Chapter 7
Development of the RTS,S/AS Vaccine Candidate from Concept to Phase III

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Abstract This review describes the developmental history of the RTS,S/AS vaccine. Selection of the circumsporozoite protein (CSP) as the target antigen was key to the successful development of the vaccine so far, from concept to the initiation of Phase III testing. CSP, a pre-erythrocytic protective antigen against *Plasmodium falciparum*, has been demonstrated to be immunodominant and protective in pre-clinical studies both in animals and humans. The vaccine antigen was designated "RTS,S"; RTS being a hybrid polypeptide consisting of a portion of the CSP antigen and S the surface antigen of Hepatitis B virus (HBsAg). The RTS,S/AS candidate vaccine has been evaluated in multiple Phase I/II studies and shown to have a favourable safety profile and to be well tolerated in both adults and children. Consistent and significant efficacy against *Plasmodium falciparum* infection and disease was observed in the target population of infants and children in a range of age groups and in different malaria transmission settings. The RTS,S/AS01$_E$ malaria vaccine candidate has recently entered Phase III testing. Reaching this important milestone is the culmination of more than 20 years of research and development by GlaxoSmithKline, their partners and collaborators. If the Phase III results confirm the observations made during Phase II testing, the RTS,S/AS01$_E$ vaccine, when broadly implemented and judiciously integrated with other malaria-prevention measures, could have a major public-health impact in sub-Saharan Africa.

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H. Mehlhorn (ed.), *Progress in Parasitology*, Parasitology Research Monographs 2,
DOI 10.1007/978-3-642-21396-0_7,

7.1 Introduction

The RTS,S/AS candidate vaccine is currently the most advanced anti-malarial vaccine in clinical development. Research into the RTS,S/AS vaccine was initiated in 1987 at GlaxoSmithKline (GSK), as part of an ongoing collaboration with the Walter Reed Army Institute of Research (USA). The RTS,S antigen is based on a large segment of the *Plasmodium falciparum* circumsporozoite protein (CSP; Amino Acids 207–395 of the CSP from the NF54 strain of *P. falciparum*) fused to the hepatitis B virus surface protein (S) produced in genetically engineered *Sacharomyces cerevisiae* yeast cells. Immune responses to the RTS,S antigen were optimised by the development of new innovative Adjuvant Systems (AS). This review article provides an overview of the major milestones that the pre-erythrocytic RTS,S/AS vaccine candidate has successfully achieved from conception to ongoing Phase III clinical assessment.

7.1.1 Circumsporozoite Protein: A Valuable Vaccine Candidate Antigen

The critical role for **CSP** as a pre-erythrocytic protective antigen against *P. falciparum* has been demonstrated in pre-clinical studies in animals and humans using sporozoites whose life cycle is stopped at the liver stage (Nussenzweig and Nussenzweig 1989; Doolan and Marinez-Alier 2006; Mueller et al. 2005). In humans, bites from mosquitoes infected with radiation-attenuated *P. falciparum* protected against a subsequent infection with non-irradiated sporozoites (sporozoite challenge model). Protection was specific for *falciparum* species, required repeated immunisation sessions and, in a few volunteers, was shown to last up to 42 weeks (Hoffman et al. 2002). In mice, sterile immunity has also been demonstrated by injection of irradiated or genetically attenuated sporozoites (Nussenzweig and Nussenzweig 1989; Mueller et al. 2005). In these different models, CSP was shown to be immunodominant and protective (Kumar et al. 2006, 2009).

The important role of **antibodies** in protection was first shown in rodent models (Potocnjak et al. 1980). The repeat domain of *P. falciparum* CSP contains a B-cell epitope composed of a four amino acids motif, $(NANP)_n$. Sera from individuals "vaccinated" with radiation-attenuated parasites contain antibodies that bind to the sporozoite surface, and neutralise its infectivity (Sinnis and Nussenzweig 1996). Sera from individuals living in endemic areas of Africa also contain low titres of antibodies to the surface of *P. falciparum* sporozoites as detected by immunofluorescence assays. In general, there is an excellent correlation between the titres measured using immunofluorescence assays and the titres of antibodies to the (NANP)n motif of CSP (Zavala et al. 1985). To date, the only antibodies that consistently neutralise sporozoite infectivity of rodent or human sporozoites are directed to the CSP repeats (Sinnis and Nussenzweig 1996). The NANP repeats were therefore logical targets for the development of *P. falciparum* vaccines.

As the sporozoites injected by mosquitoes travel rapidly to the liver to infect the hepatocytes, a short time is left for the induction of a humoral response and the time that antibodies have to neutralise sporozoites before they enter hepatocytes is limited. Protective mechanisms other than antibody responses to *P. falciparum* sporozoites were thus explored for developing pre-erythrocytic vaccines. There is evidence for a protective role of interferon-γ secreting $CD4^+$ T lymphocytes, and the major epitope that is recognised is the "promiscuous" conserved T-cell epitope at the C-terminus of CSP (Ferreira et al. 1986; Reece et al. 2004).

In order to induce **T-cell recognition**, liver stage antigens need to be processed into short peptides and presented by antigen-presenting cells. Several liver cell types have such an antigen presentation capacity: Kuppfer cells, endothelial cells, dendritic cells, and hepatocytes themselves (Frevert and Nardin 2008). The continuous shedding of CSP off the sporozoite surface upon migration through the liver has been described (Singh et al. 2007). CSP is thus found in the cytoplasm of infected hepatocytes, where it inhibits cellular metabolic pathways, is processed and antigenic peptides derived from the CSP antigen are presented on the cell surface (Singh et al. 2007). Both $CD4^+$ and $CD8^+$ T cells may then recognise these antigenic peptides, which may lead to the release of cytokines such as interferon-γ or expression of other effector functions in the proximity of the infected hepatocytes, resulting in the inhibition of the parasite liver stage development (Sinnis and Nussenzweig 1996).

7.1.2 From the Laboratory to the Clinic Setting

Although several plasmodial antigens were investigated, the major focus was the CSP of *P. falciparum*. Over a decade of research spanning from the mid-1980s to the mid-1990s, nearly a dozen vaccine formulations were tested preclinically and six candidate vaccines were tested in Phase I/II clinical trials with little success (Ballou et al. 1985, 1987; Young et al. 1985, 1987; Hockmeyer et al. 1986; Wirtz et al. 1987; Hollingdale et al. 1987; Fries et al. 1992; Brown et al. 1994; Sherwood et al. 1996; Ballou and Cahill 2007; Vekemans and Ballou 2008).

In the late 1970s and early 1980s, the recombinant hepatitis B vaccine (*Engerix-B*™[1]) was in development. This genetically engineered vaccine is based on the gene encoding the hepatitis B virus surface protein, HBsAg or S. Crucially, the S proteins produced in genetically modified *Saccharomyces cerevisiae* yeast cells spontaneously assemble into virus-like particles (Rutgers et al. 1987). This led to the use of HBsAg as a carrier matrix for *P. falciparum* CSP by fusing the part of the CSP genetic sequence that encodes the B and T-cell epitopes to the sequence of HBsAg and expressing the chimeric gene in *S. cerevisae* (Gordon et al. 1995).

[1]Engerix-B is a trademark of the GlaxoSmithKline group of companies.

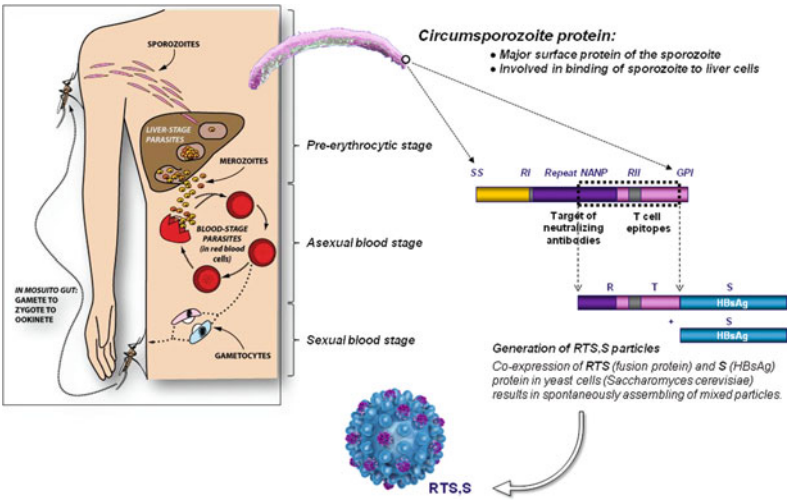

Fig. 7.1 The RTS,S vaccine antigen

The resulting fusion protein assembled into virus-like particles similar to those formed by the unfused viral HBsAg surface protein. The vaccine construct was designed to induce antibody responses against the dominant $(NANP)_n$ B-cell epitope as well as cellular immune responses against several T-cell epitopes identified in the C-terminal non-repetitive region of CSP (Gordon et al. 1995; Good et al. 1988). The vaccine antigen was designated RTS,S to indicate the presence of the CSP repeat region (R), T-cell epitopes (T) fused to the hepatitis B virus surface antigen (S), and assembled into virus-like particles with unfused copies of S antigen (Fig. 7.1).

A number of innovative **Adjuvant Systems** were assessed in extensive pre-clinical studies, in rodents and primates. This work led to the identification of a promising vaccine candidate combining the RTS,S antigen with the adjuvant system $AS02_A$ (proprietary oil-in-water emulsion with MPL® and QS21 immunostimulants) (Garçon et al. 2003). In several clinical trials involving healthy subjects experimentally challenged by the bites of *P. falciparum*-infected mosquitoes, RTS,S/$AS02_A$ consistently conferred full protection against infection in about 40% of the volunteers (Stoute et al. 1997; Kester et al. 2001, 2007, 2008). Table 7.1 provides a summary of the RTS,S/$AS02_A$ vaccine components and subsequent formulations.

7.1.3 Proof of Concept from Adult Clinical Trials in Malaria Endemic Countries

Field evaluation followed the promising results achieved in the **laboratory-based challenge trials**. Safety, immunogenicity and efficacy of RTS,S/$AS02_A$ was

Table 7.1 Formulations of RTS,S/AS

Formulation	Freeze-dried fraction	Liquid fraction				
	RTS,S (μg)		MPL (μg)	QS21 (μg)	Dose volume (mL)	Key milestones
RTS,S/AS02$_A$ (0.5 mL dose)	50	Oil-in-water emulsion	50	50	0.5	Efficacy demonstrated in adults in challenge model (Stoute et al. 1997) and in endemic countries (Bojang et al. 2001)
RTS,S/AS02$_A$ (0.25 mL dose)	25	Oil-in-water emulsion	25	25	0.25	Efficacy against clinical disease in children in Mozambique (Alonso et al. 2004, 2005; Sacarlal et al. 2009)
RTS,S/AS02$_D$	25	Oil-in-water emulsion	25	25	0.5	Efficacy in infants. Paediatric formulation (Macete et al. 2007b; Aponte et al. 2007; Abdulla et al. 2008)
RTS,S/AS01$_B$	50	Liposomes	50	50	0.5	Efficacy in adults in challenge model (Kester et al. 2009) and in endemic countries (Polhemus et al. 2009)
RTS,S/AS01$_E$[a]	25	Liposomes	25	25	0.5	Paediatric formulation. Efficacy against clinical disease in children in Kenya and Tanzania (Bejon et al. 2008)

[a]Formulation selected for Phase III clinical development

confirmed in adult men from The Gambia (Doherty et al. 1999; Bojang et al. 2001), where three doses of RTS,S/AS02$_A$ (administered according to a 0, 1, 5-month schedule) conferred significant protection against infection over a 15-week period (34%; 95% CI: 8–53; $p = 0.014$). Although efficacy appeared to wane during the surveillance period, a booster dose at 19 months demonstrated 47% (95% CI: 4–71; $p = 0.037$) efficacy over a 9-week period, which corresponds to the malaria transmission season in this region. Furthermore, the efficacy conferred by RTS,S/AS02$_A$

did not appear to be specific for the genotype of the parasite strain used to generate the vaccine construct (Allouèche et al. 2003). In this study, long-term safety and persistence of anti-CSP and anti-HBsAg antibodies induced by the RTS,S/AS02$_A$ vaccine candidate were subsequently documented over a 5-year period (Bojang et al. 2009).

As the work with the RTS,S/AS02 vaccine was progressing, additional pre-clinical studies in mice and primates identified a new Adjuvant System, AS01 (liposome suspension, MPL® and QS21), which improved humoral responses to both CSP and HBsAg antigen components of the vaccine candidate, and improved Th1-type cell-mediated immune responses against CSP (Stewart et al. 2006a, b; Mettens et al. 2008). These pre-clinical results were subsequently confirmed in malaria-naïve adults from the USA in whom anti-CSP antibody responses and multifunctional CD4$^+$ T-cell immune responses were found to be superior with RTS,S/AS01$_B$ compared to RTS,S/AS02$_A$. There was also a trend towards greater protection against infection with RTS,S/AS01$_B$ than with RTS,S/AS02$_A$ (50% vs. 32%; $p = 0.11$) following experimental sporozoite challenge (Kester et al. 2009). In a field trial conducted in adults in Kenya, RTS,S/AS01$_B$ induced greater anti-CSP humoral responses and similar efficacy compared to RTS,S/AS02$_A$ (Polhemus et al. 2009).

7.1.4 Assessment of the Vaccine in the Paediatric Population

The encouraging results obtained in studies in adults justified progression to development of the RTS,S/AS candidate vaccine in the paediatric population. This development was undertaken through a **Private/Public partnership** between GSK and the Malaria Vaccine Initiative (MVI) from the Program for Appropriate Technology in Health (PATH), funded by the Bill and Melinda Gates Foundation. Initial Phase I/II trials focussed on age de-escalation in the paediatric populations, dose optimisation, and evaluation of vaccine immunogenicity and safety profiles. Half the adult dose of RTS,S/AS02$_A$ (i.e. 0.25 mL) was shown to be highly immunogenic for both the CSP and S antigens and to have an encouraging safety profile in children aged 1–11 years from The Gambia and Mozambique (Bojang et al. 2005; Macete et al. 2007a).

Proof-of-concept of efficacy in the paediatric population was demonstrated in children aged 1–4 years from Mozambique (Table 7.2). Three doses of RTS, S/AS02$_A$ administered at monthly intervals showed that vaccine efficacy was 30% (95% CI: 11–45; $p = 0.004$) against the first clinical episode and 58% (95% CI: 16–81; $p = 0.019$) against severe malaria over a 6-month surveillance period. Vaccine efficacy was 35% (95% CI: 22–47; $p < 0.001$) and 49% (95% CI: 12–71; $p = 0.02$) over an 18-month surveillance period, against clinical episodes and severe malaria, respectively respectively (Alonso et al. 2004, 2005). Crucially, when considering the total follow-up period of 42 months, the vaccine conferred a protection of 26% against all clinical episodes of

Table 7.2 RTS,S/AS Phase II efficacy results in the paediatric population

Country/ reference	Vaccine formulation	Age subjects	Endpoints	Duration of follow-up post vaccination	Vaccine efficacy (%)	95% CI	*p*-value
Mozambique (Alonso et al. 2004, 2005)	RTS,S/AS02	1–4 years	Clinical disease	6 months	29.9	11.0–44.8	0.004
				18 months	35.3	21.6–46.6	<0.001
			All episodes	6 months	27.4	6.2–43.8	0.014
				18 months	29.8	13.8–42.8	0.001
			Severe disease	6 months	57.7	16.2–80.6	0.019
				18 months	48.6	12.3–71.0	0.020
			Hospitalised malaria	6 months	32.3	1.3–53.9	0.053
				18 months	30.5	4.1–49.9	0.032
			Infection	6 months	45.0	31.4–55.9	<0.001
Tanzania/ Kenya (Bejon et al. 2008)	RTS,S/AS01	5–17 months	Clinical disease	8 months (mean value)	52.9	28.1–69.1	<0.001
Mozambique (Aponte et al. 2007)	RTS,S/AS02	10–18 weeks	Infection	3 months	65.9	42.6–79.8	<0.001
Tanzania (Abdulla et al. 2008)	RTS,S/AS02	6–10 weeks	Infection	6 months	65.2	20.7–84.7	0.012

malaria (95% CI: 12–37; $p \leq 0.001$) and of 38% against severe malaria (95% CI: 3–61; $p = 0.045$) (Sacarlal et al. 2009). Furthermore, at the end of the follow-up period, the prevalence of *P. falciparum* parasites was 34% lower in RTS,S/AS02$_A$ recipients (12% compared to 19% in children in the control group; $p = 0.004$). Over the 42-month follow-up period, RTS,S/AS02$_A$ had an acceptable safety profile, with significantly fewer serious adverse events and a trend towards reduced all-cause mortality compared to recipients of control vaccines. As in the adult trial conducted in The Gambia, the protection conferred by RTS,S/AS02$_A$ was not strain-specific and reduced the genotypic multiplicity of infections by *P. falciparum* (Enosse et al. 2006).

A paediatric version of the RTS,S/AS02$_A$ vaccine candidate, RTS,S/AS02$_D$ (which contains half the RTS,S/AS02$_A$ dose in a volume of 0.5 mL, Table 7.1), was then developed and shown to be equivalent to RTS,S/AS02$_A$ (half dose) in terms of safety and immunogenicity (Macete et al. 2007b). The promising results obtained in field studies of RTS,S/AS02$_A$ in children led to the assessment of the RTS,S/AS02$_D$ vaccine candidate in infants within the Expanded Program for Immunisation (EPI) age range. In infants from Mozambique, staggered administration of RTS,S/AS02$_D$ at 8, 12, and 16 weeks of age and EPI vaccines diphtheria, tetanus, pertussis, and *Haemophilus influenzae* type b vaccine (DTPw/Hib) and oral polio vaccine (OPV) at 10, 14, and 18 weeks of age showed that the safety and tolerability profile of RTS,S/AS02$_D$ was similar to that of hepatitis B control vaccine (Aponte et al. 2007). In this study, vaccine efficacy against *P. falciparum* infection was demonstrated over 3-month's follow up (66%; 95% CI: 43–80; $p < 0.001$).

When co-administered with EPI vaccines (DTPw/Hib and OPV) in infants from Tanzania, low-grade fever and rash were reported more frequently in infants receiving the RTS,S/AS02$_D$ vaccine than in the control group receiving the hepatitis B vaccine, also co-administered with EPI vaccines (Abdulla et al. 2008). However, there were no cases of high-grade fever or rash in recipients of RTS, S/AS02$_D$ and there was no clinically significant difference in the incidence of any other solicited adverse events between the two groups. Remarkably, there was a trend towards fewer all-cause hospitalisations and hospitalisations due to pneumonia in the RTS,S/AS02$_D$ group than in the control group. In this study, vaccine efficacy against *P. falciparum* infection was 65% (95% CI: 21–85; $p = 0.012$) over a 6-month follow-up period. The study also demonstrated the noninferiority of responses against the EPI antigens upon RTS,S/AS02$_D$ vaccine co-administration.

Across all studies conducted in children and infants from malaria-endemic countries, the RTS,S antigen formulated with AS02 was highly immunogenic for anti-CSP and anti-HBsAg antibodies, irrespective of the age of the children or the region where the studies were conducted. Geometric mean titres for anti-CSP antibodies were generally higher in paediatric populations than in adult subjects from endemic regions of Africa.

Following the promising results with the AS01 adjuvant system in adults (see 7.1.3), comparative studies of RTS,S/AS02$_D$ and RTS,S/AS01$_E$ (the paediatric version of RTS,S/AS01$_B$; Table 7.1) were initiated. In children aged 18 months to 4 years in Gabon and in children aged 5–17 months in Ghana, vaccination with RTS,S/AS01$_E$ demonstrated better anti-CSP humoral responses than vaccination with RTS,S/AS02$_D$ (Lell et al. 2009; Owusu-Agyei et al. 2009). Both RTS,S/AS02$_D$ and RTS,S/AS01$_E$ vaccine candidates were well tolerated and had a favourable safety profile. Vaccine efficacy of RTS,S/AS01$_E$ was then assessed in children aged 5–17 months in Tanzania and Kenya over an average 8-month follow-up period and was shown to be 53% (95% CI: 28, 69; $p < 0.001$) against malaria disease (Bejon et al. 2008). Furthermore, there was a trend towards fewer serious adverse events leading to hospitalisation and fewer non-malaria morbidity among subjects in the RTS,S/AS01$_E$ group than in the control group.

7.1.5 Onwards to Phase III Testing

The encouraging Phase II efficacy, immunogenicity, and safety evaluation of RTS, S/AS01$_E$ in the paediatric population supported progression of the candidate vaccine to Phase III development.

The pivotal Phase III study of RTS,S/AS01$_E$ was initiated in May 2009 (ClinicalTrials.gov). This is a multicentre, double-blind, randomised controlled trial conducted in 11 centres in seven African countries, thereby representing diverse malaria transmission settings (Fig. 7.2). Children in two age categories, 6–12 weeks and 5–17 months at first vaccination, will participate in the study and will be followed for a total of 32 months. In February 2011, enrolment was

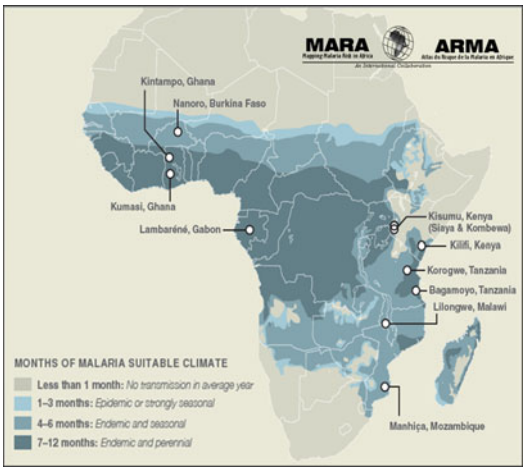

Fig. 7.2 Pivotal Phase III trial of RTS,S/AS01$_E$. The map used in this figure was adapted from the "Duration of Malaria Transmission Season" map (2001) published by MARA (Mapping Malaria Risk in Africa; http://www.mara.org.za/)

completed with 15460 children included in the study. The primary objective of the study is the evaluation of vaccine safety and its efficacy against clinical malaria disease. Secondary objectives will assess, among other endpoints of public health relevance, efficacy against severe malaria disease, severe anaemia, malaria hospitalisations, fatal malaria and all-cause mortality. Analysis by site will evaluate efficacy under different conditions of malaria transmission. Evolution of vaccine efficacy will be assessed during a 32-month follow-up period, and the potential benefit of a booster dose will be evaluated in a subgroup of subjects. Finally, the impact of RTS,S/AS01$_E$ co-administration with the current EPI vaccines and the vaccines that should be included in EPI programmes shortly, such as the rotavirus vaccine and the pneumococcal conjugate vaccine, will additionally be evaluated in Phase III clinical trials.

7.2 Conclusions

A collective effort by the malaria vaccine scientific community, coordinated by the World Health Organisation (WHO) Institute for Vaccine Research (IVR), led to the publication by WHO of a "Malaria Vaccine Technology Roadmap" in 2006 (Malaria Vaccine Technology Roadmap 2006). The document states that the communities first landmark goal is to: "by 2015, develop and license a first-generation malaria vaccine that has a protective efficacy of more than 50% against severe malaria and death and lasts longer than 1 year".

In Phase II evaluation of the RTS,S/AS vaccine, consistent and significant efficacy has been observed against *P. falciparum* infection, clinical episodes of malaria and severe malaria in different transmission settings and in the different age groups evaluated. In one study, the clinical benefit conferred by the vaccine has been demonstrated to extend up to 42 months following vaccination. Finally, it was

shown that the RTS,S/AS vaccine candidate can be co-administered with existing EPI vaccines.

In conclusion, major milestones have been achieved in the development of the RTS,S/AS vaccine over the last two decades. Provided that the results of the RTS,S/AS01$_E$ Phase III trial confirm the promising Phase II data, and that the challenges in vaccine registration, procurement and implementation are met, the ambitious goal set by the WHO in 2006 seems therefore achievable.

Acknowledgments The results reported in this review are the fruit of many years of very hard work by literally hundreds of highly dedicated individuals in research institutions and in organisations involved in the development of the RTS,S/AS malaria vaccine, in Europe, the USA, and Africa.

The authors wish in particular to acknowledge the many partner organisations that contributed to this work, including the Southern collaborating institutions: the Institut de Recherche en Science de la Santé, Nanoro, Burkina, the Kumasi Centre for Collaborative Research, Ghana, the School of Medical Sciences Kumasi, Ghana, the Kintampo Health Research Centre, Ghana, the Albert Schweitzer Hospital, Gabon, the Kenya Medical Research Institute, Kilifi, Kenya, the Wellcome Collaborative Research Programme, Kilifi, Kenya, the Kenya Medical Research Institute, Kisumu, Kenya, the University of North Carolina Project, Lilongwe, Malawi, the Centro de Investigação em Saude de Manhiça, Mozambique, the Ifakara Health Institute, Tanzania and the National Institute of Medical Research, Tanzania; and the Northern collaborating institutions: the Prince Leopold Institute of Tropical Medicine, Belgium, the University of Copenhagen, Denmark, the University of Tuebingen, Germany, the Bernhard Nocht Institute, Germany, the University of Barcelona, Spain, the Swiss Tropical Institute, Switzerland, the London School of Hygiene & Tropical Medicine, UK, the University of North Carolina at Chapel Hill, USA, the Walter Reed Army Institute of Research, USA, and the Center for Disease Control and Prevention, USA; the PATH Malaria Vaccine Initiative (PATH-MVI), the Malaria Clinical Trial Alliance (MCTA), and the Clinical Trial Partnership Committee (CTPC) which is a network of African-based research centres and Northern partners, PATH-MVI and GSK. The CTPC oversees the implementation of the RTS,S/AS clinical development plan. Most of all, the authors want to thank the thousands of volunteers who participated in the RTS,S/AS clinical trials, as well as their families and communities, for their trust and cooperation.

The authors would also like to acknowledge the significant contribution of Dr. Julia Donnelly for expert editorial assistance and Dr. Julie Harriague (4Clinics) for writing assistance.

Conflicts of Interest: J. Cohen, J. Vekemans, A. Leach and L. Schuerman are employees of GlaxoSmithKline Biologicals. A. Leach, J. Vekemans, J. Cohen, L. Schuerman and S. Benns hold shares in GlaxoSmithKline. J. Cohen is listed as an inventor of patented malaria vaccines, including RTS,S.

Comment of editor: In the mean time the clinical trial was successfully finished!

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