks after last vaccination. A separate study comparing compacted immunization schedules of 0, 1, 3 months and 0, 7, 28 days demonstrated comparable efficacy (39 and 45%, respectively) upon sporozoite challenge 3 weeks following final vaccination [31].

The bulk of preclinical and clinical experience, up to this point of development, was suggestive that the most successful RTS,S vaccine would not only produce a strong antibody response, but also a potent RTS,S-specific cellular immune response [32]. Using both murine and rhesus monkey models, WRAIR and GSK Biologicals were able to demonstrate that the novel adjuvant, designated as AS01B, was able to elicit not only antibody responses equivalent to the AS02A formulation, but also a more robust and sustained RTS,S cellular immune response [33–35]. In 2003, the WRAIR and GSK Biologicals collaborators initiated a Phase IIa trial comparing the AS02A and AS01B formulations of the RTS,S vaccine [36]. In this trial, volunteers were vaccinated with 50 µg of either formulation on a schedule of 0, 1, 2 months, and underwent sporozoite challenge 2–3 weeks following the final dose of the vaccine. Volunteers that exhibited protection during the initial challenge were offered rechallenge 5 months later. In addition to being well tolerated, the AS01B formulation appeared to be more efficacious than the AS02A formulation during the acute challenge, protecting 18 of 36 volunteers (50%) versus 14 of 44 (32%), respectively. Although not statistically significant ($p = 0.11$), this difference was felt to be a true improvement in efficacy based upon comparison of immune markers among those vaccinated. Protected volunteers exhibited higher anti-CS protein antibody responses and CS protein-specific multifunctional $CD4^+$ T-cell immune responses as compared with nonprotected volunteers. In addition, mean values for these responses were notably greater in volunteers vaccinated with RTS,S/AS01B as compared with those vaccinated with RTS,S/AS02A. Rechallenge 5 months later revealed a promising 44.4% efficacy (four out of nine protected in each vaccine group), giving credence to the notion of long-term immunity induction.

Field trials of RTS,S in semi-immune adults

Building upon the encouraging results from controlled challenges in malaria-naive adults, trials in malaria-endemic settings were pursued during the late 1990s. The first vaccine tested in the field, RTS,S/AS02A, was found to be safe, well tolerated and immunogenic in semi-immune adults living in both low- and high-transmission areas (The Gambia and Western Kenya, respectively) [37,38]. In 1998, a Phase IIb trial was initiated in The Gambia. During this trial, 131 adult men were vaccinated with RTS,S/AS02A on a schedule of 0, 1, 5 months, and compared with a control group of 119 volunteers who received rabies vaccination. A total of 2 weeks prior to receiving their third dose of vaccine, volunteers were treated with sulfadoxine/pyrimethamine to clear blood-stage *P. falciparum* infections and followed with blood smears at least weekly to detect vaccine failure as evidenced by parasitemia. As in malaria-naive subjects, the vaccine was well tolerated and highly immunogenic. By the third vaccination of the RTS,S/AS02A group, the geometric mean concentration of antibody against CS protein increased 20-fold over the baseline concentration of 1.58 mg/l, and maintained a tenfold increase over baseline during the following year. Proliferative T-cell and IFN-γ responses to RTS,S were also demonstrated in the RTS,S/AS02A vaccination group. Genetic analysis of infecting parasites revealed that the RTS,S/AS02A vaccine provided heterogenous protection against strains other than the NF54 strain it was derived from. After adjustment for

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confounders, the investigators found an overall 34% vaccine efficacy versus parasitemia. Upon further comparison of the first 9 weeks and last 6 weeks of surveillance, it appeared that duration of protection was short lived. Over this time period, vaccine efficacy versus parasitemia, adjusted for age, bednet use, village and prevaccination concentration of antibody versus CS protein, declined significantly, from 71 to 0%. This insinuated that there was no apparent boosting effect from natural infection. In addition to the observed decline in antibody titers during this period, it has been postulated that unaccounted covariates, variations in seasonal transmission and selection bias may have all played a role in this perceived decline [14,39]. The following year, a fourth dose of RTS,S/AS02A was administered to 73 volunteers and rabies vaccine was administered to 85 controls, just prior to the peak of malaria transmission; both groups were then followed for 9 weeks. Signifying the development of a cogent immune memory, mean antibody concentrations in the RTS,S/AS02A group returned to high levels; twofold higher than after their third dose. With this apparent rise in antibody concentration, vaccine efficacy also improved to 47%, adjusted for the same covariates. Given the relatively superior immunogenicity and efficacy of the RTS,S/AS01B over the RTS,S/AS02A formulation in experimental human challenge, a Phase IIb study was conducted in Western Kenyan adult volunteers [36,40]. This trial was a double-blind, randomized controlled study of 6 months duration with a subsequent 6-month single-blind follow-up. Approximately 70 volunteers in each of the three cohorts received standard doses of either RTS,S/AS01B, RTS,S/AS02A or rabies vaccine on a 0, 1, 2 month schedule and then underwent surveillance for an additional 10 months. Volunteers were treated with atovaquone/proguanil prior to vaccine dose three, and then monitored for patent parasitemia. Geometric mean anti-CS antibody titers were consistently and significantly higher in the RTS,S/AS01B group, as compared with the RTS,S/AS02A group, both immediately after vaccination and until the end of the surveillance period ($p \leq 0.001$ and $p = 0.002$, respectively). At the 12-month mark, the geometric mean titers for both vaccines were approximately 50% of their 3-month peak postinitiation of vaccination. Similar to the experience in malaria-naive volunteers, the investigators were able to demonstrate that those protected from infection had significantly higher geometric mean titers than those not protected ($p \leq 0.0007$ 1 month postdose two, 1 month postdose three, and 4.5 months postdose three). This gave further support for the superiority of the RTS,S/AS01B formulation. The observed attack rate was much lower than expected during the study period, 50% versus the expected 72%, thus the study was underpowered to measure vaccine efficacy compared with the control group. Raw efficacy reported in the RTS,S/AS01B group was 30% ($p = 0.164$) and 32% in the RTS,S/AS02A group ($p = 0.128$). Given its promising efficacy in both malaria-experienced and -naive adult subjects, improved immunogenicity compared with the AS02A formulation, and comparable safety and tolerability, it was advocated that the RTS,S/AS01B formulation be further evaluated in a pediatric population.

Pediatric trials with RTS,S

Armed with successful results in trials of malaria-naive and semi-immune adults, there was great impetus to pursue development of the RTS,S malaria vaccine candidate in infants and children. In a partnership credited for significantly furthering the pediatric clinical development process, GSK and the Malaria Vaccine Initiative of the Program for Appropriate Technology in Health (MVI-PATH) embarked upon a series of pediatric trials within endemic African countries. Key objectives for this endeavor included preventing patent parasitemia and the prevention of clinically apparent and severe disease manifestations. Preceding clinical validation of the RTS,S/AS01 vaccine candidate, the RTS,S/AS02A formulation was the first evaluated in children. With the goal of ultimately assessing the efficacy and safety of this candidate vaccine in infants, investigators initiated step-down age-de-escalation and dose-escalation safety trials in older children – a population also at risk for severe malaria-related disease. Two sequential Phase I safety and immunogenicity trials of RTS,S/AS02A were conducted in children 6–11 years of age and then 1–5 years of age. Between March 2001 and January 2002, investigators in The Gambia examined 10-, 25- and 50-µg vaccine doses versus rabies vaccine (control) on a 0, 1, 3 month schedule [41]. In both groups, the vaccine was demonstrated to be safe and immunogenic. Although there was mild-to-moderate reactogenicity with higher doses, the vaccine was, overall, well tolerated in both groups. Antibody responses to both CS and HBsAg in the 25- and 50-µg groups tended to be higher than in the 10-µg group at the 1-month follow-up postvaccination. Based upon these results, the 25-µg RTS,S dose was selected for future pediatric trials. As part of the clinical development plan for RTS,S/AS02A, a proof of concept of efficacy trial in Mozambican children aged 1–4 years was planned. Given concerns that ethnic-related genetic features and differences in parasite transmission intensity could modify vaccine safety and/or immunogenicity, a separate follow-up Phase I study in this specific population was performed. As in the other pediatric groups, the 25-µg RTS,S dose vaccine was again safe, immunogenic and well tolerated [42].

With completion of the Phase I pediatric trials for RTS,S/AS02A, in 2003, investigators from Mozambique, the University of Barcelona, GSK and MVI-PATH initiated a Phase IIb, double-blind, randomized controlled trial to assess the safety, immunogenicity and efficacy of the vaccine [43,44]. A total of 2022 children in Mozambique, 1–4 years of age, were randomized to receive either the pediatric dose of RTS,S/AS02A or a control vaccine (HBV, *Haemophilus influenzae* type b or pneumococcal) on a 0, 1, 2 month schedule. These 2022 children were separated into two cohorts: cohort one was assessed for the primary end point of clinical disease via passive case detection, while cohort two (after presumptive pharmacological clearance of parasitemia) was followed to detect new infection via both active and passive surveillance. At the 6-month analysis, cohort one revealed that vaccine efficacy for first episode of clinical disease was 29.9% ($p = 0.004$), 27.4% ($p = 0.014$) for efficacy versus all clinical episodes, and 57.7% ($p = 0.019$) for efficacy against severe malaria. Analysis of cohort two demonstrated that vaccine efficacy in extending time

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to first infection was 45% (p = 0.0001), however, by the end of this initial observation period, nearly all the children in cohort two had demonstrated patent parasitemia [45]. These cohorts were followed up at month 45 postinitiation of vaccination, where vaccine efficacy for first episode of clinical disease was 30.5% (p < 0.001), for all clinical episodes was 25.6% (p < 0.001) and for severe malaria was 38.3% (p = 0.045) [46]. Exhibiting a one-quarter reduction in malarial disease burden during the study period, the trial re-enforced the feasibility of developing a vaccine with durable and tangible effect upon clinically relevant end points.

Two separate trials next examined RTS,S/AS02D in infants. The first trial was conducted in Mozambique, from 2005 to 2007 [47]. A total of 214 infants were randomized to receive either the RTS,S/AS02D or HBV vaccine at 10, 14 and 18 weeks of age, as well as routine vaccinations at 8, 12, and 16 weeks of age (thus, RTS,S/AS02D was staggered with the Expanded Program of Immunization [EPI]). After pharmacologic clearance of parasitemia prior to the third dose of vaccine, volunteers underwent active and passive surveillance for infection and clinical disease over 6 months. Adjusted vaccine efficacy against infection was 65.9% (p < 0.0001) at 3 months, but this had effectively disappeared by the 6-month mark. Efficacy versus clinical disease was 35.5% (p = 0.093) through the end of the surveillance period. The second trial was conducted in Tanzania from 2006 to 2008 [48]. A total of 340 infants were randomly assigned to receive either the RTS,S/AS02D vaccine or HBV vaccination at 8, 12 and 16 weeks of age, and presumptive pharmacologic clearance of parasitemia was performed prior to surveillance for infection. In contrast to the Mozambique study, enrolled infants also received additional vaccinations, in accordance with the EPI, at the time of the RTS,S/AS02D administration. Anti-CS titers after vaccination, although much higher than controls, were lower than those observed in the trials utilizing staggered administration and those among 1–4-year olds. However, this had no apparent impact upon vaccine efficacy: at the end of the 6-month surveillance period, adjusted efficacy against infection was 65.2% (p = 0.01) and efficacy against clinical disease was similar to the Mozambique study, at 41.8% (p = 0.20). Noninferiority of the humoral responses to all EPI antigens was demonstrated when comparing the RTS,S/AS02D and HBV vaccination groups. Immunological and clinical assessment of RTS,S/AS02D with the EPI vaccines in these trials clearly demonstrated the feasibility of integrating this formulation into the routine EPI schedule, which would be important if millions of doses were to be eventually delivered across the endemic countries of sub-Saharan Africa.

Bolstered by the success of the RTS,S/AS02D trials and the promising adult experience with RTS,S/AS01B, clinical trials with the pediatric formulation, RTS,S/AS01E, ensued. The trials validated both the safety and superior immunogenicity of the AS01E formulation (compared with RTS,S/AS02D) in infants and small children living within malaria-endemic regions [49,50]. In 2007, a double-blind efficacy trial randomized 894 Kenyan and Tanzanian children, aged 5–17 months, to receive either RTS,S/AS01E versus rabies vaccine on a 0, 1, 2 month schedule [51]. Adjusted vaccine efficacy for first or only clinical episode, over a mean 8 months of follow-up, was 53% (p < 0.001), with a low level of asymptomatic parasitemia (2% RTS,S/AS01E group vs 3% in the rabies vaccine group). At 15 months follow-up, 58 out of 209 children in the RTS,S/AS01E group and 85 out of 206 in the rabies vaccine group had their first or only clinical malaria episode, translating into a vaccine efficacy of 45.8% (p = 0.0004) [52]. As had been demonstrated with the AS02D vaccine formulation, a follow-up Phase II trial in Ghana, Tanzania and Gabon also found RTS,S/AS01E to be safe and immunogenic when integrated into the EPI [53].

In May of 2009, GSK, MVI-PATH and a consortium of African researchers initiated the largest malaria trial to date, a Phase III trial of RTS,S/AS01E [102,103]. At the present time, it has enrolled over 15,000 children in seven countries across sub-Saharan Africa [104]. This multicenter, double-blind, randomized controlled trial includes two age groups of children. The first age group contains children aged 5–17 months at time of first vaccination, who will receive RTS,S/AS01E without co-administration of other vaccines. The second group contains children aged 6–12 weeks at time of first vaccination, who will receive coadministration of EPI vaccinations. Both groups incorporate a comparison of RTS,S/AS01E booster versus control immunization after the initial three-dose RTS,S vaccination regimen. It is anticipated that this trial will be complete in July of 2013, yielding data critical for the effort towards licensure [105].

RTS,S in combination: other antigens & prime–boost

Investigators have explored combining RTS,S with other protein targets and vaccine platforms. Combining RTS,S with another pre-erythrocytic malaria protein that would target the malaria sporozoite by blocking a critical step in cell traversal or hepatocyte invasion would theoretically potentiate protective efficacy by reducing the number of parasites entering the liver or interfering with intrahepatic development. This approach has been tested in the clinic by combining recombinant proteins RTS,S with merozoite surface protein-1 or thrombospondin-related anonymous protein and, in nonhuman primates, with liver-stage antigen-1 [54–56]. Unfortunately, enhanced protection was not observed, suggesting that immunological mechanisms, not entirely understood, cannot be underestimated when combining separate vaccines that theoretically would provide superior protection compared with each antigen alone.

Although immune correlates of protection have not been well defined, clinical trials with RTS,S have demonstrated that increased protective efficacy is associated with higher anti-CS antibody titers and increased CS-specific cellular immune responses [36]. Induction of a powerful humoral immune response has always been a strength of the RTS,S vaccine, and although greatly improved throughout its development, the cell-mediated immune response has been the comparatively weaker component. It has been hypothesized that a heterologous prime–boost vaccination approach, utilizing sequential delivery of the same antigens in different systems, may enhance both $CD8^+$ and $CD4^+$ T-cell responses. In humans, it has been demonstrated, that priming with a *P. falciparum* DNA vaccine coding for the CS protein,

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and boosting with RTS,S/AS02A 12–14 months later, provides enhanced antibody, $CD8^+$ T-cell, and $CD4^+$ T-cell immune responses against CS [57,58]. There have been no human challenge trials, to date, to test the efficacy of this regimen. In another trial of malaria-naive adults, investigating priming and boosting with either RTS,S/AS02A or modified vaccinia virus Ankara expressing CS protein, there was only a small improvement in T-cell immunogenicity, no increased antibody response and no improvement in prevention of patent parasitemia (33% in both groups) [59]. Most recently, preclinical work with nonhuman primates has shown that priming with a replication-defective adenovirus serotype 35 vector encoding CS protein (Ad35.CS), followed by boosting with RTS,S/AS01B at 1 and 3 months, resulted in a dramatically improved and sustained immune response [60]. CS protein-specific antibody response remained strong, however there was an impressive 16-fold improvement in IFN-γ T-cell responses as compared with the group that received only RTS,S/AS01B at 0, 1 and 3 months. Furthermore, this response remained 6–7.3-fold higher than either the Ad35.CS or the RTS,S/AS01B groups 6 months later. Based upon these results, a multicenter efficacy trial is planned to begin in 2011 to further investigate this very promising prime–boost regimen in malaria-naive humans.

Expert commentary & five-year view

Research and development into a series of second-generation vaccines has already begun. It has taken over 20 years of development, testing, and reformulation to reach an important threshold of efficacy that could prevent mortality and morbidity in tens of thousands of infants in sub-Saharan Africa. However, the fact remains that many of the volunteers from these studies in endemic countries still develop malaria infection and disease by the end of the study monitoring period, or likely will some time afterward. The quest for an even better malaria vaccine is not finished. Next-generation malaria vaccines need to approach 80% efficacy in order to demonstrate a significant improvement over RTS,S/AS01E alone and garner the financial support required for their further development. There are several approaches being pursued concurrently. Some vaccine developers favor improvement of a single-antigen vaccine by increasing immunogenicity through a prime–boost approach, in which one vaccine delivery platform primes the immune response, and a second vaccine of the same antigen, delivered in a different manner, boosts the response over and above that seen with a single delivery platform. As discussed, one prime–boost approach capitalizes on the critical role that adenovirus-vectored vaccines have in priming the immune system for enhanced cell-mediated immune responses followed by recombinant protein (i.e., RTS,S) booster immunizations that increase the magnitude and duration of both cellular and humoral arms of the immune system. Other prime–boost approaches use two different virus-vectored vaccines expressing the same antigen. Current formulations under study include Ad35.CS followed by Ad26.CS serotype vaccines and Simian adenovirus-vectored vaccines followed by pox-virus-vectored vaccines [61,62].

Apart from the purely scientific considerations that lead to improvements of vaccine efficacy are factors that impact the financial and logistical hurdles that face the vaccine manufacturer. These include the costs associated with manufacture of multiple antigens or virus-vectored vaccines, the logistical hurdles of delivering different platforms under different cold-chain requirements in resource-poor settings, and the complexities involved in ensuring that the proper sequence of vaccines are delivered at the appropriate time. Finally and realistically, since many of the virus-vectored vaccine delivery vectors are being developed concurrently for different pathogens, for instance malaria, TB and HIV, 'he' who gets there first with a licensed and launched vaccine will most likely place other similar but not yet licensed vaccines in a perilous situation by generating a significant degree of antivector immunity that could interfere with subsequent vaccinations.

Despite the hurdles faced, we are currently in a tremendously satisfying era of vaccine development and testing that has generated enthusiasm among young investigators entering this exciting field. More so than ever, the future is bright and perseverance the key.

"Through perseverance many people win success out of what seemed destined to be certain failure"

Benjamin Disraeli.

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Key issues

- RTS,S/AS01 represents the most developed and clinically validated malaria vaccine formulation.
- The protective efficacy of RTS,S-based vaccine formulations is associated with both production of high circumsporozoite protein-specific antibody concentrations and robust cellular immune responses.
- Increasingly more effective adjuvants have been key in improving the potency of the RTS,S vaccine formulations.
- RTS,S-based vaccine formulations have been demonstrated to be safe, well tolerated and immunogenic in both malaria-naive and -experienced adults and children.
- Further research to optimize and improve vaccine efficacy is ongoing.

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