Supplementary Methods

Investigator and sponsor roles.

The study was sponsored by GlaxoSmithKline Biologicals (GSK), the vaccine developer and manufacturer, and funded by the PATH Malaria Vaccine Initiative. The study was designed by; Philip Bejon, John Lusingu, Ally Olotu, Amanda Leach, Marc Lievens, Johan Vekemans, Trudie Lang, Marie-Claude Dubois, Chris J. Drakeley, Tonya Villafana, W. Ripley Ballou, Joe Cohen, Eleanor M Riley, Martha M. Lemnge, Kevin Marsh and Lorenz von Seidlein. Data were gathered by; John Lusingu, Ally Olotu, Salum Msham, Marie-Ange Demoitié, Patricia Njuguna, Ken Awuondo, Anangisye Malabeja, Omar Abdul, Samwel Gesase, Neema Mturi and Lorenz von Seidlein. Philip Bejon, Lorenz von Seidlein and Marc Lievens vouch for the data and analysis. The first draft of the manuscript was written by Philip Bejon. All authors contributed to revision of the manuscript, and these revisions were co-ordinated by Philip Bejon. Scientific writers employed by GSK Biologicals suggested minor revisions of the manuscript (Conor Cahill and Marie-Sylvie Remacle). The data were subject to a confidentiality agreement between the sponsor and investigators, which established full access to the study data by the investigators and included an obligation to permit publication without excessive delay.

Location and site details.

The study was carried out in two sites, recruiting similar numbers of children. In Kilifi, Kenya, children were recruited in two administrative locations (Pingilikani and

Junju), within the Chonyi area in the southern part of Kilifi District. In Tanzania, children were recruited from the catchment areas of three dispensaries (Ngombezi, Mbagai and Makuyuni) in Korogwe district, Tanga Region. Both sites are malaria endemic, with all year round transmission and two high transmission seasons [1]. The transmission intensity has previously been measured as 22-53 infective bites per year in Junju, Kilifi and 90 bites per year in Korogwe [2, 3], although the present transmission intensity is probably much lower [4, 5]. There are successful ITN distribution programmes in both countries [6, 7], and artemether/lumefantrine was the first line anti-malarial treatment. There were no insecticide spraying campaigns in the area at the time of the study. Both areas are rural, and most of the population are subsistence farmers.

Study Participants: Screening.

The participating children were aged 5-17 months old (inclusive) at the time of first vaccination, healthy, and resident in the study area. Children were screened by history, clinical examination and blood tests (full blood count, creatinine and alanine aminotransferase). Subjects with clinically significant illness, severe malnutrition (defined as weight for age z score <-3) or out of range blood tests, were excluded. Clinically evident immunosuppression was one of the criteria for exclusion, but HIV testing was not conducted. Subjects were referred to an appropriate service for management of illnesses identified at screening. Subjects were recruited following public meetings and invitations to attend the local dispensary for a screening visit. All subjects were provided with study identification cards.

Vaccines

Children were randomized to receive three doses of RTS,S/AS01E, i.e. RTS,S with the proprietary Adjuvant System AS01E comprising liposomes, MPL (3-D-deacylated Monophosphoryl Lipid A) and QS21 (a triterpene glycoside purified from the bark of Quillaja saponaria) or 3 doses of Sanofi-Pasteur's human diploid cell rabies vaccine. In the description "RTS,S/AS01E","RTS,S" describes the carboxyl terminal part of the circumsporozoite protein fused to the hepatitis B surface antigen, co-expressed in yeast with the non-fused hepatitis B surface antigen, "AS01" indicates the Adjuvant System, and the letter suffix "E"; indicates a pediatric dose formulation of the AS01 Adjuvant System. The acceptable interval between vaccinations was from 20 to 60 days.

Assessment of Safety.

After each vaccination children were observed for one hour after which solicited adverse events were recorded by trained fieldworkers. Fieldworkers then visited subjects daily for the first 6 days after vaccination to record solicited and unsolicited adverse events. All severe adverse events were assessed by a study clinician. Data relating to all malaria admissions to hospital were reviewed to define cases of severe malaria disease by five clinically qualified investigators prior to unblinding. Episodes of clinical malaria detected in surveillance to determine efficacy were not considered to be adverse events. Severe adverse events (SAEs) were categorised according to the

preferred term from the MedDRA® database, allocated before unblinding. Non-malaria SAEs were defined as those which excluded the MedDRA terms "*Plasmodium falciparum* infection", "Malaria" and "Cerebral malaria". A grade was assigned all adverse events as follows; grade 1 (easily tolerated), grade 2 (interference with everyday activities) and grade 3 (prevents everyday activities). Blood tests for routine biochemistry (plasma alanine aminotransferase and creatinine) and hematology (full blood counts) were conducted at all cross-sectional bleeds. A detailed report of safety data will be given elsewhere.

Monitoring for Clinical Malaria Episodes.

Both active and passive case detection was established. For active case detection, children were visited every week by fieldworkers. The parent/guardian was asked whether they thought the child had fever, and the axillary temperature was measured. When the temperature was greater than or equal to 37.5 degrees, a blood film was made and a rapid near-patient test (Optimal®) for malaria was conducted. Rapid test results were used to determine treatment decisions, but the blood film results (read in duplicate) were used to define the study endpoint. Treatment for episodes of malaria was with artemether-lumefantrine. Children requiring admission and too unwell to take oral medication were treated with intravenous quinine.

When the parent/guardian reported that the child was febrile but there was no objectively elevated temperature, blood films and rapid tests were not taken, but the fieldworker returned to the child after 6-12 hours. Rapid tests and blood films were taken if the temperature was elevated at the second visit.

Parents could bring their children for assessment between scheduled visits if they thought the child had developed fever, and the child was assessed in the same way. Fieldworkers were stationed in the study villages, and so were readily accessible to the parents. Passive case detection was also established in local dispensaries providing care to the population.

There was telephone contact with a clinician for all unwell children, and parents were advised to bring their children to the dispensary for assessment if indicated. Children requiring hospital admission were referred to the local district hospital. Criteria for severe malaria were derived from the WHO definition. [8] These were asexual *P falciparum* parasitemia, no other more-probable cause of illness, and one of the three following sub-groups; a) severe malaria anemia (hemoglobin<5g/dL), b) cerebral malaria (Blantyre coma score <2), c) multiple seizures (two or more generalised convulsions in 24 h), prostration, hypoglycaemia (<22 mmol/L), acidosis, or shock.

Laboratory Methods

Antibodies to the circumsporozoite protein (CS) tandem repeat epitope were assessed by ELISA. The antibody response to the *P. falciparum* CS repeat region (designated anti-CS) was measured at CEVAC, Ghent, Belgium and results were reported in EU/mL. Plates were adsorbed with the recombinant antigen R32LR that contains the sequence $[NVDP(NANP)_{15}]_2LR$.

Thick and thin films for parasite density readings were made in the laboratories in Kilifi and Korogwe and stained with giemsa. Parasite densities were calculated using

contemporaneous full blood counts. Films were read in duplicate, and by a third reader if one film was positive and the other negative or if the calculated densities differed by more than tenfold when one density was below 400 parasites/μl or if the calculated densities differed by twofold when both densities were above 400 parasite/μl. The final result was the geometric mean density of two readings. Where three positive readings were available, the geometric mean of the closest two readings was taken as the final result. Where there was a discrepancy between positive and negative readings, the majority result was taken as final. Final density results were calculated for all films before unblinding the study. A 3rd slide reading was required on 4% of all films. 51% of these instances were among RTS,S/AS01E vaccinees. An external quality assurance process was used to accredit slide readers throughout the trial. Blood films were made for febrile children, and for all children on the second cross-sectional bleed.

Data Analysis

An analysis plan was agreed by the DSMB, Sponsor and investigators prior to unblinding. The primary analysis was the hazard ratio of first or only episode of malaria meeting the primary case definition (defined as fever with parasitemia above 2500/μL), according to vaccination group. The according to protocol (ATP) cohort included all children who were eligible to be enrolled according to the protocol, and who received all three vaccinations within acceptable time limits (defined prior to unblinding). Data from the ATP cohort were analyzed from two and a half months after randomization (i.e. two weeks after a complete vaccination course) until the final cross-sectional bleed. Data from the intention to treat (ITT) cohort were analysed

from the time of randomization (i.e. at first vaccination) until the final cross-sectional bleed. As pre-specified in the analysis plan, the calculated time at risk for Cox and Poisson regression excluded absences from the study area and 7-28 days after administering anti-malarials (depending on the estimated duration of action). Home visits were used to identify absences. Secondary analyses included the secondary case definition (any parasitemia with fever), primary and secondary case definitions for the intention to treat (ITT) cohort (i.e. all children randomized to receive vaccinations) and multiple episodes (by Poisson regression). The hazard ratios and 95% confidence intervals for ATP analyses were estimated by Cox regression, adjusted for covariates known to influence the risk of presentation with malaria in the study area; i.e. (a) age of the subject, (b) bednet use (in two categories; (i) sleeping under a treated net every night, or an untreated net with less than three holes into which a finger could comfortably fit, versus (ii) sleeping under an untreated net with three holes or more or not using a bednet[9]), (c) geographical location (defined by 7 clusters of villages across the two sites) and (d) distance from the dispensary. Hazard ratios were transformed to give vaccine efficacy.

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