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REVIEW ARTICLE

# RTS,S MALARIA VACCINES: TARGETING THE CIRCUMSPOROZOITE PROTEIN

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**ABSTRACT**

*Malaria is a mosquito-borne disease transmitted by inoculation with Plasmodium sporozoites. With approximately 2.4 billion people at risk, Plasmodium falciparum infection continues to be a major cause of mortality and morbidity, mainly in the tropics and subtropics. Although malaria-related morbidity and mortality have decreased in some areas due to improved treatment and the use of insecticide-treated mosquito nets, a vaccine is needed for sustained, effective, long-term control. Immunization with high doses of irradiated sporozoites has induced protective responses against malaria in human volunteers. An understanding of the immune mechanisms underlying the protection induced by irradiated sporozoites will pave the way for the development of subunit vaccines directed at the pre-erythrocytic stage of Plasmodium. To date, RTS,S (GlaxoSmithKline), in combination with an effective adjuvant therapy, is the only vaccine candidate to demonstrate protection in humans in artificial challenge and natural environment trials. However, the protection afforded by this vaccine does not approach the level of sterile protection that is achievable with irradiated sporozoites. Strategies for improving its efficacy are under development and it is likely that we will soon have a second-generation multitarget malaria vaccine capable of inducing more powerful and long-lasting immune responses.*

## INTRODUCTION

Malaria is a life-threatening disease caused by parasites transmitted through the bites of infected mosquitoes. Approximately half of the world's population is at risk of malaria, particularly those living in lower-income countries (Fig. 1) (1). Severe and complicated malaria kills more than 2 million people annually in sub-Saharan Africa; children under the age of 5 years, pregnant women and people living with HIV/AIDS are particularly vulnerable to life-threatening anemia and cerebral malaria (2). Therefore, an effective vaccine against this disease is urgently needed (3, 4).

*Plasmodium* spp. transmission occurs by the injection of infectious sporozoites while an infected female Anopheles mosquito is probing for a blood meal (5). Once injected into the skin, sporozoites actively move away from the site of injection. Some enter a capillary and can reach the liver within minutes, where they invade hepatocytes (6). Others invade lymphatic vessels and end up in the proximal draining lymph node (7). This observation is of importance to understand the mechanism by which irradiated sporozoites can initiate antimalarial immune responses (8).

In a hepatocyte, the parasite replicates and differentiates, giving rise to many thousands of merozoites that are released into the blood and infect red blood cells (RBCs) (6). After penetrating RBCs, the merozoites assume ring forms known as trophozoites. While growing, the trophozoites metamorph into schizonts and produce new merozoites inside the RBCs (9). A subset of merozoites differentiate into male and female gametocytes (10). These gametocytes, taken up in the blood meal by the mosquito, develop into gametes that fuse in the midgut of the mosquito to form a motile zygote, the ookinete, where meiosis occurs. The ookinete crosses the midgut wall and forms an oocyst in which sporozoites develop and then exit in order to enter the mosquito salivary gland, thereby completing the cycle (Fig. 2).

Natural exposure to the bites of malaria-infected mosquitoes is unable to induce protective immunity in people living in endemic areas, leaving them susceptible to lifelong reinfection (8). However, in 1967, Nussenzweig et al. demonstrated for the first time that partial protective immunity against malaria can be achieved in mice by i.v. injection of irradiated *Plasmodium berghei* sporozoites (11-13). More importantly, in 1973 and 1974, respectively, Clyde et al. (14) and Rieckmann et al. (15) published studies performed at two different institutions, reporting that immunization with the bites of irradiated mosquitoes carrying *Plasmodium falciparum* sporozoites induced complete protection against challenge with infected mosquitoes (14, 15). Furthermore, during 1989-1999, 11 volunteers were immu-

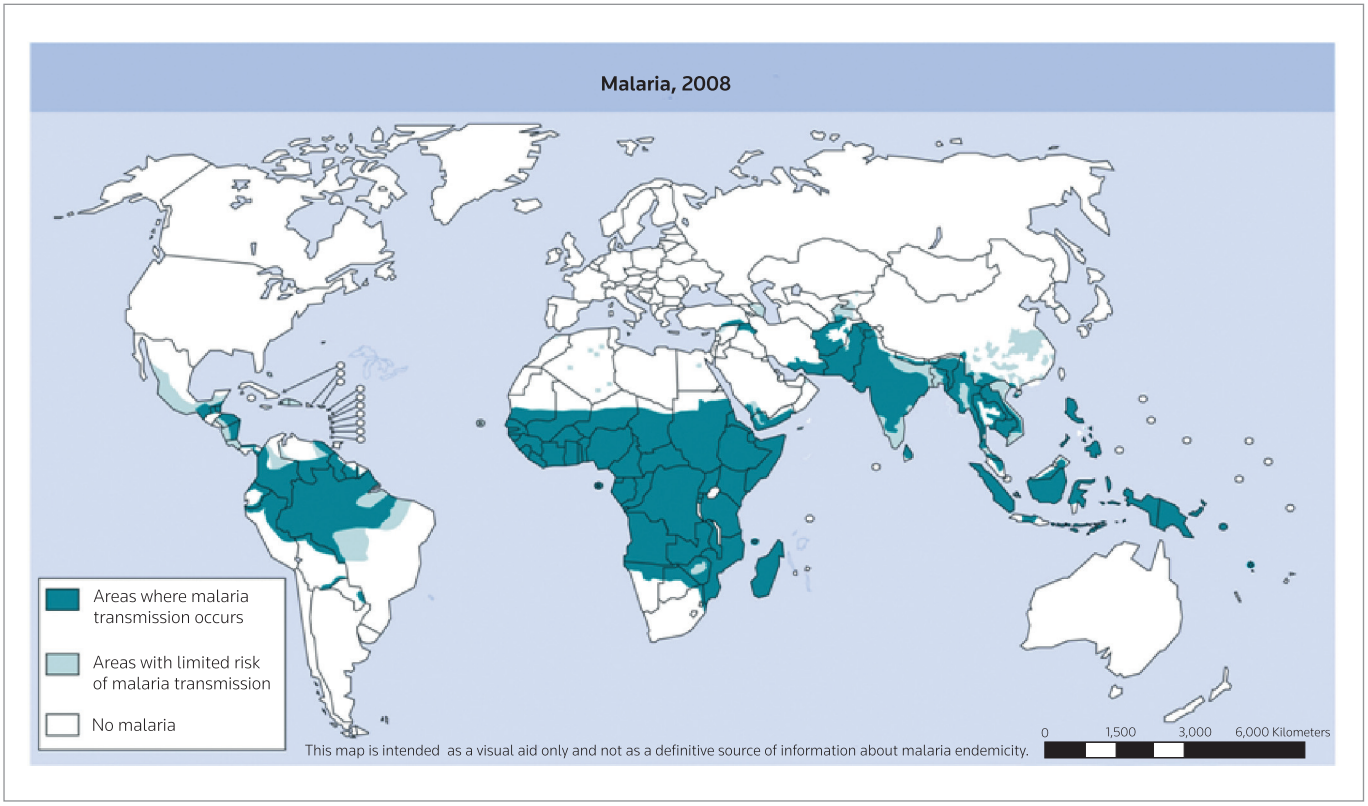


**Figure 1.** The spatial distribution of *Plasmodium falciparum* malaria endemicity. Reproduced with permission from World Health Organization. Map Production: Public Health information and Geographic Information Systems (GIS).

nized with bites from 1,001 to 2,927 irradiated mosquitoes, following which there was protection in 24 of 26 challenges with virulent sporozoites for at least 10 months (16).

## IRRADIATED SPOROZOITES AS MALARIA VACCINE: THE ROLE OF THE CIRCUMSPOROZOITE PROTEIN

Sporozoites are irradiated when infected mosquitoes are subjected to radiation (14). As with normal sporozoites, upon inoculation into a susceptible host, the irradiated sporozoites penetrate the hepatocytes and begin intracellular development. Unlike their normal counterparts, however, irradiated sporozoites are not capable of nuclear division and do not develop further, but persist for several weeks or months (17). Interestingly immunization with heat-killed sporozoites failed to induce protective immune responses, indicating, as has been demonstrated for other pathogens (18, 19), the requirement for live parasites (17).

As previously indicated, a large number of mosquito bites are required to produce protective immunity against malaria, and thus the development of an effective vaccine based on immunization with irradiated sporozoites was considered a difficult task. For this reason, considerable research has been carried out to reveal the mechanisms of this protection and identify the antigens involved (20). Antibodies are effective at limiting the number of parasites that successfully reach the liver, but this protection is hard to achieve, requiring high titers of high-affinity antibodies from long-lasting durable memory B cells to be effective (21, 22). $CD8^+$ T cells are important for eliminating parasites that successfully invade and replicate within hepatocytes (23-25), and thus, the liver stage is the primary target for vaccine-inducible T-cell responses and is the presumed target of the irradiated sporozoite vaccination model (10). It was long believed that $CD8^+$ T-cell priming by irradiated sporozoites took place in the liver. However, after inoculation of irradiated sporozoites, certain antigens remain in the skin, and a proportion of the inoculum goes to the draining lymph node, where its antigens are presented to naïve $CD8^+$ T cells, initiating an antisporozoite T-cell protective immune response (26).

Thus, antibody and T-cell responses appeared to be involved in irradiated sporozoite-induced protection. Following this discovery, which antigen or antigens are directly implicated in the induction of this protective immune response remained to be elucidated. In 1969, Vanderberg et al. demonstrated that sera from mice immunized with irradiated sporozoites precipitated a repetitive surface antigen that densely covered the sporozoite surface –the circumsporozoite (CS) protein (27). For this reason, antibodies against CS were thought to be primary in the protective immune response induced by irradiated sporozoites. In agreement with this, passive transfer of monoclonal and polyclonal antibodies against the CS protein protected against *P. berghei* and *Plasmodium yoelii* sporozoite-induced malaria (28-31). Furthermore, passive transfer of monoclonal antibodies against CS protein to Saimiri monkeys induced protection

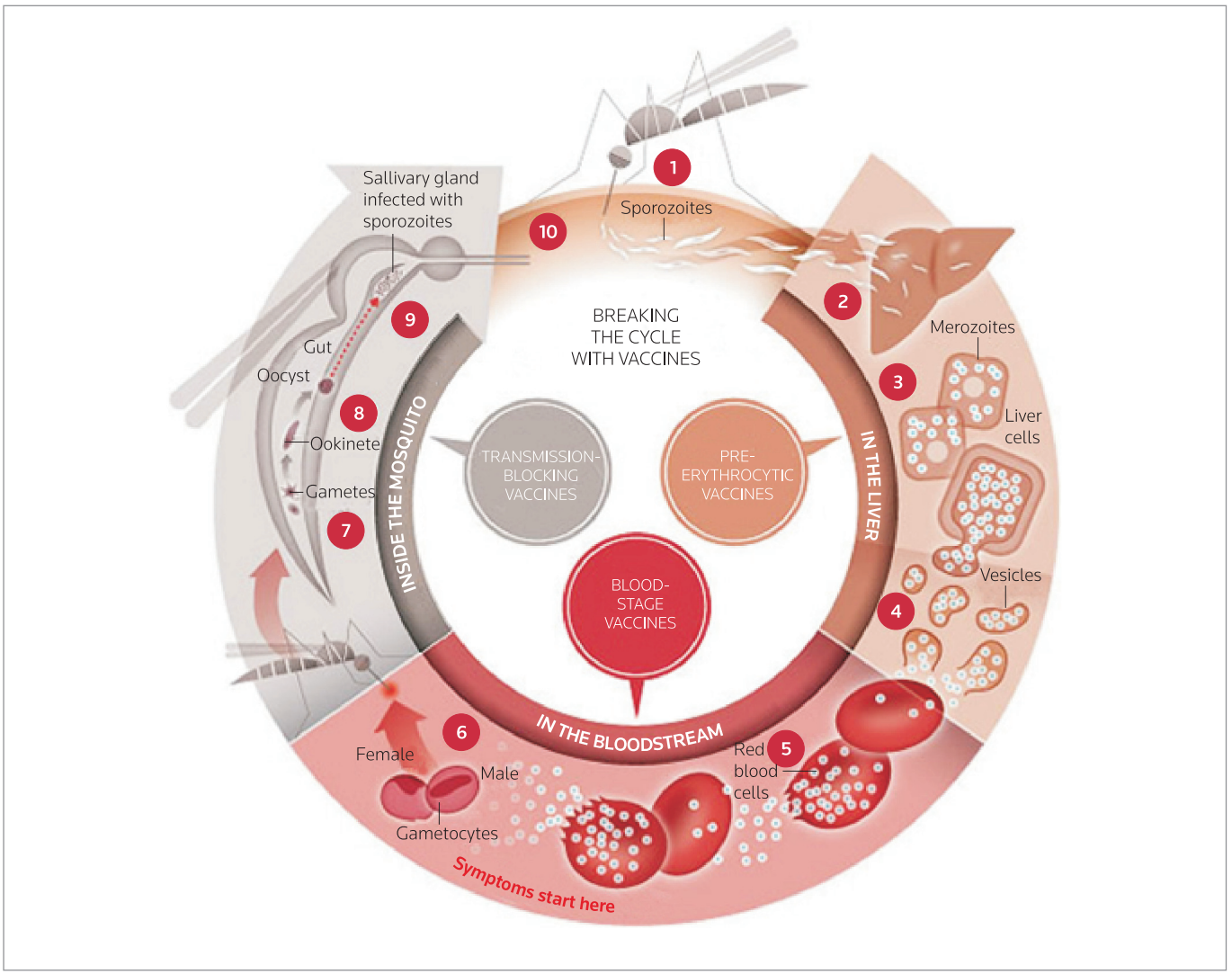


**Figure 2.** The life cycle of the malaria parasite. Reproduced with permission from Malaria Vaccine Initiative (MVI). http://www.malariavaccine.org/mal-what_is_malaria.htm.

against *P. yoelii* sporozoite challenge (32). Numerous studies in rodents have demonstrated that a T-cell immune response against CS protein is indispensable for irradiated sporozoite-induced protection (33-35). Elegant studies performed by the research groups of Nussenzweig and Zavala in transgenic mice expressing CS demonstrated that the CS protein is an immunodominant protective antigen in irradiated sporozoites (36).

The development of a CS-based synthetic vaccine was made possible due to the cloning of the CS gene from different *Plasmodium* species by 1980 (37, 38). B-cell and MHC class I-restricted epitopes were identified in the CS protein (39). Synthetic peptides and recombinant protein vaccines based on the repeat region of the CS protein, as well as bacterial and viral vectors encoding *Plasmodium* CS protein, have induced protective immunity (30, 40-45). Experimental vaccines based on *P. falciparum* CS protein were the first to be tested in humans (46-51). The best protection was achieved with a recombinant fusion protein composed of the CS *P. falciparum* repeat region and a carrier protein encapsulated in liposomes containing monophosphoryl lipid A (MPL) and adsorbed to alum (25% protective efficacy) (52). The composition of this vaccine is very similar to the RTS,S/AS02A (GlaxoSmithKline) vaccine which is the focus of this review.

CS-based vaccines are likely to induce immune responses that either inactivate the sporozoite or prevent further development in the liver. Sporozoite transmission is one of the bottlenecks in the *Plasmodium* life cycle, since only a few dozen sporozoites are injected into the skin and not all of them are able to reach the liver (53). However, pre-erythrocytic-stage vaccines must be 100% effective because the escape of a single sporozoite generates tens of thousands of merozoites. Indeed, this may explain why CS-based vaccines thus far only partially protect against *Plasmodium* sporozoite infection in humans (54).

## MALARIA SUBUNIT VACCINES: RTS,S

Over the last 20 years, despite considerable efforts and resources, only 22 *P. falciparum* antigens have been under research and development for malaria vaccines (55). Current research and development efforts are focused on only 4 pre-erythryocytic-stage antigens (CS protein CSP1, liver stage-specific antigen LSA-1, liver stage antigen-3 [LSA-3] and thrombospondin-related adhesive protein [TRAP]), 13 blood-stage antigens (apical membrane antigen AMA-1, merozoite surface proteins MSP-1, MSP-3 and MSP-4, glutamate rich protein GLURP, ring-infected erythrocyte surface antigen [RESA], serine-repeat antigen 5 [SERA], erythrocyte-binding antigen EBA-175, erythrocyte-binding protein EBP-2, chimeric erythrocyte-binding protein MAEBL, rhoptry-associated protein 2 [RAP2], erythrocyte membrane protein EMP1 and DBL a protein) and 5 sexual-stage surface antigens (Pfs25, Pfs27, Pfs28, Pfs45/48 and Pfs230) of *P. falciparum*.

Four malaria vaccine candidates, SPf66 and MSP/RESA (against the asexual stages of the *Plasmodium* parasite) and CS-NANP and RTS,S (against the sporozoite stages), have been tested in randomized, controlled trials in endemic areas (56). Currently, the RTS,S vaccine is the most advanced candidate (Fig. 3).

The RTS,S vaccine was created in 1987 by scientists at GlaxoSmith-Kline (GSK) Biological laboratories. RTS,S is a hybrid molecule expressed in yeast that consists of the central tandem repeat (R) and carboxyl-terminal region containing major T-cell epitopes (T) of the CS protein fused to the *N*-terminus of the S-antigen of hepatitis B virus (S) in a particle that also includes non-fused S-antigen. Experiments performed in animal models demonstrated that immunization with RTS,S led to production of high titers of antibodies and a robust T-cell response against CS protein (57).

A preliminary evaluation of the RTS,S vaccine in individuals who had never been exposed to malaria demonstrated that the immunogenicity of the RTS,S vaccine depends on the adjuvant formulation (58-61). Gordon et al. (59) and Stoute et al. (60, 61) tested four different formulations of the vaccine: one formulation containing alum; a second with alum and MPL; the third, an oil-in-water emulsion; and the fourth, an oil-in-water emulsion plus the immunostimulants 3-deacylated MPL (3D-MPL) and the saponin derivative QS21. When RTS,S is formulated with the oil-in-water emulsion plus MPL and QS21 (adjuvant AS02A; GSK), the vaccine is highly immunogenic and confers partial protection against a challenge from mosquitoes infected with *P. falciparum*. Moreover, a consistent delay in patency in those individuals who became infected indicated that the vaccine eliminated 90% of the sporozoite inoculum (61). Protected individuals tended to have higher antibody titers against tandem repeat epitopes than those who developed malaria (60). Furthermore, strong T-cell responses to both the whole protein antigen and peptides from CS were detected (60, 61). Importantly, independently of adjuvant formulation, the vaccine was well tolerated (58-60).

The vaccine candidate RTS,S/AS02A is being developed and tested by GSK and the Programme for Appropriate Technology in Health (PATH) Malaria Vaccine Initiative (MVI) with support from the Bill & Melinda Gates Foundation. MVI works with government, industry and academic partners on five continents within PATH. Its mission is to accelerate the development of malaria vaccines and ensure their availability for developing countries (http://www.malariavaccine.org). MVI is accelerating the development of malaria vaccines by testing multiple candidates simultaneously (Fig. 3).

After initial successful evaluation of RTS,S/AS02A in naïve participants, a phase I trial was performed in a small number of adults living in The Gambia in areas hypo- and meso-endemic for malaria who had been previously exposed to malaria infection. The vaccine was demonstrated to be safe and produced significant increases in antibody titer against CS and hepatitis B virus (HBV) surface proteins in semi-immune individuals (62). A phase I trial was later conducted in a small number of semi-immune participants in an area in Kenya hyperendemic for malaria. Once again the vaccine was safe and well tolerated and induced high antibody titers against the CS protein (63).

In subsequent phase II trials (Table I), vaccine efficacy was assessed using time-to-event analysis. Investigators usually compare the proportion of vaccinated participants observed during a certain interval of time that become cases versus the proportion of unvaccinated individuals observed during the same period of time that become cases. However, when a vaccine has a partial protective effect, other parameters should be considered to assess the efficacy of a vaccine, for example, the duration of the illness and the duration of the infection. For a vaccine such as RTS,S, which delays but does not necessarily prevent infection or clinical malaria, time-to-event analysis was needed.

The first randomized phase II trial was performed in The Gambia to test the efficacy of RTS,S/AS02A against natural *P. falciparum* infection. A total of 250 adult men were enrolled and 131 received the RTS,S vaccine. In vaccinated individuals there was a 34% increase in time to first infection, the primary endpoint of the trial (64). Furthermore, a trend towards a reduction of clinical malaria was observed in the vaccinated group in comparison with the control group. High antibody titers against CS protein, as well as cellular immunity, were induced in RTS,S-immunized subjects (65, 66). An important observation from this study was that protection was not limited to the NF54 parasite genotype from which the vaccine was derived, indicating that the protective efficacy of the RTS,S vaccine is not strain-specific (67).

The evaluation of the safety and efficacy of malaria vaccines in infants and children is of utmost importance because most deaths and illness from malaria occur in these age groups. Therefore, based on the promising results obtained in adult volunteers, a pediatric vaccine dose was selected and used in a phase I trial in Mozambican and Gambian children. The vaccine was safe, well tolerated and immunogenic (68, 69). A phase IIb trial was then conducted in 2,022 children aged 1-4 years in Mozambique, where malaria is endemic. This trial produced the best results that have ever been obtained with a candidate malaria vaccine (70-73). The overall vaccine efficacy, based on time to first infection, was 45% and the vaccine had an excellent safety profile. The rate of efficacy against the more clinically relevant endpoint of clinical malaria was 35.3%. The findings were very encouraging, since the vaccine induced a strong antibody response and stimulated Th1 cellular immunity (69). The vaccine also reduced the incidence of severe disease by 58%, leading to a reduction in mortality. Interestingly, the efficacy was higher when children under 24 months of age were considered separately (76.9%).

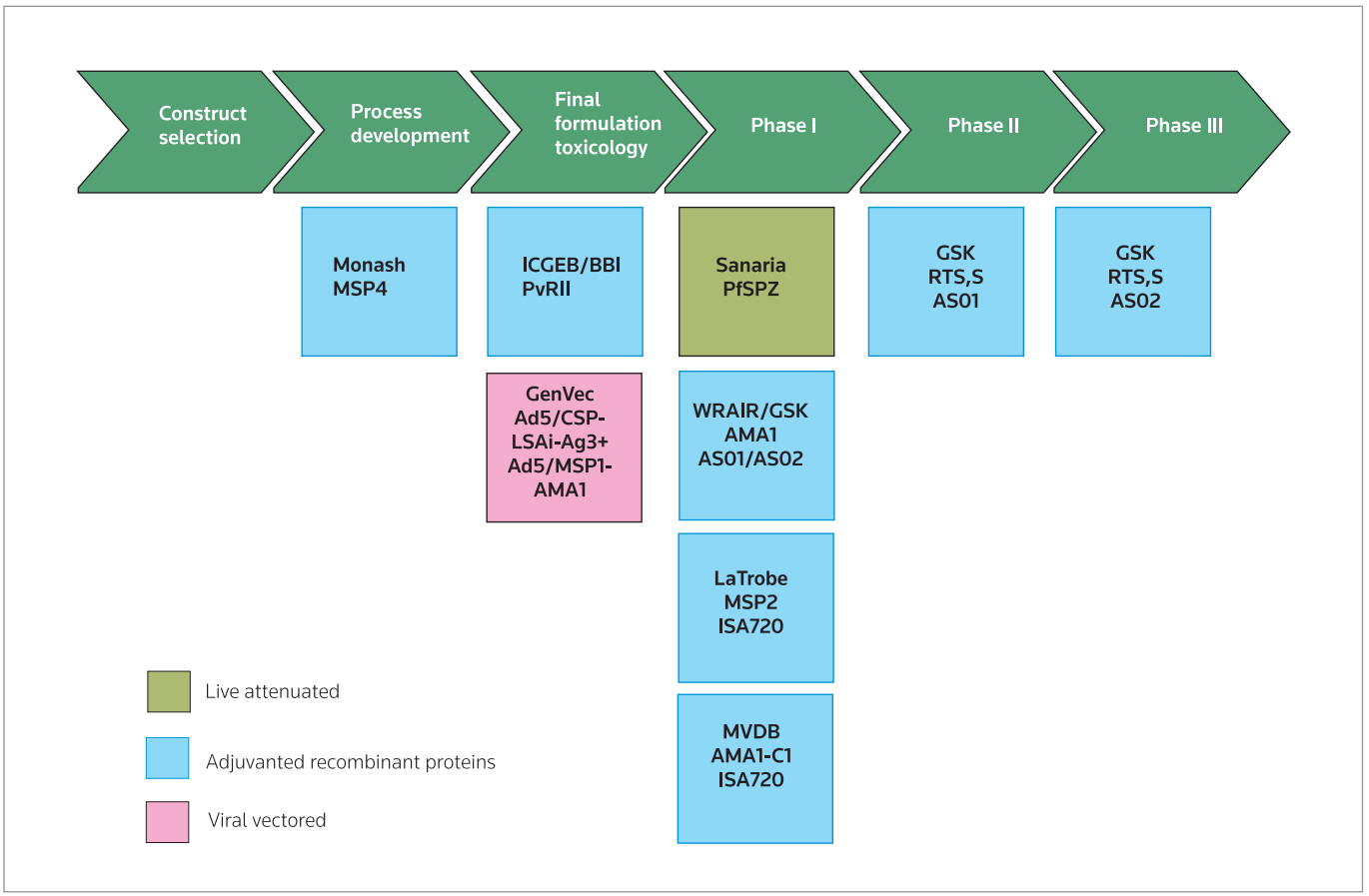


**Figure 3.** Malaria vaccine candidates that are being developed by the Malaria Vaccine Initiative (MVI). http://www.malariavaccine.org/.

***Table I.*** *RTS,S + adjuvant phase II clinical trials.*

| RTS,S + adjuvant | RTS,S/AS02 | | | | | | | | RTS/AS01E | |
|---|---|---|---|---|---|---|---|---|---|---|
| Recipients | Gambian adults | | Mozambican children | | | | Tanzanian children | | Tanzanian/ Kenyan children | |
| | | | Cohort 1 | | Cohort 2 | | | | | |
| Groups | Controls‡ | Vaccinees | Controls‡ | Vaccinees | Controls‡ | Vaccinees | Controls‡ | Vaccinees | Controls‡ | Vacinees |
| n | 119 | 131 | 745 | 745 | 178 | 189 | 153 | 153 | 402 | 407 |
| Age | 18-45 | | 1-4 years | | | | 7-9 months | | 5-17 months | |
| Efficacy of the vaccine against first infection | 34% (95% CI: 8.0-53) | | 29.9% (95% CI: 11.0-44.8) | | 45% (95% CI: 31.4-55.9) | | 65.2% (95% CI: 20.7-84.7) | | 53% (95% CI: 28.7-69) | |
| Efficacy of the vaccine against clinical malaria | 35.3% (95% CI: 21.6-46.6) | | | | | | | | | |
| Efficacy of the vaccine against severe malaria | 48.6% (95% CI: 12.3-71) | | | | | | | | | |
| Anticircumsporozoite antibody titer | 1276 mg/L (95% CI: 727-2,237) | | 158 EU/mL (95% CI: 142-176) | | | | 199 EU/mL (95% CI: 150.9-26,457) | | 39.6% EU/mL (95% CI: 500.7-581.6) | |

The figures include only the individuals who received three doses of the RTS,S/adjuvant vaccine. ‡Control was three doses of a human diploid cell rabies vaccine (rabies vaccine BP). †Control children aged 24 months and older received three pediatric doses of HBV vaccine (Engerix-B™). Children under 24 months received two pediatric doses of a pneumococcal vaccine (Prevnar™). #Control was three pediatric doses of HBV vaccine (Engerix-B™). Both control and vaccine groups received the DTPw/Hib vaccine. CI, confidence interval; EU, enzyme-linked immunosorbent assay units

Subsequent phase II trials in children have been completed or are ongoing. In countries where a malaria vaccine is needed most, the current immunization schedule for infants, the WHO Expanded Program on Immunization (EPI), would provide an optimal delivery platform for the RTS,S vaccine. The EPI vaccine contains antigens for diphtheria (D), tetanus (T), whole-cell pertussis (Pw) and *Haemophilus influenzae* B (Hib). A study in 340 infants under 12 months of age in Tanzania found that RTS,S/AS02A coadministered with EPI antigens at 8, 12 and 16 weeks of age did not interfere with the protective immune responses to each of the vaccine components. These data show for the first time that the vaccine candidate can be administered as part of existing African national immunization programs. Additionally, the study reported a 65% reduction in time to first infection from malaria in those infants who received three doses of the RTS,S/AS02A vaccine and were followed over a 6-month period (74). This study confirms results obtained in a trial in Mozambican children, which found a similar level of efficacy for RTS,S/AS02A when it was administered in a staggered fashion with the DTPw/Hib vaccine (73).

A trial enrolling 894 children aged between 5 and 17 months in Kenya and Tanzania was designed to evaluate the safety and efficacy of RTS,S combined with another GSK proprietary adjuvant system, AS01 (75-77). This was a double-blind, randomized clinical trial in which children received either three doses of the RTS,S/AS01 vaccine candidate or a rabies vaccine (78, 79). The candidate RTS,S/AS01 reduced the risk of clinical episodes of malaria by 53% over an 8-month follow-up period and had a promising safety profile. This vaccine formulation induced 10-fold higher antibody titers than RTS,S/AS02A.

Regarding the mechanism of protection, the trials of RTS,S/AS02A in malaria-naïve adults demonstrated an association between anti-CS antibody concentrations and protection against malaria challenge (60, 61). The role of anti-CS antibodies in efficacy studies without artificial malaria challenge conducted with different endpoints and over different periods of time is less clear (70, 72, 78). A potential explanation for the lack of correlation in some field studies is the use of a different efficacy endpoint. Furthermore, the analysis of cellular immune responses in vaccinated individuals demonstrated a strong association between the frequency of multifunctional CS-specific $CD4^+$ and $CD8^+$ T cells and complete protection against malaria challenge (67, 80, 81).

The first clinical studies carried out in naïve and semi-immune adult volunteers demonstrated that the efficacy of the vaccine wanes with time (64, 65). CS-specific T cells and total concentrations of antibody against CS decline with time. In naïve volunteers, the protection against sporozoite challenge was also lost, although it was recovered by boosting immunization (64). These data raised concerns about the possible short duration of protection. However, the research group of Alonso recently published the long-term safety and efficacy results of the phase IIb trial in Mozambican children over 45 months (82). There was evidence that RTS,S/AS02A maintained protection during the 45-month surveillance period, which indicated the feasibility of developing an effective vaccine against malaria. These results strengthened the rationale for advancing towards a phase III trial aiming to register RTS,S/AS as the first malaria vaccine.

The phase III trial of RTS,S/AS02A began on May 26, 2009 in Tanzania. The trial is expected to start in other countries across sub-Saharan Africa and will enroll up to 16,000 children and infants. It has been designed to demonstrate how the vaccine performs in a large group of children and infants in different transmission settings across a wide geographic region. It will evaluate the efficacy of the vaccine in two groups of children. One group, aged 6-12 weeks, will be vaccinated as part of their regular schedule of infant immunizations. The second group includes children aged 5-17 months (http://www.malariavaccine.org/). If this trial is successful, the projection is that the RTS,S/AS02A vaccine (Mosquirix™) could be available in 5 years (http://www.malariavaccine.org/). The development and implementation of a malaria vaccine would constitute a major breakthrough for global health.

## PLANNING FOR THE FUTURE

Results from the RTS,S/AS02A phase III trial will provide more information on efficacy, although phase II trials have proven that RTS,S is only partially effective and cannot eradicate malaria alone (70, 72, 78). To improve efficacy, additional antigens could be selected to add to the CS-based vaccine. Several research groups are evaluating additional antigens and combined vaccine platforms with the goal of developing a next-generation RTS,S-based malaria vaccine.

Recently, experimental data indicated that sporozoite antigens other than the CS protein might play an important role in irradiated sporozoite-induced protection. Studies performed in transgenic mice expressing CS protein (which are therefore tolerant to the CS protein) demonstrated that immunization with three doses of irradiated sporozoites induced sterile protection that was mediated by $CD8^+$ T cells. This demonstrated that other sporozoite antigens are also capable of inducing protective immune responses and could be included in future vaccines (36).

Furthermore, the RTS,S vaccine exclusively targets the pre-erythrocytic stages of *Plasmodium* and only confers protection against sporozoite and liver stages. Thus, if immune responses induced by RTS,S fail to block just a single sporozoite from invading or developing in the hepatocyte, then a blood-stage infection will follow. A potential solution to this problem will come from the use of a multi-component vaccine targeting different developmental stages of *Plasmodium* (83, 84). Studies are under way to evaluate the protective effect of adjuvanted recombinant MSP-1 or AMA-1 for combination with adjuvanted RTS,S (85, 86).

A different strategy to improve the effectiveness of the RTS,S vaccine is the use of other vaccine platforms to boost the immune response induced by RTS,S. Several recombinant viral and nonviral vectors expressing CS protein are currently being tested, such as plasmids, modified Ankara virus (MVA) or adenovirus (87-90).

## OTHER VACCINE EFFORTS: WHOLE SPOROZOITE-BASED VACCINES

Irradiated sporozoites remain the only vaccines to confer sterile immunity against infectious sporozoite challenge in humans. The main problem of this experimental immunization strategy is that over 1,000 cumulative infectious mosquito bites were needed to confer sterile protection, which is equivalent to the injection of approximately 100,000 sporozoites. At that time, the production of sporozoites for mass vaccination was unthinkable for technical rea-

sons. However, in the last 10 years, Sanaria has developed processes to establish mosquito-based production for parenteral administration of a live attenuated sporozoite vaccine that meets regulatory requirements (16). Phase I trials to assess safety and protective efficacy of irradiated *P. falciparum* sporozoites began in May 2009.

## CONCLUSIONS

An effective human malaria vaccine has been sought for over 70 years. That RTS,S in combination with the proper adjuvant system is a safe malaria vaccine in infants and children and has reasonable efficacy has been discussed. This vaccine reduced the risk of clinical malaria, delayed the time to new infection and reduced the number of episodes of severe malaria, thus reducing infant mortality. Further optimization of vaccine composition is required to increase the effectiveness of the vaccine. Nevertheless, the availability of a vaccine with nearly 50% efficacy in conjunction with other control interventions (91) would help reduce the considerable global disease burden caused by malaria.

## ACKNOWLEDGMENTS

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## DISCLOSURE

The author states no conflicts of interest.

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