

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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1. AUTHORS AND AFFILIATIONS

Submitted by the RTS,S Clinical Trials Partnership.

2. SUPPLEMENTARY METHODS

2.1. Ethical considerations

This phase III, double-blind (observer-blind), randomized, controlled multi-center study is currently being undertaken in 11 centers across sub-Saharan Africa. The study design and rationale for selection of endpoints have been described previously.¹ The study is being conducted in accordance with the current Declaration of Helsinki, International Committee on Harmonization Good Clinical Practice guidelines² and with the local rules and regulations of each country. The study is monitored by the sponsor, GlaxoSmithKline, and overseen by a formally constituted Independent Data Monitoring Committee (IDMC), that reviewed, among other information, unblinded comprehensive safety data every 3 months to authorize trial continuation. The IDMC conferred before the initiation of the study and has had 3-monthly teleconferences and 1 annual meeting thereafter. A Local Safety Monitor was available at each study center. The study protocol, and amendments 1 to 3, consent forms, and other information that required pre-approval were reviewed and approved by a national, regional, or research center IEC or IRB as per local requirements. A list of all IEC/IRBs is provided in supplementary table 1.

2.2. Roles of investigators and sponsor

The study is sponsored by GlaxoSmithKline Biologicals (GSK), the vaccine developer and manufacturer, and funded by both GSK Biologicals and the PATH Malaria Vaccine

Initiative (MVI). The data generated by the trial are subject to a confidentiality agreement between the sponsor and investigators, which allows the investigators full access to the study data at the end of the study and includes an obligation to permit publication without excessive delay.

2.3. Study sites

The study is being conducted in 11 sites located in 7 countries in sub-Saharan Africa:

- Institut de Recherche en Science de la Santé, Nanoro, Burkina Faso
- Albert Schweitzer Hospital, Lambarene, Gabon
- School of Medical Sciences, Kumasi, Ghana.
- Kintampo Health Research Center, Kintampo, Ghana
- KEMRI - Walter Reed Project, Kombewa, Kenya
- KEMRI - Wellcome Trust Research Program, Kilifi, Kenya
- KEMRI/CDC Research and Public Health Collaboration, Siaya, Kenya
- University of North Carolina Project, Lilongwe, Malawi
- Centro de Investigação em Saúde de Manhiça, Manhiça, Mozambique
- Ifakara Health Institute, Bagamoyo, Tanzania
- National Institute of Medical Research, Korogwe, Tanzania

These sites represent the range of malaria transmission seen across sub-Saharan Africa (Supplementary figure1).

2.4. Screening and informed consent

Two groups of children were eligible for inclusion in the trial. One group comprised children who were between 6 to 12 weeks of age (inclusive) at the time of first vaccination who had not previously received a dose of vaccine against diphtheria, tetanus, pertussis or *Haemophilus influenzae* type b and the other group comprised children between 5 to 17 months of age at the time of first vaccination. Screening procedures included a review of the child's medical history, a physical examination and a blood test for assessment of hemoglobin concentration. The main exclusion criteria were: moderate or severe illness at the time of enrolment, major congenital defects, malnutrition requiring hospitalization, severe anemia - defined as a hemoglobin concentration < 5.0 g/dL or a hemoglobin concentration < 8.0 g/dL associated with clinical sign of heart failure or severe respiratory distress, or a past history of a neurological disorder or atypical febrile seizure. A febrile seizure is atypical if it meets one of the following criteria: not associated with fever; lasts > 5 minutes; focal (not generalized); followed by transient or persistent neurological abnormality; occurs in a child < 6 months of age. 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.

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 the community meetings, and a positive recommendation from community leaders, the parent(s)/guardian(s) of children in the eligible age groups were approached. The need for a vaccine against malaria was discussed and the objectives of the study were explained. The study procedures were carefully described including immunization and blood collection. Parent(s)/guardian(s) interested in enrolling their child into the study were invited to the screening visit.

Formal consent was obtained from each child's parent(s) or guardian(s) prior to the performance of any study-specific procedures. The site investigator or his/her designate described the protocol to the parent(s)/guardian(s) of potential participating children face to face or the informed consent information was presented to groups at an initial information session. Information was provided in both oral and 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. 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.

2.5. Randomization and blinding

After verification of eligibility criteria, and prior to the first vaccination, a unique treatment number was assigned to each participating child. Participating children from each age-category were randomized into one of 3 study groups according to a 1:1:1 ratio (R3R, R3C or C3C) using a randomization algorithm at GSK Biologicals using SAS version 9.1 (Supplementary figure 2). Randomization was stratified for age-category using center as a minimization factor, ensuring balanced treatment allocation within each study center. 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 (RDE) and electronic case report forms (eCRF).

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 control 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 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.

2.6. Study vaccines

Each child received 3 doses of either the candidate malaria vaccine RTS,S/AS01 or the control vaccine; in the 6-12 weeks age group the control vaccine was a Meningococcal C conjugate vaccine Menjugate™ (Novartis) while in the 5-17 months age group the control vaccine was a rabies vaccine VeroRab™ (Sanofi-Pasteur) (Supplementary figure 2). Vaccines were administered intramuscularly into the left deltoid for the 5-17 months age-category and into the left anterolateral thigh for the 6-12 weeks age-category. The choice of control vaccines was guided by the principles of benefit to the control group without compromising the evaluation of clinical study endpoints.

The RTS,S/AS01 candidate vaccine has been developed and manufactured by GSK Biologicals and is designed to protect against *P. falciparum* malaria. “RTS,S” comprises the carboxyl terminal portion of the circumsporozoite protein 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-D-deacylated Monophosphoryl Lipid A) and QS21 (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 QS21 with liposomes.⁴

Sanofi Pasteur’s chromatographically purified Vero cell culture rabies vaccine VeroRab™ is based on 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 Menjugate contains 10 µg *Neisseria meningitidis* (strain C11) Group C oligosaccharide conjugated to 12.5-25 µg *Corynebacterium diphtheriae*

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.

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 doctor accredited in pediatric resuscitation was available at all vaccination sessions.

2.7. Bednets and indoor residual spraying (IRS)

The research team ensured that insecticide treated bednet use was optimized in each study population: in 2 centers (Kilifi, Kenya and Bagamoyo, Tanzania) this was achieved through close collaboration with the National Malaria Control Programs. In the other centers, impregnated bednets were distributed by the study teams to all children who consented for screening, regardless of whether they were eligible for the trial.

Data were collected on malaria control measures used by the participants' families during the period of surveillance. Bednet usage and indoor residual spraying (IRS) were documented 12 months after the third vaccine dose had been given. 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.

2.8. Safety assessments

During the study, investigators or their colleagues were responsible for documenting and reporting events meeting the criteria and definition of an adverse event (AE) or 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 (AE) was defined as any untoward medical occurrence in a child participating in the clinical trial, temporally associated with vaccination whether or not considered related to the vaccine. An AE could, therefore, be any unfavorable 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 serious adverse event (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 within 30 days of vaccination. Abnormal laboratory findings that were judged by the assessing clinician to be clinically significant were recorded as SAEs if they met the previously defined criteria.

Seizures and immune-mediated disorders were reported as SAEs in order to ensure availability of full case narrative descriptions.¹ Data on seizures occurring within 7 days following vaccination were collected according to the Brighton Collaboration guidelines.⁵

Because pediatric auto-immune diseases are rare and may be underestimated in sub-Saharan Africa, training material on pediatric 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 locally available.

All solicited AEs were reported for 7 days (day of vaccination and 6 subsequent days) following each vaccine dose for the first 200 children enrolled at each center. Local AEs solicited were: pain at injection site; swelling at injection site and redness at injection site. Solicited general AEs were: drowsiness, fever; irritability/fussiness and loss of appetite. Intensity of AEs was assessed as described in supplementary table 4.

All unsolicited AEs were reported for 30 days following each vaccine dose for the first 200 participating children enrolled at each center.

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 with the participating children. 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. The 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 is easily tolerated by the child, causing minimal discomfort and not interfering with everyday activities; grade 2 (moderate): an AE which is sufficiently discomforting to interfere with normal everyday activities and grade 3 (severe): an AE which prevents 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 to ascribe the cause of death 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 be locally appropriate.⁶ To support the timely reporting of SAEs, diagnoses were made according to the usual processes of each center.

2.9. 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 trial. All participating children who presented to a health facility in the study area were evaluated as potential cases of malaria using a standardized algorithm. All parents were asked whether the child 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 a history of fever during the prior 24 hours or who had a measured axillary temperature greater or equal to 37.5°C at time of presentation.

Children who needed inpatient care were provided transport to a study hospital. All participating children who presented for admission were evaluated as potential cases of severe malaria disease following a predefined algorithm (Supplementary table 3).

Detection and management of severe malaria have been described in detail by Vekemans et al.⁷ During any hospitalization, the child's course was monitored to capture the signs and blood parameters indicative of a progression to severe malaria. If a child's condition deteriorated following admission then additional investigation was performed.

Treatment of malaria was performed in accordance with national guidelines. In 9 of the 11 study centers, the first line treatment for uncomplicated malaria was a 6-dose regimen of artemether-lumefantrine whilst in two, both in Ghana, it was artesunate-amodiaquine. Children who required inpatient care were admitted to the hospital and received treatment with intravenous quinine, according to national guidelines,

2.10. Chest X-rays

Chest X-rays were performed as part of the standardized evaluation of study children 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.⁷

A digital X-ray system was provided to each study site to facilitate radiological assessment of study participants. The radiographer and the physician who read the images for the trial endpoints received standardized technical training by the manufacturer of the X-ray equipment and training on interpretation by expert radiologists and physicists. To ensure a robust and verifiable X-ray data base, quality control systems comprising 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 Biologicals via a satellite internet connection.

To ensure accurate diagnosis of pneumonia, a process developed by WHO¹⁰ was followed. Each X-ray was read independently by a clinician attached to the center where the X-ray was taken, and by an external radiologist. GSK Biologicals reviewed all readings made by the centers and by the external radiologists and any images with discordant readings were sent to another panel of radiologists for a final reading.

Clinicians and external radiologists were trained in chest X-ray interpretation according to WHO guidelines.¹⁰

2.11. Anthropometry

Length/height, weight and mid-upper arm circumference were measured at screening and one month after the third dose of vaccine. The methodologies used for anthropometry were adapted from Cogill.¹¹

2.12. Laboratory analysis

Development of standardized laboratory methods and quality control processes for this trial have been described fully in a separate publication¹² and are summarized briefly here.

- **Hematology and biochemistry**

Automated biochemical and hematologic methods were used. All biochemistry automated analyzers were initially enrolled with International External Quality

Assessment (EQA) but later switched to the program run by the Royal College of Pathologists of Australia, because the latter was more appropriate for the study requirements at the time. All hematology automated analyzers were enrolled in EQA. Each laboratory had to demonstrate method qualification for biochemistry and hematology, including analysis of repeatability, reproducibility, linearity, QC stability and accuracy between main and back-up analyzers. Data were sent to GSK Biologicals for analysis and feedback was provided to laboratories.

Daily internal QC was performed at each site, and external quality control was performed monthly for biochemistry and hematology samples.

- **Microbiology**

Standard microbiology methods for blood and cerebrospinal fluid (CSF) culture were followed using automated Bactec™ incubators and pediatric bottles (Bactec BD Diagnostic Systems, USA). Positive cultures were sub-cultured using standard methods.^{13,14} For the purpose of trial analysis, as opposed to clinical care, results were classified by standardized case definitions based on an established methodology.¹⁵ A blood culture was considered positive if a definite pathogen was isolated (e.g. *Streptococcus pneumoniae*, *S. agalactiae*, *S. pyogenes*, *Haemophilus 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.¹⁵

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

Bacterial meningitis was defined as the presence of a CSF white cell count of $\geq 50 \times 10^6/L$, 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*.^{7, 16}

Microbiology quality assessment included evaluation of microscopy, culture and identification and antimicrobial susceptibility testing. Each laboratory received 6 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 (ATCC) control strains for species identification every week or 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 programs for both clinical and laboratory staff and more intense quality evaluation when there was a high contamination rate.

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

All slides were read twice by two microscopists. A third independent microscopist read the slide if there were any of the following discrepancies between the first two readings: (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 the 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 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 (in log scale). As a quality measure, agreement between the 2 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.

2.13. Immunological assessment

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.¹⁷ Briefly, R32LR protein was coated onto a 96-well polystyrene plate. Serial dilutions of serum samples 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 color 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 tested 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 titer below the cut-off value were given a value of 0.25 EU/mL.

2.14. Data collection and management and statistical analysis

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

In order to preserve the blinding of the ongoing trial, all data cleaning processes were blinded to study group and analyses were conducted by external statisticians, Lisa Allamassey (Keyrus) and Catherine Dettori (4Clinics), who performed the analyses using SAS v9.2 SDD (SAS Institute Inc., Cary, NC, USA) based on a cleaned dataset and quality controlled programs provided by GSK.

2.15. Concordance in blood slide readings

In the 6 weeks to 17 months combined age-categories, 78,937 blood slides were read independently by two microscopists. In total, 78,800 (99.8%) were read according to the slide reading algorithm, that is each slide was read independently by 2 qualified microscopists and a 3rd reading was performed if indicated according to the protocol.

Agreement between microscopists was high: kappa=90.4 (95%CI: 90.1, 90.8).

3. MAJOR PROTOCOL DEVIATIONS

- **Mozambique - Temperature Deviation**

In the center in Mozambique, study vaccines (RTS,S/AS01 or VeroRab™) were exposed to temperatures outside the acceptable storage range. Temperature deviations occurred in the fridges where the vaccines were stored in Manhiça and also during the transport to the study clinics. These temperature deviations were discovered after most of the participating children enrolled in Mozambique had been vaccinated. It was not possible to identify specifically which vaccines were exposed to these out-of-range temperatures, due to lack of information on the storage location of vaccine lots. Therefore, the most cautious approach was taken, and 996 children out of 1002 children enrolled in the 5-17 months age-category were considered to have received at least one dose of vaccine potentially exposed to an out-of range temperature. These 996 children were excluded from the ATP population for efficacy and ATP population for immunogenicity but were included in the ITT population for the analysis of safety. The IRBs, ECs and the IDMC were informed. The IDMC reviewed the safety information and no safety concerns were raised. The parents of enrolled children were also informed, and the parents/guardians of each child who had not yet received three doses of vaccine were asked to give consent for

their child to receive subsequent vaccinations. Among the 136 children who had not yet received the third dose, parents of 24 children did not give their consent for the third vaccine administration. All other children received the last dose as requested by the IRB, but these vaccinations occurred outside of the per-protocol schedule with a delay ranging from 3.5 to 4.5 months.

- **Kombewa and Korogwe - Provision of Bednets**

Two study centers, Kombewa, Kenya and Korogwe, Tanzania, deviated from the protocol requirement for bednet provision. Both site specific documents specified that an insecticide treated bednet (ITN) would be provided for each child who presented for screening, regardless of whether the parent chose to enroll their child in the study.

Recruitment began in Kombewa in August 2009 and in February 2010 in Korogwe. Both teams omitted to distribute bednets to all screened children. The respective IRBs and ECs and the IDMC were notified when this was ascertained. The team at Kombewa purchased ITNs and started to distribute bednets in February 2010. In June 2010, Korogwe received bednets donated by the Tanzania National Malaria Control Program and started their distribution. Both study teams provided a net to all children who had presented for screening, regardless of whether or not they took part in the trial and to all new children who presented for screening.

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6. DETAILED CONTRIBUTIONS TO THE STUDY

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John Aponte and Marc Lievens vouch for the data and analysis;

Salim Abdulla, Tsiri Agbenyega, Selidji Agnandji Todagbe, Ali Mohamed Ali, Daniel Ansong, John Aponte, Terrell Carter, Joe Cohen, Umberto D'Alessandro, Samwel

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Salim Abdulla, John Aponte, Brian Greenwood, Mary J Hamel, Didier Leboulleux, Amanda Leach, Marc Lievens, Patricia Njuguna, David Schellenberg, Marcel Tanner, and Johan Vekemans wrote the paper;

All authors decided to publish the paper, reviewed all manuscript drafts, and approved the final version of the manuscript.

7. CONFLICTS OF INTEREST

The study was sponsored by GSK, the vaccine developer and manufacturer, and funded by both GSK Biologicals and the MVI. All centers declare receiving a grant from MVI for running the trial. Author travel and accommodation related to this trial were financed by MVI. GlaxoSmithKline Biologicals 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 and in forms available with the full text of this article at NEJM.org.

Pedro Aide declares having received a grant from GSK Biologicals as a contribution to post-graduate training, John Aponte has received for the center consultancy fees from

GSK Biologicals for membership of the DSMB Board for a pneumococcal vaccine.

Patricia Njuguna, Kevin Marsh, Pauline Akoo, Philip Bejon, Charity Maingi, Ally Olotu, Trudie Lang, Jesse Gitaka, Christine Kerubo, and Norbert Peshu declare that their institution has received grants from MVI for other malaria studies. Philip Bejon declares having received compensation for travel and accommodation related to other malaria studies. Roma Chilengi and Benjamin Tsofa declare that their institution receives infrastructural support from MVI. Brian Greenwood declares that the London School of Hygiene and Tropical Medicine, at which he is employed, receives a grant from GSK for work on a pneumococcal protein vaccine. John Lusingu, Samwel Gesase, Anangisy Malabeja, Omari Abdul, Hassan Kilavo, Coline Mahende, Edwin Liheluka, and Martha Lemnge declare that their institution receives additional infrastructural support from MVI for this trial. John Lusingu declares receiving a salary supplement from MVI for conducting this trial, and his institution has received funds from MVI for GCLP training to staff conducting this clinical trial and to strengthen the infrastructure. John Lusingu declares having received consultancy fees from the Task Force on Immunization in the WHO-AFRO region outside the submitted work, and having grants pending from DANIDA-ENRECA and AMANET. AInstitut de Recherche en Science de la Santé, Nanoro, Burkina Faso

AAAll GSK authors are employed by GSK Biologicals. W. Ripley Ballou, Joe Cohen, Didier Lapierre, Amanda Leach, Opokua Ofori-Anyinam and Johan Vekemans have shares/stock options in GSK. W. Ripley Ballou and Joe Cohen declare that they have patents assigned to GSK.

Terrell Carter, Didier Leboulleux, Christian Loucq, Afiya Radford, Barbara Savarese, Marla Sillman, and Preeti Vansadia are 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. Christian Loucq holds GSK shares.

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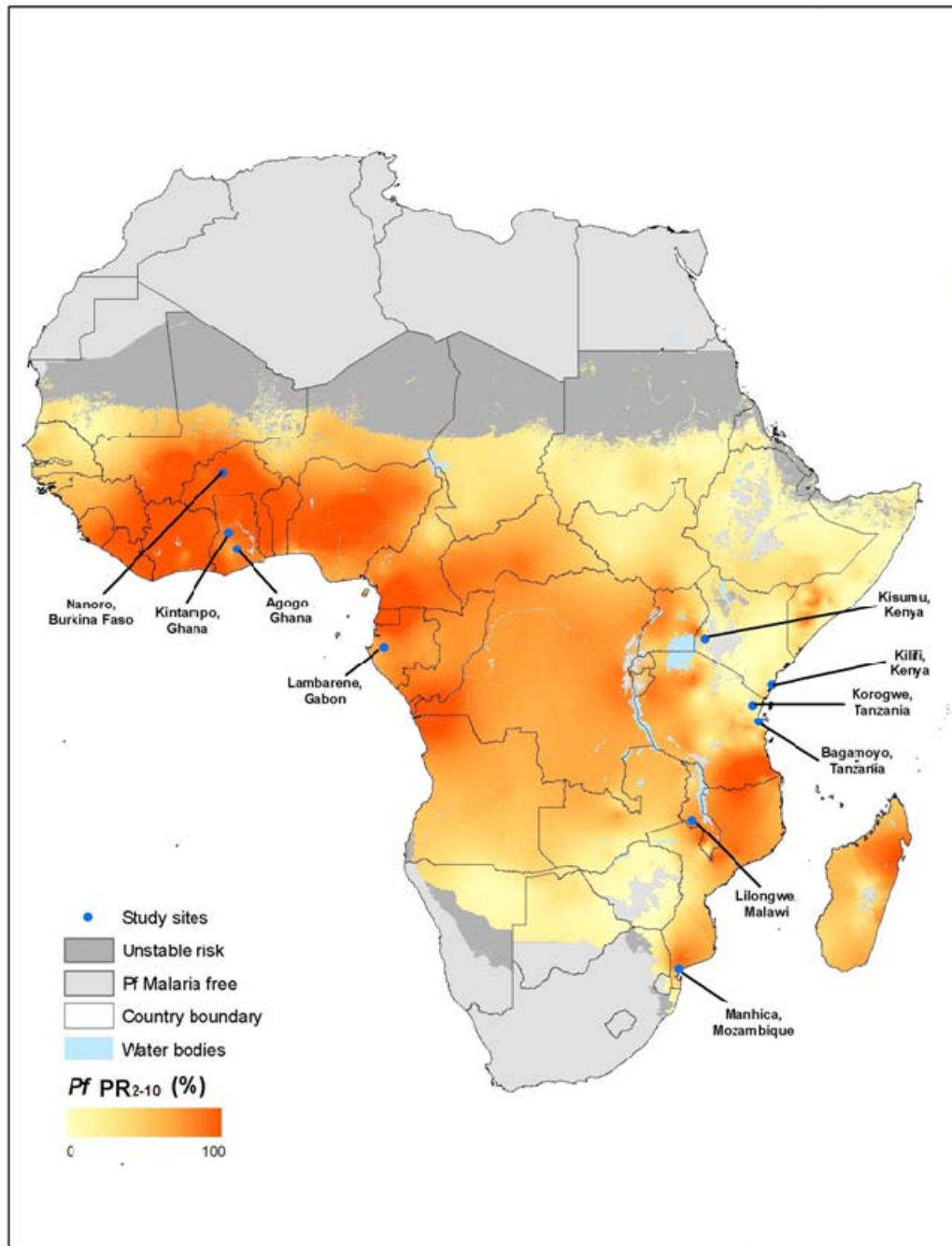
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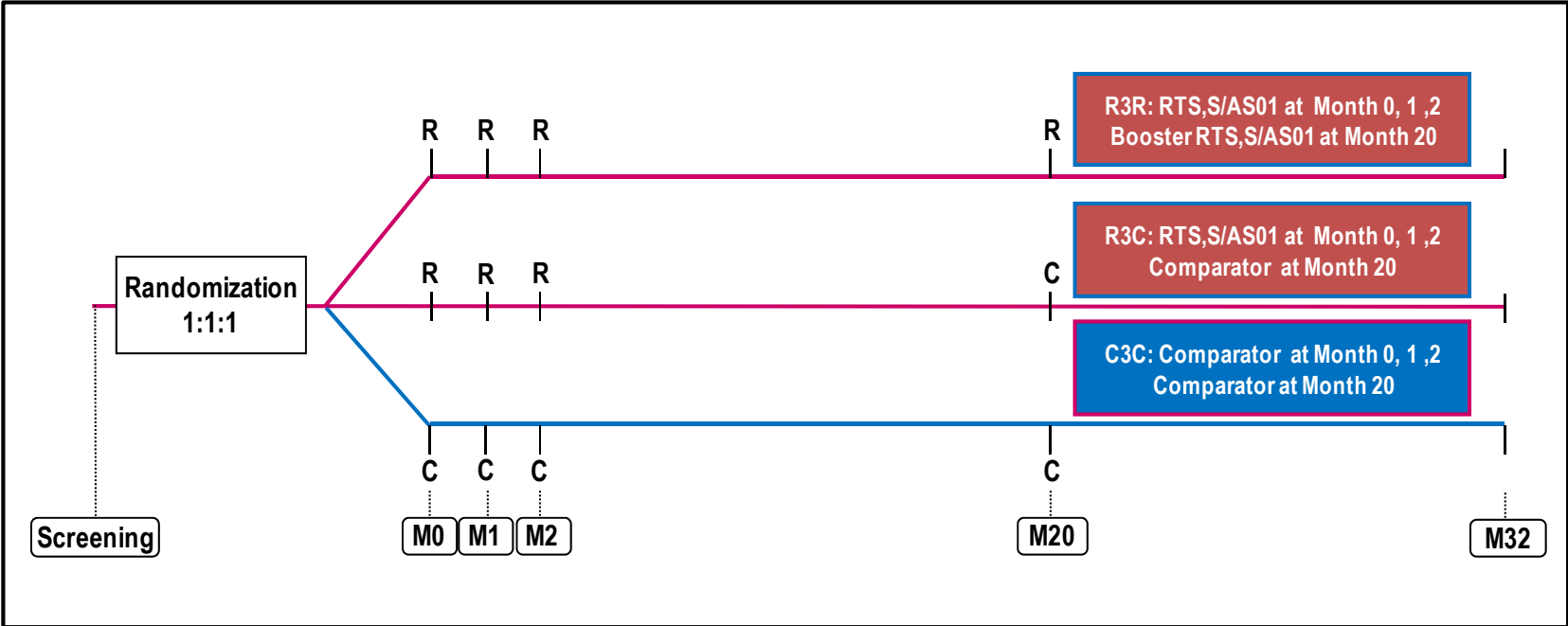
9. SUPPLEMENTARY TABLES AND FIGURES

Supplementary figure 1. Study sites and malaria endemicity



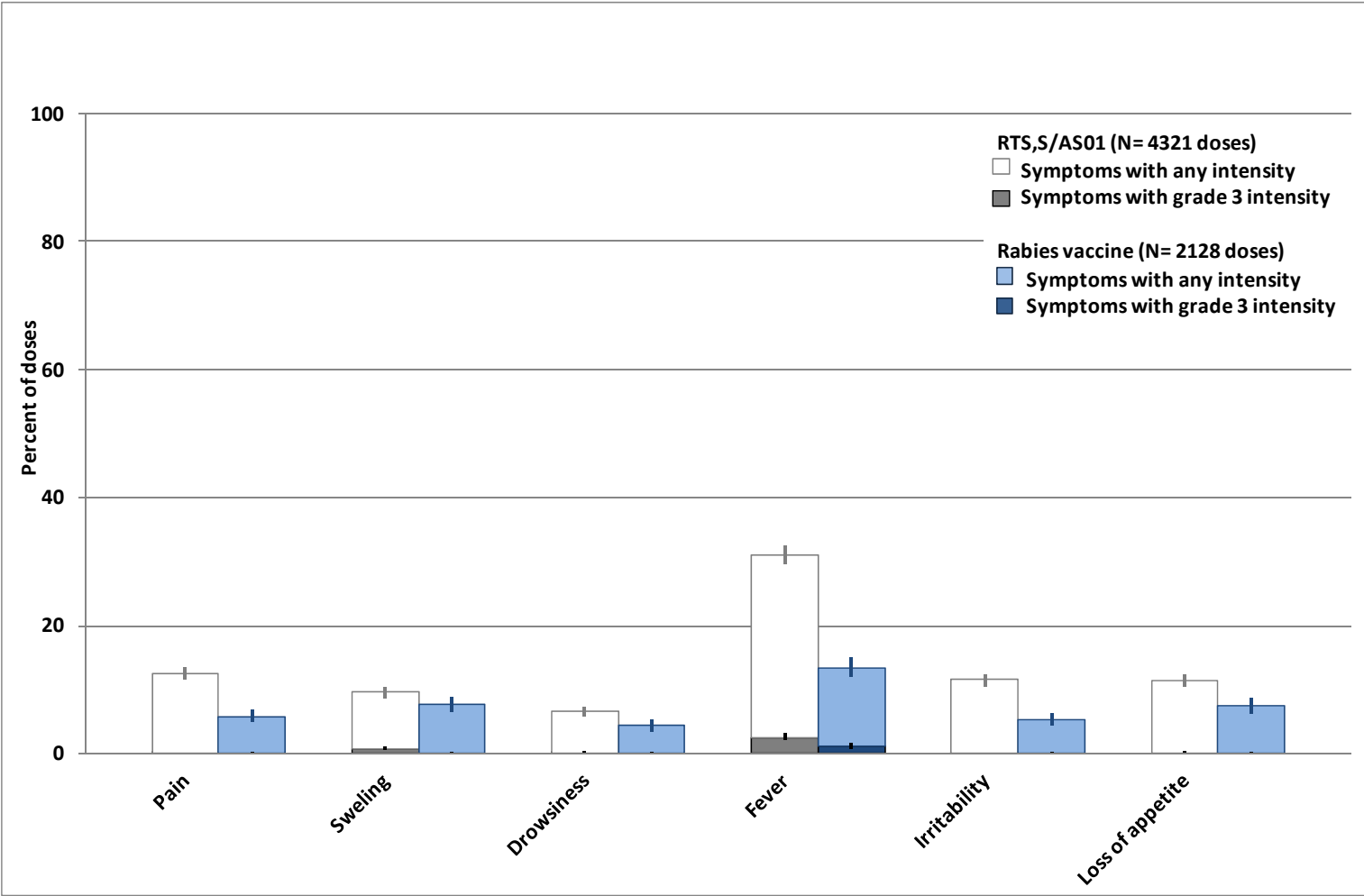
Adapted from Hay et al, 2009.¹⁸ The location of each participating center has been added to this previously published map showing the spatial distribution of *P. falciparum* malaria endemicity. Two centers are located in Kisumu, Kenya. The data are the model-based geostatistical point estimates of the annual mean *P. falciparum* parasite rate age-standardized for 2-10 years for 2007 within the stable spatial limits of *P. falciparum* malaria transmission, displayed as a continuum of yellow to red from 0%–100% (see map legend). The rest of the land area was defined as unstable risk (medium grey areas) or no risk (light grey). Nanoro, Burkina Faso has high seasonal malaria transmission.

Supplementary figure 2. Study design



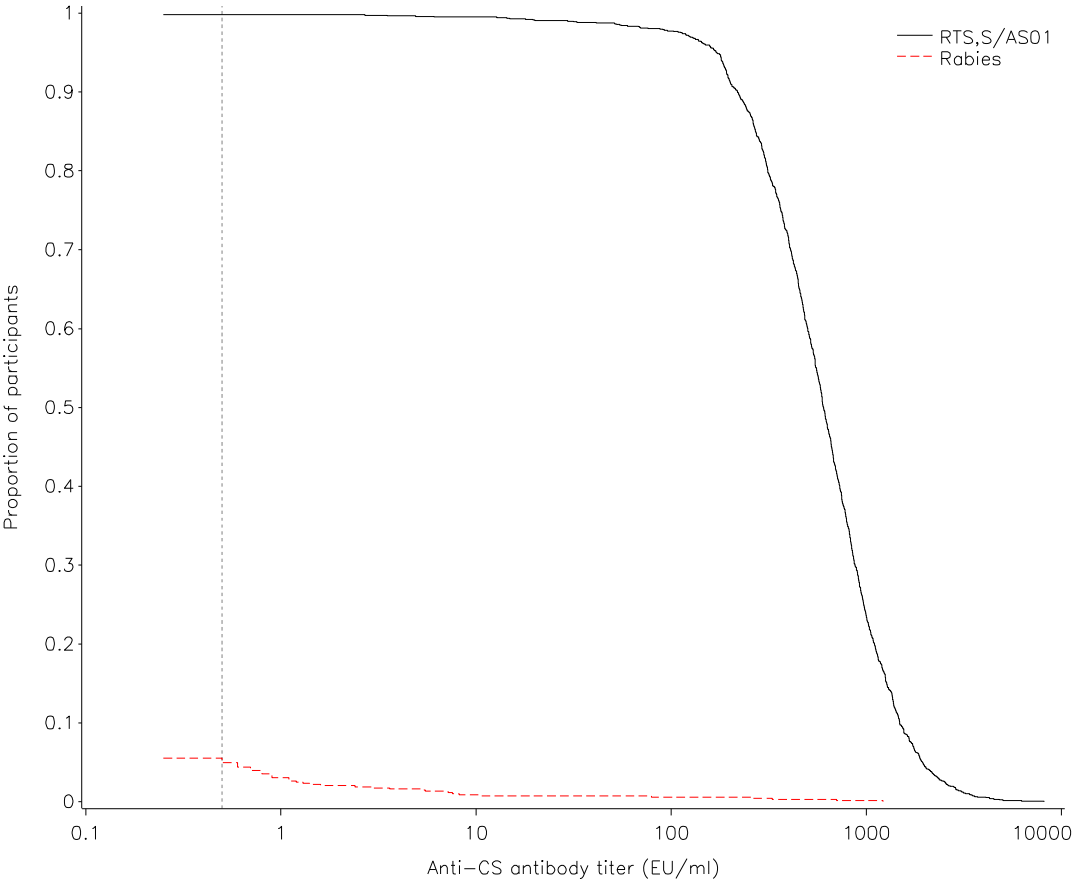
M = Study Month

Supplementary figure 3. Incidence of solicited local and general adverse events reported during the 7-day post vaccination period following each dose in children 5-17 months of age at enrollment (ITT)



Solicited adverse events during the 7-day post-vaccination period were recorded and analyzed only in the first 200 subjects enrolled at each site.

Supplementary figure 4. Reverse cumulative distribution curve for Anti-CS antibody titers in children 5-17 months at 1 month post-Dose 3 (ATP)



Supplementary table 1. List of Ethic Committees and Review Boards

Study Sites	Ethics Review Body
Institut de Recherche en Science de la Santé, Nanoro, Burkina Faso	Western Institutional Review Board (WIRB)
	Comité d’Ethique Institutionnel du Centre Muraz (Institutional Ethics Committee of Muraz Center)
	Comite d’Ethique pour la Recherche en Santé (Ethics Committee for Health Research)
Albert Schweitzer Hospital, Lambarene, Gabon	Western Institutional Review Board (WIRB)
	Comité d’Ethique Régional Indépendant de Lambaréné (CERIL) (Independent Regional Ethics Committee of Lambaréné)
	Comité National d’Ethique pour la Recherche (National Ethics Committee for Research The Board)
School of Medical Sciences, Kumasi (Agogo), Ghana	Western Institutional Review Board (WIRB)
	Ghana Health Service (GHS) Ethical Review Committee (ERC) Research and Development Division
	Committee on Human Research Publication and Ethics (CHRPE)
Kintampo Health Research Center, Kintampo, Ghana	Western Institutional Review Board (WIRB)
	Kintampo Health Research Centre (KHRC) Institutional Ethics Committee (IEC)
	London School of Hygiene and Tropical Medicine Ethic Committee
	Ghana Health Service (GHS) Ethical Review Committee (ERC) Research and Development Division
KEMRI - Walter Reed Project, Kombewa, Kenya	Western Institutional Review Board (WIRB)
	Kenya Medical Research Institute (KEMRI) National Ethics Review Committee
	Walter Reed Army Institute of Research (WRAIR) IRB
KEMRI - Wellcome Trust Research Program, Kilifi, Kenya	Western Institutional Review Board (WIRB)
	Kenya Medical Research Institute (KEMRI) National Ethics Review Committee
KEMRI/CDC Research and Public Health Collaboration, Siaya, Kenya	Western Institutional Review Board (WIRB)
	Kenya Medical Research Institute (KEMRI) National Ethics Review Committee
	Centers for Disease Control and Prevention(CDC) – IRB
University of North Carolina Project, Lilongwe, Malawi	Western Institutional Review Board (WIRB)
	National Health Sciences Research Committee
	Office of Human Research Ethics
Centro de Investigação em Saúde de Manhiça, Manhiça, Mozambique	Western Institutional Review Board (WIRB)
	Comitè Etic Investigació Clínica (Hospital Clinic (Barcelona University) Ethics Committee)
	Comité Nacional de Bioética para a Saúde (National Bioethical Health Committee, Mozambique)
Ifakara Health Institute, Bagamoyo, Tanzania	Western Institutional Review Board (WIRB)
	National Institute for Medical Research (NIMR)
	Ethikkommission beider Basel (EKBB) (Ethics Committee of the Swiss Tropical Institute)
	Ifakara Health Institute research training services - IRB
National Institute of Medical Research, Korogwe, Tanzania	Western Institutional Review Board (WIRB)
	London School of Hygiene and Tropical Medicine
	Tanzania Medical Research Coordinating Committee (MRCC) operating within National Institute for Medical Research (NIMR)
	The Danish National Committee on Biomedical Research Ethics

Supplementary table 2. Case definitions of severe malaria

Primary definition	<i>P. falciparum</i> > 5000 parasites per μ L	AND with one or more marker of disease severity: <ul style="list-style-type: none"> • Prostration • Respiratory distress • Blantyre score ≤ 2 • Seizures 2 or more • Hypoglycemia < 2.2 mmol/L • Acidosis BE ≤ -10.0 mmol/L • Lactate ≥ 5.0 mmol/L • Anemia < 5.0 g/dL AND without diagnosis of a co-morbidity: <ul style="list-style-type: none"> • Radiographically proven pneumonia • Meningitis on CSF examination • Positive blood culture • Gastroenteritis with dehydration
Secondary definition	<i>P. falciparum</i> > 5000 parasites per μ L with co-morbidity	with one or more marker of disease severity

Prostration: in an acutely sick child, the inability to perform previously-acquired motor function: in a child previously able to stand, inability to stand; in a child previously able to sit, inability to sit and in a very young child, inability to suck.

Respiratory distress: lower chest wall indrawing or abnormally deep breathing.

2 or more seizures: occurring in the total time period including 24 hours prior to admission, the emergency room and the hospitalization.

Radiographically proven pneumonia: a consolidation or pleural effusion defined per protocol on a chest x-ray taken within 72 hours of admission.

Meningitis on CSF examination: WC $\geq 50 \times 10^6$ /L or positive culture of compatible organism or latex agglutination positive for Hib, pneumococci or meningococci.

Gastroenteritis with dehydration: history of 3 or more loose or watery stools in previous 24 hours and an observed watery stool with decreased skin turgor (> 2 seconds for skin to return following skin pinch).

Positive blood culture: defined per protocol on a blood culture taken within 72 hours of admission.

Supplementary table 3. Algorithm for the evaluation of a hospital admission as a potential case of severe malaria

For all acute hospital admissions (i.e. except planned admissions for medical investigation/care or elective surgery and trauma admissions), a blood sample was taken for evaluation of:

- Malaria parasite density
- Blood culture
- Hemoglobin
- Blood glucose, lactate and base excess

Lumbar Puncture was indicated by the presence of:

- Seizure except simple febrile seizure (defined as associated with fever, lasts for 5 minutes or less, generalized as opposed to focal, not followed by transient or persistent neurological abnormalities, occurring in a child ≥ 6 months of age, with full recovery within 1 hour)
- Blantyre Coma Score < 5 (children ≤ 9 months of age < 4 [in association with best motor response of 1]) 8
- Prostration in child < 3 year of age
- Meningism/stiff neck/bulging fontanelle
- Clinician's judgment

Chest X-ray (CXR) was indicated by the presence of:

- Tachypnea (≥ 50 breaths per minute in a child < 1 year and ≥ 40 breaths per minute in a child ≥ 1 year) 9
 - Lower chest wall indrawing
 - Abnormally deep breathing
 - Clinician's judgement
-

Supplementary table 4. Grading of solicited adverse events (AEs)

Adverse Event	Intensity grade	Parameter
Pain at injection site	0	Absent
	1	Minor reaction to touch
	2	Cries/protests on touch
	3	Cries when limb is moved/spontaneously painful
Swelling at injection site	0	Absent
	1	<5 mm
	2	5-20 mm
	3	>20 mm
Redness at injection site	0	Absent
	1	<5 mm
	2	5-20 mm
	3	>20 mm
Fever	0	<37.5°C
	1	37.5-38°C
	2	>38-39°C
	3	>39°C
Irritability/Fussiness	0	Behavior as usual
	1	Crying more than usual/ no effect on normal activity
	2	Crying more than usual/ interferes with normal activity
	3	Crying that cannot be comforted/ prevents normal activity
Drowsiness	0	Behavior as usual
	1	Drowsiness easily tolerated
	2	Drowsiness that interferes with normal activity
	3	Drowsiness that prevents normal activity
Loss of appetite	0	Appetite as usual
	1	Eating less than usual/ no effect on normal activity
	2	Eating less than usual/ interferes with normal activity
	3	Not eating at all

Supplementary table 5a. First 6000 children aged 5-17 months enrolled, number by site

	ATP				ITT			
	RTS,S/AS01 N = 2830		Rabies vaccine N = 1466		RTS,S/AS01 N = 3997		Rabies vaccine N = 2003	
Study sites	n	%	n	%	N	%	n	%
Agogo	366	12.9	193	13.2	393	9.8	200	10.0
Bagamoyo	353	12.5	185	12.6	467	11.7	237	11.8
Kilifi	308	10.9	157	10.7	365	9.1	187	9.3
Kintampo	336	11.9	172	11.7	372	9.3	191	9.5
Kombewa	595	21.0	306	20.9	652	16.3	325	16.2
Korogwe	0	0	0	0	0	0	0	0
Lambarene	182	6.4	99	6.8	244	6.1	120	6.0
Lilongwe	87	3.1	52	3.5	189	4.7	90	4.5
Manhica	0	0.0	0	0.0	664	16.6	336	16.8
Nanoro	141	5.0	60	4.1	143	3.6	61	3.0
Siaya	462	16.3	242	16.5	508	12.7	256	12.8

N = number of subjects

n = number of subjects in a given category

% = n / Number of subjects with available results x 100

Supplementary table 5b. Number of children aged 6 weeks - 17 months enrolled by site

	ATP				ITT			
	RTS,S/AS01 N = 8597		Control vaccine N = 4364		RTS,S/AS01 N = 10307		Control vaccine N = 5153	
Study sites	n	%	n	%	n	%	n	%
Agogo	795	9.2	417	9.6	858	8.3	430	8.3
Bagamoyo	967	11.2	483	11.1	1138	11.0	567	11.0
Kilifi	528	6.1	273	6.3	597	5.8	307	6.0
Kintampo	803	9.3	396	9.1	889	8.6	444	8.6
Kombewa	1001	11.6	516	11.8	1089	10.6	542	10.5
Korogwe	951	11.1	478	11.0	1006	9.8	499	9.7
Lambarene	527	6.1	261	6.0	628	6.1	302	5.9
Lilongwe	865	10.1	442	10.1	1086	10.5	540	10.5
Manhica	381	4.4	188	4.3	1087	10.5	550	10.7
Nanoro	832	9.7	425	9.7	850	8.2	431	8.4
Siaya	947	11.0	485	11.1	1079	10.5	541	10.5

N = number of subjects

n = number of subjects in a given category

% = $n / \text{Number of subjects with available results} \times 100$

Supplementary table 6. Malaria prevention in the first 6000 children aged 5-17 months at enrollment at 14 months post Dose-1 (ITT)

		RTS,S/AS01 N = 3997		Rabies vaccine N = 2003	
Characteristics	Categories	n	%	n	%
Bednet use, measured at Month 14 post Dose-1	ITN no holes	1473	42.9	736	41.3
	ITN holes	1116	32.5	590	33.1
	Untreated w/o holes	130	3.8	64	3.6
	Untreated with holes	97	2.8	60	3.4
	No bednet	619	18.0	331	18.6
	Missing	562	-	222	-
Indoor residual spraying	N	3173	92.1	1651	92.6
	Y	274	7.9	131	7.4
	Missing	550	-	221	-

N = number of subjects

n = number of subjects in a given category

% = $n / \text{Number of subjects with available results} \times 100$

ITN no holes = insecticide treated bednet with no hole large enough to admit three fingers

ITN holes = insecticide treated bednet with at least one hole large enough to admit three fingers

Untreated w/o holes = untreated bednet with no hole large enough to admit three fingers

Untreated with holes = untreated bednet with at least one hole large enough to admit three fingers

Supplementary table 7. composition of the cohort that included both the younger and older age-categories

Cohort of analysis	Number of children per age category	Duration of follow-up post dose-3	Reported efficacy post dose 3
6000	6000 5-17 months	All subjects followed to 12 months post dose-3 (or death or withdrawal)	12 months per subject
15460	8923 5-17 months 6537 6-12 weeks	All subjects followed to 31st May (or booster at 18 months or death or withdrawal)	Overall: 11 months average range (0 - 22) 5-17 months: 16 months average range (0-22) 6-12 weeks: 7 months average range (0-15)

Supplementary table 8a. Baseline characteristics in the first 6000 children aged 5-17 months at enrollment (ITT)

		RTS,S/AS01 N = 3997	Rabies vaccine N = 2003
Age in months at first dose [5-17 months]	Mean ± SD	10.9 ± 3.8	10.9 ± 3.7
Male Gender	n (%)	1965 (49.2)	980 (48.9)
Distance outpatient [km]	Mean ± SD	3.4 ± 4.2	3.6 ± 4.7
Distance inpatient [km]	Mean ± SD	12.7 ± 11.3	12.8 ± 11.2
Height for age z-score	Mean ± SD	-1.1 ± 1.4	-1.1 ± 1.3
Weight for age z-score	Mean ± SD	-0.8 ± 1.2	-0.8 ± 1.1
Hemoglobin [g/dL]	Mean ± SD	9.8 ± 1.4	9.8 ± 1.3
Moderate anemia [Hb <8g/dL]	n (%)	380 (9.5)	173 (8.6)

N = number of subjects

n = number of subjects in a given category

SD = Standard deviation

None of the baseline characteristics differed by group ($p > 0.05$)

Supplementary table 8b. Baseline characteristics in children aged 6 weeks - 17 months at enrollment (ITT)

		RTS,S/AS01 N = 10307	Control vaccine N = 5153
Age in months at first dose [5-17 months]	N	5949	2974
	Mean ± SD	10.6 ± 3.8	10.6 ± 3.7
Age in weeks at first dose [6-12 weeks]	N	4358	2179
	Mean ± SD	7.1 ± 1.4	7.1 ± 1.4
Male Gender	n (%)	5216 (50.6)	2550 (49.5)
Distance outpatient [km]	Mean ± SD	4.2 ± 4.5	4.2 ± 4.6
Distance inpatient [km]	Mean ± SD	14.2 ± 12.4	13.9 ± 12.0
Height for age z-score	Mean ± SD	-1.2 ± 1.4	-1.2 ± 1.3
Weight for age z-score	Mean ± SD	-0.7 ± 1.2	-0.7 ± 1.1
Hemoglobin [g/dL]	Mean ± SD	10.3 ± 1.6	10.3 ± 1.6
Moderate anemia [Hb <8g/dL]	n (%)	668 (6.5)	345 (6.7)

N = number of subjects

n = number of subjects in a given category

SD = Standard deviation

None of the baseline characteristics differed by group ($p > 0.05$)

Supplementary table 8c. Baseline characteristics in the first 6000 children aged 5-17 months at enrollment (ATP)

		RTS,S/AS01 N = 2830	Rabies vaccine N = 1466
Age in months at first dose [5-17 months]	Mean ± SD	10.9 ± 3.8	10.9 ± 3.7
Male Gender	n (%)	1417 (50.1)	742 (50.6)
Distance outpatient [km]	Mean ± SD	3.7 ± 4.6	3.9 ± 5.2
Distance inpatient [km]	Mean ± SD	11.8 ± 11.7	11.9 ± 11.7
Height for age z-score	Mean ± SD	-1.1 ± 1.3	-1.1 ± 1.3
Weight for age z-score	Mean ± SD	-0.8 ± 1.2	-0.8 ± 1.1
Hemoglobin [g/dL]	Mean ± SD	9.8 ± 1.4	9.8 ± 1.4
Moderate anemia [Hb <8g/dL]	n (%)	323 (11.4)	144 (9.8)

N = number of subjects

n = number of subjects in a given category

SD = Standard deviation

None of the baseline characteristics differed by group ($p>0.05$)

Supplementary table 9. Vaccine efficacy: model selection using Cox regression with time dependent covariates for clinical malaria disease (primary case definition) (ATP)

Model	-2 log likelihood	Parameters	Akaike's criterion	Schwarz Bayesian criterion [§]
No time-varying covariates	19837.4	1	19839.4	19844.9
group*time ⁻²	19834.7	2	19838.7	19849.6
group*time ⁻¹	19825.8	2	19829.8	19840.7
group*time ^{-0.5}	19807.9	2	19811.9	19822.8
group*log(time)	19787.6	2	19791.6	19802.5
group*time	19779.1	2	19783.1	19793.9
group*time ^{0.5}	19778.6	2	19782.6	19793.4
group*time ²	19789.7	2	19793.7	19804.6
Piecewise Cox regression ^{§§}	19788.5	3	19794.5	19810.8

[§]Lowest Swartz Bayesian Criterion corresponds to best model fit among pre-specified models evaluated

^{§§}Piecewise Cox regression splitting the time at risk in three periods, allowing in each one a third of the cases

Supplementary table 10a. Clinical features of children with severe malaria aged 6 weeks - 17 months at enrollment (ATP)

		All episodes primary case definition N = 282	
Frequency of markers of disease severity		n	%
Prostration		63	22.3
Respiratory distress		24	8.5
BCS ≤ 2		20	7.1
Seizures 2 or more		100	35.5
Hypoglycemia < 2.2 mmol/L		12	4.3
Acidosis BE ≤ -10 mmol/L		147	52.1
Lactate ≥ 5 mmol/L		83	29.4
Anemia < 5 g/dL		51	18.1
Number of disease markers	1	162	57.4
	2	62	22.0
	3	34	12.1
	4	14	5.0
	5	4	1.4
	6	6	2.1
Number of cases per site	Agogo	28	9.9
	Bagamoyo	7	2.5
	Kilifi	0	0.0
	Kintampo	62	22.0
	Kombewa	55	19.5
	Korogwe	2	0.7
	Lambarene	7	2.5
	Lilongwe	10	3.5
	Manhica	1	0.4
	Nanoro	21	7.4
	Siaya	89	31.6
Number of cases per age	5-17 months	216	76.6
	6-12 weeks	66	23.4

N = number of events meeting the primary case definition

n = number of events meeting the primary case definition in a given category

% = n / Number of events meeting the primary case definition with available results

A total of 265 children experienced at least one episode of severe malaria. Overall, 282 cases of severe malaria occurred in these 265 children.

Supplementary table 10b. Frequency of co-morbidities in children with severe malaria 6 weeks - 17 months of age at enrollment (ATP)

	All episodes, secondary case definition N = 339	
	n	%
No co-morbidity detected	282	83.2
Pneumonia	6	1.8
Meningitis	1	0.3
Bacteremia*	17	5.0
Gastroenteritis	1	0.3
Co-morbidity not excluded (Algorithm not completed)	39	11.9

N = number of events meeting the secondary case definition

n = number of events meeting the case definition in a given category

% = n / Number of events meeting the secondary case definition with available results

* Salmonella 10 (2.9%), *Streptococcus pneumoniae* 1 (0.3%), other pathogens 6 (1.8%)

The total percentage exceeds 100% because a child could have more than one co-morbidity during a severe malaria episode.

Supplementary table 11a. Percentage of children aged 5-17 months at enrollment reporting a fatal serious adverse event over an average follow-up period 18 months (up to 24 months) post Dose-1 by MEDRA Preferred Term (ITT)

	RTS,S/AS01 N = 5949				Rabies vaccine N = 2974			
	n	%	95% CI		n	%	95% CI	
Overview								
At least one symptom	56	0.9	0.7	1.2	28	0.9	0.6	1.4
At least one symptom excluding Malaria	56	0.9	0.7	1.2	28	0.9	0.6	1.4
Fatal SAEs by Preferred Term								
Anaemia	8	0.1	0.1	0.3	9	0.3	0.1	0.6
Disseminated intravascular coagulation	*1*				*1*			
Neutropenia	*2*				*2*			
Atrial septal defect	*1*				*1*			
Cerebral palsy	*1*				*1*			
Enteritis	*1*				*1*			
Death	1	0.0	0.0	0.1	1	0.0	0.0	0.2
Drowning	3	0.1	0.0	0.1	1	0.0	0.0	0.2
Hypothermia	*1*				*1*			
Pyrexia	1	0.0	0.0	0.1	1	0.0	0.0	0.2
Bronchitis	*1*				*1*			
Bronchopneumonia	3	0.1	0.0	0.1	1	0.0	0.0	0.2
Disseminated tuberculosis	*1*				*1*			
Dysentery	*2*				*2*			
Encephalitic infection	*1*				*1*			
Gastroenteritis	16	0.3	0.2	0.4	6	0.2	0.1	0.4
Gastroenteritis shigella	*1*				*1*			
Hiv infection	12	0.2	0.1	0.4	7	0.2	0.1	0.5
Klebsiella sepsis	*1*				*1*			
Lobar pneumonia	*1*				*1*			
Lower respiratory tract infection	*1*				*1*			
Lymph node tuberculosis	*1*				*1*			
Malaria	3	0.1	0.0	0.1	6	0.2	0.1	0.4
Measles	*1*				*1*			
Meningitis	4	0.1	0.0	0.2	1	0.0	0.0	0.2
Oral candidiasis	*2*				*2*			
Oropharyngeal candidiasis	*1*				*1*			
Otitis media	1	0.0	0.0	0.1	1	0.0	0.0	0.2
Pneumococcal sepsis	*2*				*2*			
Pneumocystis jiroveci infection	*1*				*1*			
Pneumocystis jiroveci pneumonia	*1*				*1*			
Pneumonia	16	0.3	0.2	0.4	5	0.2	0.1	0.4
Salmonella sepsis	1	0.0	0.0	0.1	2	0.1	0.0	0.2
Sepsis	8	0.1	0.1	0.3	5	0.2	0.1	0.4
Skin bacterial infection	*1*				*1*			
Upper respiratory tract infection	*1*				*1*			
Urinary tract infection	*1*				*1*			
Herbal toxicity	2	0.0	0.0	0.1	1	0.0	0.0	0.2
Hypoglycaemia	3	0.1	0.0	0.1	1	0.0	0.0	0.2
Kwashiorkor	2	0.0	0.0	0.1	1	0.0	0.0	0.2
Malnutrition	8	0.1	0.1	0.3	3	0.1	0.0	0.3
Marasmus	7	0.1	0.0	0.2	2	0.1	0.0	0.2
Brain neoplasm	*1*				*1*			
Convulsion	4	0.1	0.0	0.2	8	0.3	0.1	0.5
Encephalitis	*1*				*1*			

Febrile convulsion	*2*				*2*			
Asphyxia	*1*				*1*			
Pneumonia aspiration	2	0.0	0.0	0.1	1	0.0	0.0	0.2
Pulmonary oedema	*1*				*1*			
Shock	*2*				*2*			

At least one symptom = at least one symptom experienced, regardless of the MedDRA Preferred Term

At least one symptom excluding Malaria= at least one symptom experienced (regardless of the MedDRA Preferred Term), excluding Malaria, *P. falciparum* infection and Cerebral malaria.

N = number of subjects with at least one administered dose

n/% = number/percentage of subjects reporting the symptom at least once

95% CI= exact 95% confidence interval; LL = Lower Limit, UL = Upper Limit

Tabulations that present single or multiple SAEs in one study group (RTS,S/AS01 or comparator) are presented in both study groups as *n*, indicating that there are n events in one of the study groups, to preserve the blind of the study.

Supplementary table 11b. Percentage of children aged 6-12 weeks at enrollment reporting a fatal serious adverse event over an average follow-up period of 9 months (up to 17 months) post Dose-1 by MEDRA Preferred Term (ITT)

	RTS,S/AS01 N = 4358				MenC vaccine N = 2179			
	n	%	95% CI		n	%	95% CI	
Overview								
At least one symptom	49	1.1	0.8	1.5	18	0.8	0.5	1.3
At least one symptom excluding Malaria	49	1.1	0.8	1.5	18	0.8	0.5	1.3
Fatal SAEs by Preferred Term								
Anaemia	5	0.1	0.0	0.3	1	0.0	0.0	0.3
Haemolytic anaemia	*1*				*1*			
Congenital megacolon	*1*				*1*			
Falot's tetralogy	*1*				*1*			
Enteritis	1	0.0	0.0	0.1	1	0.0	0.0	0.3
Intussusception	*1*				*1*			
Death	1	0.0	0.0	0.1	1	0.0	0.0	0.3
Drowning	*1*				*1*			
Pyrexia	*2*				*2*			
Bronchitis	*1*				*1*			
Bronchopneumonia	5	0.1	0.0	0.3	2	0.1	0.0	0.3
Febrile infection	*1*				*1*			
Gastroenteritis	13	0.3	0.2	0.5	4	0.2	0.1	0.5
Hiv infection	9	0.2	0.1	0.4	2	0.1	0.0	0.3
Malaria	*1*				*1*			
Meningitis	*3*				*3*			
Meningitis pneumococcal	*1*				*1*			
Pneumococcal sepsis	1	0.0	0.0	0.1	2	0.1	0.0	0.3
Pneumocystis jiroveci pneumonia	*3*				*3*			
Pneumonia	19	0.4	0.3	0.7	6	0.3	0.1	0.6
Pneumonia primary atypical	*1*				*1*			
Salmonella sepsis	*1*				*1*			
Sepsis	7	0.2	0.1	0.3	3	0.1	0.0	0.4
Septic shock	*1*				*1*			
Tuberculosis	*1*				*1*			
Urinary tract infection	*1*				*1*			
Head injury	*2*				*2*			
Herbal toxicity	*1*				*1*			
Hypoglycaemia	1	0.0	0.0	0.1	2	0.1	0.0	0.3
Kwashiorkor	*1*				*1*			
Marasmus	2	0.0	0.0	0.2	1	0.0	0.0	0.3
Convulsion	3	0.1	0.0	0.2	2	0.1	0.0	0.3
Encephalitis	*2*				*2*			
Loss of consciousness	*1*				*1*			
Pneumonia aspiration	*2*				*2*			

At least one symptom = at least one symptom experienced, regardless of the MedDRA Preferred Term

At least one symptom excluding Malaria= at least one symptom experienced (regardless of the MedDRA Preferred Term), excluding Malaria, *P. falciparum* infection and Cerebral malaria.

N = number of subjects with at least one administered dose

n/% = number/percentage of subjects reporting the symptom at least once

95% CI= exact 95% confidence interval; LL = Lower Limit, UL = Upper Limit

Tabulations that present single or multiple SAEs in one study group (RTS,S/AS01 or comparator) are presented in both study groups as *n*, indicating that there are n events in one of the study groups, to preserve the blind of the study.

Supplementary table 12. Percentage of children aged 5-17 months at enrollment reporting adverse events (AEs) within the 30-day post-vaccination period (ITT)

	RTS,S/AS01 N = 1479				Rabies vaccine N = 721			
	n	%	95% CI		n	%	95% CI	
LL			UL	LL			UL	
Overview								
At least one AE	1273	86.1	84.2	87.8	626	86.8	84.1	89.2
At least one symptom excluding Malaria	1251	84.6	82.6	86.4	612	84.9	82.1	87.4
AEs with an incidence ≥ 5% by Preferred Term								
Conjunctivitis	111	7.5	6.2	9.0	64	8.9	6.9	11.2
Diarrhea	188	12.7	11.1	14.5	88	12.2	9.9	14.8
Enteritis	124	8.4	7.0	9.9	62	8.6	6.7	10.9
Pyrexia	200	13.5	11.8	15.4	67	9.3	7.3	11.7
Bronchitis	79	5.3	4.3	6.6	32	4.4	3.1	6.2
Gastroenteritis	372	25.2	23.0	27.4	170	23.6	20.5	26.9
Malaria	265	17.9	16.0	20.0	173	24.0	20.9	27.3
Nasopharyngitis	115	7.8	6.5	9.3	58	8.0	6.2	10.3
Pneumonia	166	11.2	9.7	12.9	71	9.8	7.8	12.3
Rhinitis	95	6.4	5.2	7.8	34	4.7	3.3	6.5
Upper respiratory tract infection	638	43.1	40.6	45.7	326	45.2	41.5	48.9
Cough	107	7.2	6.0	8.7	41	5.7	4.1	7.6

At least one AE = at least one AE experienced, regardless of the MedDRA Preferred Term
 At least one symptom excluding Malaria= at least one symptom experienced (regardless of the MedDRA Preferred Term), excluding Malaria, *P. falciparum* infection and Cerebral malaria.
 N = number of subjects with at least one administered dose. AEs during the first 30 days post-vaccination were recorded and analyzed only in the first 200 subjects enrolled at each site.
 n/% = number/percentage of subjects reporting the symptom at least once
 95% CI= exact 95% confidence interval; LL = Lower Limit, UL = Upper Limit

Supplementary table 13a. Incidence of solicited local adverse events in children aged 5-17 months at enrollment reported during the 7-day post-vaccination period following each dose and overall (ITT)

Symptom	Type	RTS,S/AS01				Rabies vaccine			
		n	%	95 % CI		N	%	95 % CI	
				LL	UL			LL	UL
		Dose 1 N = 1479				Dose 1 N = 721			
Pain	All	247	16.7	14.8	18.7	61	8.5	6.5	10.7
	Grade 3	0	0.0	0.0	0.2	0	0.0	0.0	0.5
Redness (mm)	All	66	4.5	3.5	5.6	26	3.6	2.4	5.2
	Grade 3	2	0.1	0.0	0.5	0	0.0	0.0	0.5
Swelling (mm)	All	140	9.5	8.0	11.1	77	10.7	8.5	13.2
	Grade 3	6	0.4	0.1	0.9	0	0.0	0.0	0.5
		Dose 2 N = 1435				Dose 2 N = 708			
Pain	All	179	12.5	10.8	14.3	41	5.8	4.2	7.8
	Grade 3	3	0.2	0.0	0.6	0	0.0	0.0	0.5
Redness (mm)	All	26	1.8	1.2	2.6	18	2.5	1.5	4.0
	Grade 3	3	0.2	0.0	0.6	0	0.0	0.0	0.5
Swelling (mm)	All	140	9.8	8.3	11.4	50	7.1	5.3	9.2
	Grade 3	15	1.0	0.6	1.7	0	0.0	0.0	0.5
		Dose 3 N = 1407				Dose 3 N = 699			
Pain	All	108	7.7	6.3	9.2	22	3.1	2.0	4.7
	Grade 3	0	0.0	0.0	0.3	0	0.0	0.0	0.5
Redness (mm)	All	42	3.0	2.2	4.0	13	1.9	1.0	3.2
	Grade 3	2	0.1	0.0	0.5	0	0.0	0.0	0.5
Swelling (mm)	All	134	9.5	8.0	11.2	35	5.0	3.5	6.9
	Grade 3	9	0.6	0.3	1.2	0	0.0	0.0	0.5
		Overall/dose N = 4321				Overall/dose N = 2128			
Pain	All	534	12.4	11.4	13.4	124	5.8	4.9	6.9
	Grade 3	3	0.1	0.0	0.2	0	0.0	0.0	0.2
Redness (mm)	All	134	3.1	2.6	3.7	57	2.7	2.0	3.5
	Grade 3	7	0.2	0.1	0.3	0	0.0	0.0	0.2
Swelling (mm)	All	414	9.6	8.7	10.5	162	7.6	6.5	8.8
	Grade 3	30	0.7	0.5	1.0	0	0.0	0.0	0.2

N= number of administered doses. AEs during the first 30 days post-vaccination were recorded and analyzed only in the first 200 subjects enrolled at each site.

n/%= number/percentage of doses followed by at least one type of symptom

95%CI= Exact 95% confidence interval; LL = lower limit, UL = upper limit

Supplementary table 13b. Incidence of solicited general adverse events in children aged 5-17 months at enrollment reported during the 7-day post-vaccination period following each dose and overall (ITT)

Symptom	Type	RTS,S/AS01				Rabies vaccine			
		n	%	95 % CI		n	%	95 % CI	
		Dose 1 N = 1479				Dose 1 N = 721			
		LL	UL	LL	UL	LL	UL	LL	UL
Drowsiness	All	91	6.2	5.0	7.5	27	3.7	2.5	5.4
	Grade 3	3	0.2	0.0	0.6	0	0.0	0.0	0.5
Irritability	All	165	11.2	9.6	12.9	41	5.7	4.1	7.6
	Grade 3	0	0.0	0.0	0.2	0	0.0	0.0	0.5
Loss of appetite	All	202	13.7	11.9	15.5	71	9.8	7.8	12.3
	Grade 3	3	0.2	0.0	0.6	0	0.0	0.0	0.5
Temperature	All	385	26.0	23.8	28.3	108	15.0	12.5	17.8
	Grade 3	29	2.0	1.3	2.8	7	1.0	0.4	2.0
		Dose 2 N = 1435				Dose 2 N = 708			
Drowsiness	All	99	6.9	5.6	8.3	37	5.2	3.7	7.1
	Grade 3	1	0.1	0.0	0.4	0	0.0	0.0	0.5
Irritability	All	192	13.4	11.7	15.3	45	6.4	4.7	8.4
	Grade 3	2	0.1	0.0	0.5	0	0.0	0.0	0.5
Loss of appetite	All	151	10.5	9.0	12.2	47	6.6	4.9	8.7
	Grade 3	0	0.0	0.0	0.3	0	0.0	0.0	0.5
Temperature	All	503	35.1	32.6	37.6	100	14.1	11.6	16.9
	Grade 3	42	2.9	2.1	3.9	10	1.4	0.7	2.6
		Dose 3 N = 1407				Dose 3 N = 699			
Drowsiness	All	97	6.9	5.6	8.3	29	4.1	2.8	5.9
	Grade 3	1	0.1	0.0	0.4	0	0.0	0.0	0.5
Irritability	All	138	9.8	8.3	11.5	27	3.9	2.6	5.6
	Grade 3	1	0.1	0.0	0.4	0	0.0	0.0	0.5
Loss of appetite	All	138	9.8	8.3	11.5	40	5.7	4.1	7.7
	Grade 3	2	0.1	0.0	0.5	0	0.0	0.0	0.5
Temperature	All	457	32.5	30.0	35.0	77	11.0	8.8	13.6
	Grade 3	39	2.8	2.0	3.8	7	1.0	0.4	2.1
		Overall/dose N = 4321				Overall/dose N = 2128			
Drowsiness	All	287	6.6	5.9	7.4	93	4.4	3.5	5.3
	Grade 3	5	0.1	0.0	0.3	0	0.0	0.0	0.2
Irritability	All	495	11.5	10.5	12.4	113	5.3	4.4	6.3
	Grade 3	3	0.1	0.0	0.2	0	0.0	0.0	0.2
Loss of appetite	All	491	11.4	10.4	12.3	158	7.4	6.3	8.6
	Grade 3	5	0.1	0.0	0.3	0	0.0	0.0	0.2
Temperature	All	1345	31.1	29.7	32.5	285	13.4	12.0	14.9
	Grade 3	110	2.5	2.1	3.1	24	1.1	0.7	1.7

N= number of administered doses. AEs during the first 30 days post-vaccination were recorded and analyzed only in the first 200 subjects enrolled at each site.

n/%= number/percentage of doses followed by at least one type of symptom

95%CI= Exact 95% confidence interval; LL = lower limit, UL = upper limit

Supplementary table 14. Seropositivity rates and geometric mean titers (GMTs) for Anti-CS antibodies (ATP)

			≥ 0.5 EU/mL				GMT				
					95% CI			95% CI			
Group	Timing	N	n	%	LL	UL	value	LL	UL	Min	Max
RTS,S/AS01	Screening	1034	132	12.8	10.8	15.0	0.3	0.3	0.3	<0.5	7.7
	1 month post-Dose 3	1033	1032	99.9	99.5	100	621.2	591.7	652.1	<0.5	8147.2
Rabies vaccine	Screening	524	46	8.8	6.5	11.5	0.3	0.3	0.3	<0.5	16.2
	1 month post-Dose 3	528	31	5.9	4.0	8.2	0.3	0.3	0.3	<0.5	329.9

GMT = geometric mean antibody titer calculated on all subjects

N = number of subjects with available results. Immunogenicity data were analyzed only in the first 200 subjects enrolled at each site.

n = number of subjects with antibody titer greater than or equal cut-off

n/% = number/percentage of subjects with concentration within the specified range

95% CI = 95% confidence interval; LL = Lower Limit, UL = Upper Limit

Min/Max = Minimum/Maximum