**Supplementary Materials**

This appendix has been provided by the authors to provide readers with additional information.

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# Authors and Affiliations

The paper was submitted by the RTS,S Clinical Trials Partnership.

# Supplementary Methods

This supplement provides additional information about the study population and the methods employed in the conduct of the trial.

## Ethical considerations

This phase III, double-blind (observer-blind), individually randomized, controlled multicentre trial was performed in 11 sites across sub-Saharan Africa. The study design and rationale for selection of endpoints have been described previously.[1]Overall this study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki, the principles of Good Clinical Practice [2] and with the local rules and regulations of each country. The study was monitored by the sponsor, GlaxoSmithKline (GSK) Biologicals SA (GSK monitors or outsourced monitors from Quintiles [Quintiles, Centurion, South Africa] contracted by GSK Biologicals SA), and overseen by a formally constituted Independent Data Monitoring Committee (IDMC), that reviewed, among other information, unblinded comprehensive safety data every three months to authorize study continuation. The IDMC conferred before the initiation of the study and had three-monthly teleconferences and one annual meeting thereafter. A Local Safety Monitor, who was an experienced clinician not taking part in the study, was available at each study site to support the clinical investigators and to act as a link between the investigators and the IDMC. The study protocol and amendments, consent forms, and other information that required pre-approval were reviewed and approved by a national, regional, or research centre ethics committee (EC) or institutional review board (IRB) in accord with local requirements. A list of all EC/IRBs is provided in Table S1a.

## Roles of investigators and sponsor

The study was sponsored by GSK Biologicals SA, the vaccine developer and manufacturer, and funded by both GSK Biologicals SA and the PATH Malaria Vaccine Initiative (MVI).The study was designed by the Clinical Trials Partnership Committee (CTPC), consisting of representatives of all contributing research centres, study sponsor and study funders (as detailed in [1]). All authors were involved in data collection. All data were analysed following a pre-defined analysis plan. The CTPC had full access to the study data, made the decision to publish the manuscript in its current form, and supervised the writing of the manuscript.

## Study sites and affiliated partners

The study was conducted in 11 study sites located in seven countries in sub-Saharan Africa together with their partner institutions. The study sites represent the range of malaria transmission seen across sub-Saharan Africa (Figure 1 in the paper). The list of study sites and their partners is provided in Table S1b.

## Screening and informed consent

Two age categories of children were eligible for inclusion in the trial. One age category comprised infants who were 6-12 weeks of age (inclusive) at the time of first vaccination and who had not previously received a dose of vaccine against diphtheria, tetanus, pertussis or *Haemophilus influenzae* type b. The other age category comprised children 5-17 months of age (inclusive) at the time of first vaccination. Screening procedures included a review of a child’s medical history, a physical examination and a blood test for assessment of haemoglobin concentration. The main exclusion criteria were: moderate or severe illness at the time of enrolment, a major congenital defect, malnutrition requiring hospitalization, severe anaemia - defined as a haemoglobin concentration < 5.0 g/dL or a haemoglobin concentration < 8.0 g/dL associated with clinical signs of heart failure or severe respiratory distress, or a past history of a neurological disorder or of an atypical febrile seizure. A past history of a simple febrile seizure was not an exclusion criterion. Children with active HIV disease of Stage III or Stage IV severity, as defined by the World Health Organization, at the time of screening were excluded.[3] A previous history of active Stage III or Stage IV HIV disease was not an exclusion criterion. Routine testing for HIV was not done in this study. HIV positivity was reported on the general medical history taken at screening or identified by morbidity surveillance during the study. The decision to report a new HIV infection depended on the investigators judgment as to whether it met the criteria for a serious adverse event. Likewise, it was at the investigators discretion whether to perform antibody or PCR confirmatory testing. Voluntary counselling and testing, highly active anti-retroviral therapy (HAART) and prevention of mother to child transmission (PMCT) were available at all study sites according to national policies.

Prior to enrolment, study teams conducted a series of information activities. Study teams held discussion meetings with the administrative leaders and/or community leaders. They described the outline of the proposed study, paying particular attention to study procedures, including screening of children, immunization, blood collection, follow-up and their associated risks. Following community meetings, and a positive recommendation from community leaders, the parent(s)/guardian(s) of children in the eligible age categories were approached. The need for a vaccine against malaria was discussed and the objectives of the study were explained. The study procedures were described carefully, including the blinding of study treatment, the immunization and blood collection. Parent(s)/guardian(s) interested in enrolling their child into the study were invited to the screening visit. At the screening visit, the site investigator or his/her designate described the protocol to the parent(s)/guardian(s) face to face or the informed consent information was presented to groups at an initial information session. Information was provided in both an oral and a written form in a language fully comprehensible to the child’s family. Each child’s family had the opportunity to inquire about details of the study and ask any questions individually in a private place. Formal informed consent was obtained from each child’s parent(s) or guardian(s) prior to the performance of any study-specific procedures. Literate parent(s)/guardians willing to let their child enter into the study were asked to sign and date the informed consent form (ICF). If the parents or guardians were illiterate, the study and the ICF were explained point by point in the presence of an impartial witness. The impartial witness could be a friend or family member accompanying the parents or any other literate person independent from the study team. Parent(s)/guardian(s) confirmed their consent for their child to take part in the study by marking the ICF with their thumbprint and the impartial witness personally signed and dated the ICF.

During the course of the study, the protocol was amended to extend the follow-up of study participants. The study includes a primary phase of approximately 32 months for each subject (+ one month of screening) and an extension phase that continued the follow-up until end December 2013. Parents/guardians of subjects who had received at least one dose of study vaccine or comparator vaccine in the primary study phase and whose first extension visit took place before (and including) 30 September 2013 were invited to enrol their child into the extension phase. Freely given informed consent was obtained from subjects’ parent(s)/guardian(s) prior to participation in the extension.

## Randomization and blinding

After verification of eligibility criteria, and prior to first vaccination, a unique treatment number was assigned to each participating child. Participating children from each age category were randomized into one of three study groups according to a 1:1:1 ratio (R3R, R3C or C3C) using a randomization algorithm with SAS version 9.1. Randomization was stratified for age category using study site as a minimization factor, ensuring balanced treatment allocation within each study site. All children’s parent(s)/guardian(s) were provided with a study identification card with a photo of their child, the child’s name and a unique subject number. All data were collected using remote data entry and electronic case report forms.

Data were collected in a double-blinded (observer-blind) manner; the vaccinated children and their parent(s)/guardian(s) as well as those responsible for the evaluation of study endpoints were unaware of whether RTS,S/AS01 or a comparator vaccine had been administered to a particular child. The vaccines used in this study were of different appearance. The content of the syringe was, therefore, masked with an opaque tape to ensure that parent(s)/guardian(s) were blinded. The only members of study staff who knew of the vaccine assignment were those responsible for preparation and administration of vaccines; these staff played no other role in the study except screening or collection of biologic specimens.

## Study vaccines

Each child received a primary schedule of three doses of either the candidate malaria vaccine RTS,S/AS01 or a comparator vaccine. In the 5-17 months age category, the comparator vaccine for the primary series was a rabies vaccine VeroRab™ (Sanofi-Pasteur) and in the 6-12 weeks age category the comparator vaccine was a meningococcal C conjugate vaccine Menjugate™ (Novartis). Participants in both age categories received a booster dose of either RTS,S/AS01 (in the R3R group) or Menjugate™ (R3C and C3C groups) 18 months after the third dose of the primary schedule. Vaccines for the primary series were administered intramuscularly into the left deltoid of children in the 5-17 months age category and into the left anterolateral thigh of infants in the 6-12 weeks age category. Booster doses were administered into the left deltoid for all participants. The choice of comparator vaccines was guided by the principles of benefit to the control group without compromising the evaluation of clinical study endpoints. Infants enrolled in the 6-12 weeks age category received the RTS,S/AS01 or comparator vaccine at the same time as DTPwHepB/Hib pentavalent vaccine (Tritanrix™ HepB/Hib, GSK group of companies), which was administered into the right anterolateral thigh, and an oral polio vaccine containing serotypes 1, 2 and 3 (Polio Sabin™, GSK group of companies).

The RTS,S/AS01 candidate vaccine has been developed and manufactured by GSK Vaccines and is designed to protect against *Plasmodium falciparum* malaria. Manufacturing and quality control are performed in line with current Good Manufacturing Practices. No quality issues in the vaccines used in this study were recorded. “RTS,S” comprises the carboxyl terminal portion (amino acids 207 to 395) of the circumsporozoite protein from the NF54 strain of *P. falciparum* fused to the hepatitis B surface antigen, co-expressed in yeast with non-fused hepatitis B surface antigen."AS01" describes the Adjuvant System comprising liposomes, MPL (3-O-desacyl-4’-monophosphoryl lipid A) and QS-21[[1]](#footnote-2) (a triterpene glycoside purified from the bark of *Quillaja saponaria*). Each dose of reconstituted RTS,S/AS01 (0.5 mL) contains approximately 25 μg of antigen, 25 μg of MPL and 25 μg of QS-21 with liposomes.[4]

Sanofi-Pasteur’s chromatographically purified Vero cell culture rabies vaccine VeroRab™ is based on the inactivated Wistar Rabies PM/W138 1503-3M strain and it is given in a ≥2.5 IU/0.5 mL dose.

One dose (0.5 mL) of Novartis’s meningococcal C conjugate vaccine contains 10 μg *Neisseria meningitidis* (strain C11) group C oligosaccharide conjugated to 12.5‑25 μg *Corynebacterium diptheriae* CRM197 protein adsorbed on aluminum hydroxide (1.0 mg). The excipients of the reconstituted vaccine include mannitol, sodium phosphate monobasic monohydrate, sodium phosphate dibasic heptahydrate, sodium chloride and water for injections.

GSK Vaccines’ DTPwHepB/Hib vaccine is prepared by reconstitution of the Hiberix™ pellet with the Tritanrix™ HepB suspension. Each 0.5 mL dose contains not less than 30 IU of adsorbed diphtheria toxoid, not less than 60 IU of adsorbed tetanus toxoid, not less than 4 IU of whole cell pertussis, 10 μg of recombinant hepatitis B antigen (HBsAg) protein and 10 μg of purified capsular polyribosyl ribitol phosphate covalently bound to approximately 30 μg tetanus toxoid. Tritanrix™ HepB also contains 2-phenoxyethanol, polysorbate 20, sodium chloride, thiomersal and water for injection. Hiberix™ also contains lactose.

The oral polio vaccine obtained from GSK Vaccines is a stabilized suspension of types 1, 2 and 3 live attenuated polioviruses (Sabin strains): Type 1 (strain LSc, 2ab), Type 2 (strain P 712 ch, 2ab), Type 3 (strain Leon 12a, 1b). The excipients comprise magnesium chloride, L-arginine, polysorbate 80, neomycin sulphate (residual), polymyxin B sulphate (residual) and purified water.

Children were observed closely for at least 30 minutes after vaccination, with appropriate medical treatment and equipment readily available in case of an anaphylactic reaction. A study clinician accredited in paediatric resuscitation was available at all vaccination sessions.

## Bednets and indoor residual spraying

The research team ensured that insecticide treated bednet use was optimized in each study population. At study start in two study sites (Kilifi, Kenya and Bagamoyo, Tanzania) this was achieved through close collaboration with the respective National Malaria Control Programmes. In the other sites, impregnated bednets were distributed by the study teams to all children who underwent screening, regardless of whether they were eligible for the study. During the course of the study three sites (Agogo, Siaya and Lilongwe) replaced any damaged nets upon the parents’ request. Other centres relied upon the National Malaria Control Programme for the ongoing replacement of bednets.

Data were collected on malaria control measures used by the participants’ families during the period of surveillance. Bednet usage and indoor residual spraying were documented 12 months and 29 months after the third vaccine dose had been given and also one month before the end of the extension phase. Study children’s parents were asked if their house had been sprayed with a residual insecticide and, if so, when this was done. Then they were asked if their child sleeps under a bednet. During a home visit, a field worker inspected the child’s bednet and the integrity of the net was recorded as follows: 1- no bednet; 2- impregnated bednet with no hole large enough to admit three fingers; 3- impregnated bednet with at least one hole large enough to admit three fingers; 4- untreated bednet with no hole large enough to admit three fingers; 5- untreated bednet with at least one hole large enough to admit three fingers.

## Safety assessment

During the study, investigators or their designates were responsible for documenting and reporting events meeting the criteria and definition of an adverse event (AE) or a serious adverse event (SAE). Parents/guardians of children participating in the study were requested to contact study personnel immediately if their child showed any signs or symptoms they perceived as serious.

An adverse event was defined as any untoward medical occurrence in a child participating in the study temporally associated with vaccination whether or not it was considered to be related to the vaccine. An AE could, therefore, be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with vaccination.

For the purpose of this study, a SAE was defined as any untoward medical occurrence that resulted in death, was life-threatening, required hospitalization or prolongation of existing hospitalization, resulted in disability/incapacity, or a seizure that occurred within 30 days of vaccination. Abnormal laboratory findings that were judged by the assessing clinician to be clinically significant were recorded as an SAE if they met the criteria for an SAE as defined above.

Seizures occurring within 30 days of vaccination and immune-mediated disorders occurring at any time during the study were reported as SAEs in order to ensure availability of full case narrative descriptions.[1] Data on seizures occurring within seven days following a dose of the primary vaccination series were collected and analysed according to the Brighton Collaboration guidelines [5] and have been published previously.[6,7]

Because paediatric auto-immune diseases are rare and may be underestimated in sub-Saharan Africa, training material on paediatric auto-immune disease presentation and diagnosis was provided by the study sponsor. A specific, standardized clinical data collection questionnaire was generated. Collaborations with reference laboratories in South Africa were initiated so that serum samples or histopathologic specimens could be sent to South Africa for analyses not available locally.

Diagnosis of all adverse events, including the diagnosis of meningitis, was based on all available clinical evidence and was not bound by stringent laboratory or diagnostic criteria. Efforts have been made both prospectively and retrospectively to confirm a diagnosis of meningitis on cerebrospinal fluid (CSF) samples whenever available, using biochemical, microbiologic and molecular testing as described in section 2.12 below. The IDMC also reviewed unblinded safety reports containing specific sections on seizures and meningitis.

In addition, the case histories of all participants with reported meningitis or other central nervous system (CNS) infections or inflammation were reviewed by two independent experts. Based on their judgement the experts classified the cases as definite meningitis or not meningitis; in case of no clear clinical picture and/or laboratory results interpretation the case was labelled undetermined.

Solicited AEs were reported for the period up to seven days after vaccination (day of vaccination and six subsequent days) following each vaccine dose for the first 200 infants enrolled at each study site. Local AEs solicited were: pain at the injection site; swelling at the injection site and redness at the injection site. Solicited general AEs were: drowsiness, fever; irritability/fussiness and loss of appetite. Grading for solicited AEs are presenting in Table S33. All unsolicited AEs were reported for 30 days following each vaccine dose for the first 200 infants enrolled at each study site.

SAEs were collected for all participating children throughout the study period, from the time of parental consent. At every visit/contact, information was sought on the occurrence of AEs/SAEs. SAEs were identified by surveillance at health facilities in the study area and through monthly home visits. All AEs that were observed directly or that were observed by a clinical collaborator, those that were identified through surveillance at health facilities in the study area or those reported by the child’s parent/guardian spontaneously or in response to a direct question were evaluated. Assessments were made of the maximum intensity of all unsolicited AEs and SAEs during the period of the event. This assessment was based on the attending clinician’s medical judgment. A grade was assigned to all adverse events as follows; grade 1 (mild) - an AE which was easily tolerated by the child, causing minimal discomfort and not interfering with everyday activities; grade 2 (moderate) - an AE which was sufficiently discomforting to interfere with normal everyday activities and grade 3 (severe) - an AE which prevented normal, everyday activities. SAEs were coded according to the MedDRA (Medical Dictionary for Drug Regulatory Activities). Non-malaria SAEs were defined as those which excluded the MedDRA terms “*Plasmodium falciparum* infection”, “Malaria” and “Cerebral malaria”.

Verbal autopsies were carried out on all children who died outside a health facility using a questionnaire based on the International Network for the Demographic Evaluation of Populations and Their Health in Developing Countries (INDEPTH) standard questionnaire, adapted to belocally appropriate.[8] To support the timely reporting of SAEs, diagnoses were made according to the usual processes of each study site.

At the end of the extension phase, all deaths were reviewed using all available information including SAE forms, verbal autopsy forms and information on meningitis cases provided by a panel composed of three investigators who are experienced verbal autopsy reviewers. Each death was reviewed by each of the three reviewers independently. They recorded 1) the disease or condition directly leading to death, 2) any morbid conditions leading to the condition that directly caused death and 3) any other significant conditions contributing to the death, but not related to the disease or condition causing it. Final diagnoses were based on the reviewer’s medical/clinical judgment and were coded according to the ICD10 code at the three digit level. At the end of the independent review by the three panel reviewers, all records of individual reviewers were examined centrally by the Clinical Research and Development Lead (CRDL) and the Lead CRDL at GSK Vaccines. If a minimum of two reviewers were in agreement, a cause of death was ascribed. If there was no agreement between the three reviewers, a consensus meeting was held where an agreement was reached wherever possible. If the joint panel was unable to reach a consensus the cause of death was recorded as unknown (coded R99).

## Surveillance for clinical and severe malaria episodes

During the informed consent process, parents were asked to bring their child to a study health facility as soon as possible if their child fell sick during the study. Malaria was captured by passive case detection. Passive case detection (PCD) is the detection of malaria disease by self-presentation to health facility in the study area. All participating children who presented to a health facility in the study area were evaluated as potential cases of malaria using a standardised algorithm. All parents were asked whether the child had had a fever within the previous 24 hours and all children had their temperature measured*.* A blood sample was taken for testing for malaria parasites in all children who had had a history of fever during the previous 24 hours or who had a measured axillary temperature ≥ 37.5°C at the time of presentation.

Children who needed inpatient treatment were provided transport to a hospital participating in the study. All participating children who presented for admission were evaluated as a potential case of severe malaria following a predefined algorithm (Table S2). Methods for detection and management of severe malaria in children enrolled in the trial have been described in detail by Vekemans et al.[9] During any hospitalization, the child’s course was monitored to capture the clinical signs and blood parameters indicative of progression to severe malaria. If a child’s condition deteriorated following admission, additional investigations were performed.

Treatment of malaria was conducted in accordance with national guidelines. Overall, 99% of children and young infants who presented with confirmed malaria to study clinics received treatment with artemisinin combination therapy (ACT) (Figure S3). In eight of the 11 study sites, the first line treatment for uncomplicated malaria was artemether-lumefantrine whilst in the three other sites (Agogo and Kintampo, Ghana; Nanoro, Burkina Faso), it was artesunate-amodiaquine. The study protocol specified that children admitted to hospital with severe malaria would receive intravenous quinine. During the course of the study, information on the superiority of artesunate over quinine for the treatment of severe malaria became available and as this change in treatment was introduced at country level, artemisinin preparations were used in preference to quinine, [10].

## Chest radiographs

Chest radiographs were obtained as part of the standardized evaluation of study participants brought to a healthcare facility with tachypnea, lower chest wall indrawing, abnormally deep breathing, or if a study clinician considered this to be an appropriate investigation.[9] A digital radiography system was provided to each study site to facilitate radiological assessment of study participants. The radiographers and the physicians who read the images for the study endpoints received standardized technical training by the manufacturer of the radiography equipment and training on interpretation of chest radiograph images was provided by expert radiologists and physicists. To ensure a robust and verifiable data base of radiographs, quality control systems that included local on-site training, development of quality manuals, quality control checks, on-site radiology committees and external audits were implemented. Digital images were anonymized and sent to a central repository at GSK Vaccines via a satellite internet connection.

For the purpose of endpoints assessment, and to ensure accurate diagnosis of pneumonia, a process developed by WHO [11] was followed. Each radiograph was read independently by a clinician attached to the centre where the radiograph was taken, and by an external radiologist. GSK Vaccines reviewed all readings made by the centres and by the external radiologists and any images with discordant readings were sent to another panel of radiologists for a final reading. The reporting of pneumonia as a SAE was made based on clinicians’ judgment and independent of this protocol-specific assessment. Clinicians and external radiologists were trained in chest radiograph interpretation according to WHO guidelines.[11] Physician /radiologist who read the x-ray image had to pass by 80% the WebAims test before reading images for endpoint assessment.

## Anthropometry

Length/height, weight and mid-upper arm circumference were measured at screening, one month, 18 months and 30 months after the third dose of vaccine in the primary study phase, at 42 months post third dose and at the last visit of the extension phase. Anthropometry was also measured during inpatient admissions. The methodologies used for anthropometry were adapted from Cogill.[12]

## Laboratory analyses

The development of standardized laboratory methods and quality control processes for this study have been described fully in a separate publication [13] and are only summarized briefly here.

* ***P. falciparum* counts by blood smear**

All slides were read independently by two trained microscopists. A third independent microscopist read the slide if any of the following discrepancies between the first two readings occurred: (1) a positive reading by one microscopist and a negative reading by the other; (2) both microscopists recorded a parasitemia >400 parasites/μL but the higher count divided by the lower count was >2; (3) at least one microscopist recorded a parasitemia ≤400 parasites/μL but the higher reading was more than 10 times the lower reading. If the initial two readings gave concordant results, the final parasite density was considered to be the geometric mean of these two readings. If the readings were discordant, then the following principles were applied: (1) where one reading was positive and the other negative, the majority decision obtained following the reading by the third microscopist was adopted and, when the slide was considered positive, the parasite density was recorded as the geometric mean of the two positive results; (2) when all three readings were positive, the final result was the geometric mean of the two closest readings (on a log scale). As a quality measure, agreement between the two microscopists was calculated by means of the Kappa statistic. Internal QC was performed on one negative and one positive slide for each batch of stain. The External QA process for slide reading comprised species identification and parasite quantification. Three assessments per year were carried out, including 20 samples per microscopist. Microscopists who were below the level defined as competent were considered to be 'in training' and were not allowed to read study slides until they were retrained and re-assessed.

* ***Haematology and biochemistry***

Automated biochemical and haematological methods were used. All biochemistry automated analysers were enrolled initially with International External Quality Assessment (EQA) but later switched to the programme run by the Royal College of Pathologists of Australia, because the latter was more appropriate for the study requirements at the time. All haematology automated analysers were enrolled in EQA. Each laboratory had to demonstrate method qualification for biochemistry and haematology, including analysis of repeatability, reproducibility, linearity, QC stability and accuracy between main and back-up analysers. Data were sent to GSK Vaccines for analysis and feedback was provided to laboratories. Daily internal QC was performed at each laboratory, and external quality control was performed monthly for biochemistry and haematology samples.

* ***Microbiology***

Standard microbiology methods for blood and CSF culture were followed using automated BactecTM incubators and paediatric bottles (Bactec BD Diagnostic Systems, USA). Positive cultures were sub-cultured using standard methods.[14, 15] For the purpose of study analysis, as opposed to clinical care, results were classified by standardised case definitions based on an established methodology.[16] A blood culture was considered positive if a definite pathogen was isolated (e.g. *Streptococcus pneumoniae*, *S. agalactiae*, *S. pyogenes*, *H. influenzae*, Salmonella species) or if a bacterium that could be either a pathogen or a contaminant was isolated within 48 hours of incubation (e.g. *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis*). A blood culture was considered to be contaminated if a known contaminant was isolated or if a bacterium that could be either a pathogen or a contaminant was isolated after 48 hours of incubation.[16]

CSF was examined by Gram stain and a white cell count was performed using a haemocytometer. Direct agglutination methods using commercial kits (Remel Wellcogen Bacterial Meningitis Antigen Latex Kit or BIO-RAD Pastorex Meningitis Kit) were used for early detection in CSF of specific organisms like *S. pneumoniae,* group B streptococci*, H. influenzae* type b*, E. coli* and *Neisseria meningitidis* in CSF*.* In parallel, CSF was inoculated directly onto recommended culture media and into the same bottles used for blood culture in automated incubators to allow for bacterial growth, identification and antimicrobial sensitivity testing using the disk diffusion method.

For the assessment of protocol endpoints, bacterial meningitis was defined as the presence of a CSF white cell count of ≥50 x 106/L, a positive CSF culture of compatible organisms or a positive CSF latex agglutination test for either *H. influenzae* type b (Hib), *N. meningitidis* or *S. pneumoniae*.[9, 17] The reporting of a meningitis case as an SAE was independent of this definition. SAE diagnoses were made by the study clinicians using clinical judgment based on the clinical and laboratory evidence available. Microbiology quality assessment included evaluation of microscopy, culture, identification and antimicrobial susceptibility testing. Each laboratory received six samples (with at least two meningeal and two enteric organisms) three times per year, and the criteria of acceptability were defined by the National Institute of Communicable Disease (NICD, South Africa). Internal quality control was performed using American Type Culture Collection control strains for species identification every week, when a new batch of reagent was received or when discordant results were obtained. The contamination rate of the clinical specimens was evaluated monthly by internal assessment. Continuous assessment allowed re-training programmes for both clinical and laboratory staff and more intense quality evaluation when there was a high contamination rate.

## Immunological assessment

During the primary study phase, anti-circumsporozoite (anti-CS) antibody titres were measured in the first 200 participants enrolled at each study site in each age category. During the extension phase, blood samples for the assessment of anti-CS response were collected in the first 200 participants in each age category in three study sites: Siaya, Agogo and Lilongwe.

Antibodies specific for the circumsporozoite protein tandem repeat epitope were assessed by a standard, validated ELISA with plates adsorbed with the recombinant antigen R32LR that contains the sequence [NVDP(NANP)15] 2LR as described previously.[18] Briefly, R32LR protein was coated onto a 96-well polystyrene plate. Serial dilutions of serum were added to the 96-well plate and, after incubation, the plates were washed and horseradish peroxidase conjugated polyclonal rabbit anti-human IgG was added. After a final washing step, a colour reaction was developed with 3, 3',5,5' tetramethylbenzidine and the plates were read in an ELISA reader. Antibody concentrations were calculated from a standard curve with the software SoftMax® Pro (using a four parameters equation) and expressed as EU/mL. Anti-CS antibodies were measured at the CEVAC Laboratory, University of Ghent, Belgium. The cut-off for the anti-CS ELISA was 0.5 EU/mL. Serum samples with a titre below the cut-off value were given a value of 0.25 EU/mL for the purpose of calculation of geometric mean titres.

## Data collection and data management

At each study site, data were remotely entered on electronic case report forms and transferred to GSK Vaccines for data management. External monitors reviewed medical records, sample storage, and laboratory procedures to ensure data integrity.

## Contribution to the per-protocol analyses

To be included in the per-protocol analysis of efficacy, participants enrolled in each age category must have received three doses of RTS,S/AS01 or comparator vaccine according to protocol procedures within specified intervals, and contributed to the time at risk in the follow-up period starting 14 days post dose-3. Participants unblinded by the safety department were also excluded from the per-protocol population for efficacy. In addition, participants in the 6-12 weeks age category must have received three doses of co-administered vaccine (DTPwHepB/Hib and OPV).

* Per-protocol population [M2.5-M32/SE]: N = number of subjects in the per-protocol population (as above) who received the primary schedule according to protocol.
* Per-protocol population [M21-M32/SE]: N = number of subjects in the per-protocol population [M2.5-M32/SE] who received the booster dose according to protocol.

To be included in the per-protocol analysis of immunogenicity, participants must have received all vaccinations according to protocol procedures. Subjects must also have followed protocol defined intervals for vaccinations and blood sampling schedules. Participants with protocol deviations in terms of administration of concomitant vaccinations (in the 6-12 weeks age category), screening procedures or participants unblinded by the safety department or investigators were excluded from the per-protocol analysis of immunogenicity.

## Statistical methods for the analysis of efficacy at Month 32 and at the end of the extension

* ***Presentation of results by transmission intensity***

The incidence of clinical malaria meeting the secondary case definition (a measured or reported fever within the previous 24h and a parasite density >0 parasites per cubic millimetre) in infants in the control group measured over 12 months of follow-up was used to categorize malaria transmission across study sites. This measure was used because it most closely reflects force of infection and is less influenced by acquired immunity that might reduce the incidence of clinical malaria in older children. For all tables and figures, study sites are presented from the lowest to the highest incidence of clinical malaria.

* ***Vaccine efficacy against clinical malaria***

Vaccine efficacy against all episodes of clinical malaria was estimated as 1-IR where IR is the incidence ratio (total number of events/follow-up time in the RTS,S/AS01 group over the total number of events/follow-up time in the control group) calculated by negative binomial regression, allowing for interdependence between episodes within the same subject (mixed model with over-dispersion parameter estimated from the random effect) and presented together with 95% confidence interval (CI) and p-values calculated from this model. The data were structured so that each subject in the analysis has one record with follow-up time and the number of episodes observed. Then we fitted a negative binomial model by estimating the over-dispersion parameter as a random effect on the subject. As a result, we model between subject variation, and individual subjects are not forced to an overall over-dispersion parameter. VE estimates were unadjusted for covariates. Fourteen days following an episode which met the case definition under evaluation were subtracted from the follow-up time. Results are presented per site and overall. Overall estimates were adjusted for study site as a fixed effect, whereas site estimates were unadjusted for covariates. The p-value for the interaction term between site and group allocation was calculated.

* ***Vaccine efficacy against severe malaria, incident anaemia, malaria hospitalisation, fatal malaria and against other serious illnesses***

The incidence of severe malaria, malaria anaemia, malaria hospitalisation, fatal malaria, sepsis, hospitalised pneumonia, all-cause hospitalisation, all-cause mortality and blood transfusions in children in each study group was determined. VE was estimated as 1-RR where RR is the risk ratio (proportion of participants reporting the event in the RTS,S/AS01 group over the proportion in controls) over the entire follow-up period, and presented together with 95% CIs and p-values. Vaccine efficacy estimates were unadjusted for covariates.

* ***Vaccine efficacy against prevalent parasitaemia and prevalent anemia***

VE against prevalent endpoints (parasitemia, moderate and severe anaemia) was estimated as 1-RR where RR is the risk ratio (proportion of participants reporting events in the RTS,S/AS01 group over the proportion in controls) and presented together with 95% CIs and p-values. VE estimates were unadjusted for covariates.

* ***Vaccine impact***

The number of cases of clinical malaria, severe malaria, malaria hospitalisations, fatal malaria, all-cause hospitalisation, all-cause mortality, severe anaemia and blood transfusions averted overall was calculated. The number of cases of clinical and severe malaria averted was also calculated for each site. Cases averted were calculated in the ITT population. The number of cases averted was calculated as the difference in cases between the control group and the vaccine group (R3R+R3C up to the time of booster dose and R3R and R3C separately after the booster dose) with a 95% confidence interval. The number of cases averted over time were calculated as the difference of the estimated cases between the control group and the RTS,S/AS01 group. Estimated cases in each group were calculated as the area under the curve of the three-month incidence (all episodes) over time as:



where *T* is the total follow-up time, *t* represents each one of the three-months periods, *all episodest* is the total number of episodes in the period of time *t*, *person time at riskt* is the person follow-up time during the period *t* and Δ*timet* is the duration of the period *t* (three months).

The number of cases averted was expressed as cases averted per 1000 subjects followed-up during the study period. Fourteen days following an episode were subtracted from the time at risk and no malaria events were counted during this period. The 95% confidence intervals of the difference in cases were estimated using bootstrap methodology using the 2.5 and 97.5 centiles of 1000 replicates. [19] Replicates were made sampling subjects stratified by category of the intensity of malaria transmission. To calculate the cases averted until Month 32, 11 periods of three months were used. To calculate the cases averted until the end of the extension (SE), 16 periods of three months were used in the 5-17 months age category, and 13 periods of three months were used in the 6-12 weeks age category. These correspond to the median follow-up times until the end of the extension in these age categories. The more sensitive secondary case definitions of clinical malaria (a measured or reported fever within the previous 24 hours and a parasite density >0 parasites per cubic millimetre) was used for evaluation of the impact of RTS,S/AS01 on the burden of malaria because, in clinical practice, these children would receive treatment for malaria.

To evaluate the effect on growth, height for age, weight for age and mid arm circumference, z-scores for each age category as well as the absolute height at Month 32, Month 44 and at end of extension were tabulated and the mean values were compared between study groups using a t-test. For growth parameters, the evaluation at the end of extension was stratified between children who made their Month 32 visit on or before 30 June 2012 (SE [late]) and those whose Month 32 visit was after 30 June 2012 (SE [early]). Subjects in the SE early group did not undertake the Month 44 visit but progressed directly to end of extension scheduled around December 2013 for all subjects regardless time of enrolment.

## Major protocol deviations

Deviations related to defaults in bednet distribution at screening and exposure of study vaccines to temperatures outside the recommended ranges were described in detail when the first results of this phase III study were reported.[6] These deviations did not pertain to participants enrolled in the 6-12 weeks age category. Also, during monitoring, it was found that one subject belonging to the 5-17 months age category was enrolled twice at two different clinics under two different subject numbers. This deviation was reported to the site EC/IRB. The subject was excluded from the per-protocol analyses. Due to the removal of one subject number from the database, the total number of subjects enrolled into the study changed from 15460 subjects (8923 in 5-17 months), as reported in previous analyses, to 15459 subjects (8922 in 5-17 months) in the final analyses reported here. Two field workers from Bagamoyo assigned to perform monthly home visits for the detection of unreported SAEs were suspected of not performing these visits and falsifying the visit reports. Probing the SAEs reporting rates in the potentially affected subjects and the non-affected subjects showed no evidence of under-reporting of SAEs. A sensitivity analysis (not shown here) excluding the potentially affected subjects has not resulted in any clinically meaningful difference and has, therefore, no impact on the overall interpretation of the relative incidence between RTS,S/AS01 recipients and controls in either age category or in the subgroups analyses affected (low weight for age, very low weight for age and pre-term infants) for any SAE MedDRA PT term.

## Trademarks

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# Groups that have contributed to the delivery of this study

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# Conflict of interest

The trial was sponsored by GlaxoSmithKline Biologicals SA, the vaccine developer and manufacturer, and funded by both GSK Biologicals SA and the PATH Malaria Vaccine Initiative (MVI). All centres declare receiving a grant from MVI for running the trial. Author travel and accommodation related to this trial were financed by MVI. GlaxoSmithKline Biologicals SA received a grant from MVI to run the trial. MVI received a grant from the Bill and Melinda Gates Foundation to run this trial and to compensate MVI authors for trial-related travel. Other conflicts of interest are disclosed below.

Pedro Alonso declares that his institution received grants from the Catalan government, from the Spanish government, from MMV and BGMF. Pedro Alonso declares that he received personal fees from MMV. Dorothy Mabunde, Charity Maingi, Patricia Njuguna and Lucas Otieno declare that their institutions have received grants from MVI for other malaria studies. Ali Mohammed declares that he received personal fees from MMV and GSK. Chris Odero and Kephas Otieno declare that their institutions received a grant from the Malaria Clinical Trial Alliance. Lucas Otieno declares that he received financial support from GSK to participate to scientific congresses and to set up the “Trust in Science” grant. Marcel Tanner is a board member of the Optimus Foundation, and his institution is reimbursed for his activities on the Scientific Advisory Board of the Novartis Institute for Tropical Diseases. He also has received for his institution other grants from MVI and from the Bill and Melinda Gates foundation, and travel reimbursements from MVI and Sanaria corp.

All GSK Vaccines authors are, or were at the time of the study, employed by the GlaxoSmithKline group of companies. Joe Cohen now works as an independent consultant for GSK Vaccines. Joe Cohen, Erik Jongert, Didier Lapierre and Opokua Ofori-Anyinam have shares/stock options in the GlaxoSmithKline group of companies. Joe Cohen and W. Ripley Ballou declare that they are named inventors on patents for which the rights have been assigned to GlaxoSmithKline group of companies.

David Kaslow, Didier Leboulleux, Chris Odero and Barbara Savarese are or were at the time of the study employees at PATH-MVI. David Schellenberg is employed by the London School of Hygiene and Tropical Medicine, and his consultancy activities for MVI are funded as a grant to the LSHTM by MVI.

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