



Immunogenicity and safety of the candidate RTS,S/AS01 vaccine in young Nigerian children: A randomized, double-blind, lot-to-lot consistency trial



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ABSTRACT

Background: For regulatory approval, consistency in manufacturing of vaccine lots is expected to be demonstrated in confirmatory immunogenicity studies using two-sided equivalence trials. This randomized, double-blind study (NCT01323972) assessed consistency of three RTS,S/AS01 malaria vaccine batches formulated from commercial-scale purified antigen bulk lots in terms of anti-CS-responses induced.

Methods: Healthy children aged 5–17 months were randomized (1:1:1:1) to receive RTS,S/AS01 at 0–1–2 months from one of three commercial-scale purified antigen bulk lots (1600 litres-fermentation scale; commercial-scale lots), or a comparator vaccine batch made from pilot-scale purified antigen bulk lot (20 litres-fermentation scale; pilot-scale lot). The co-primary objectives were to first demonstrate consistency of antibody responses against circumsporozoite (CS) protein at one month post-dose 3 for the three commercial-scale lots and second demonstrate non-inferiority of anti-CS antibody responses at one month post-dose 3 for the commercial-scale lots compared to the pilot-scale lot. Safety and reactogenicity were evaluated as secondary endpoints.

Results: One month post-dose-3, anti-CS antibody geometric mean titres (GMT) for the 3 commercial scale lots were 319.6 EU/ml (95% confidence interval (CI): 268.9–379.8), 241.4 EU/ml (207.6–280.7), and 302.3 EU/ml (259.4–352.3). Consistency for the RTS,S/AS01 commercial-scale lots was demonstrated as the two-sided 95% CI of the anti-CS antibody GMT ratio between each pair of lots was within the range of 0.5–2.0. GMT of the pooled commercial-scale lots (285.8 EU/ml (260.7–313.3)) was non-inferior to the pilot-scale lot (271.7 EU/ml (228.5–323.1)). Each RTS,S/AS01 lot had an acceptable tolerability profile, with infrequent reports of grade 3 solicited symptoms. No safety signals were identified and no serious adverse events were considered related to vaccination.

Conclusions: RTS,S/AS01 lots formulated from commercial-scale purified antigen bulk batches induced a consistent anti-CS antibody response, and the anti-CS GMT of pooled commercial-scale lots was non-inferior to that of a lot formulated from a pilot-scale antigen bulk batch.

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Abbreviations: AE, adverse event; ATP, according-to-protocol; CI, confidence interval; CS, circumsporozoite; ELISA, enzyme-linked immunosorbent assay; GMT, geometric mean titre; HBs, hepatitis B surface antigen; Ig, immunoglobulin; MPL, monophosphoryl lipid A; QS21, *Quillaja saponaria* Molina, fraction 21; SAE, serious adverse event; SAS, Statistical Analysis System.

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1. Introduction

The RTS,S/AS01 candidate malaria vaccine targets the *Plasmodium falciparum* circumsporozoite (CS) protein, therefore acting at the pre-erythrocytic stage of the parasite life cycle [1]. This is a partially efficacious vaccine, which has shown protection against both clinical and severe malaria in young children and infants in a large phase 3 trial in Africa [2,3], and has an acceptable safety profile when co-administered with vaccines included in the routine Expanded Programme on Immunization [2–4].

For regulatory approval of a new vaccine, it is necessary to demonstrate the quality of the manufacturing process, including consistency in the manufacturing of vaccine lots [5–7]. The assessment is expected to be performed in confirmatory immunogenicity studies using two-sided equivalence trials [8,9]. This study evaluated the consistency and safety of three different RTS,S/AS01 vaccine lots formulated from commercial-scale purified antigen bulk lots. The co-primary objectives were to demonstrate lot-to-lot consistency in terms of anti-CS antibody responses and, if reached, subsequently to demonstrate non-inferiority of the commercial-scale lots to a RTS,S/AS01 vaccine lot derived from pilot-scale purified antigen bulk material.

2. Methods

2.1. Study design and ethics

This was a phase III, randomized, double-blind study (ClinicalTrials.gov, NCT01323972) conducted at two sites between May 2011 and May 2012: University of Nigeria Teaching Hospital in Enugu, which is located in south-east Nigeria, and Jos University Teaching Hospital in Jos, which is in north-central Nigeria.

The production scale of the RTS,S purified bulk antigen was increased from 20 litres-fermentation (pilot-plant scale, produced in January 2010; hereafter referred to as pilot-scale lot) to 1600 litres-fermentation (commercial-scale scale in commercial facilities, produced in October/November 2010; hereafter referred to as commercial-scale lots). The same starting material was used at both manufacturing scales, and the components of the final vaccine, including the adjuvant system, remained identical. Eligible children were randomized (1:1:1:1) to receive one of three different commercial-scale lots (lot 1, 2 or 3) or the pilot-scale lot (comparator) of RTS,S/AS01 vaccine according to a 0, 1 and 2 month schedule.

A randomization list was generated by the study sponsor via an internet-based system, and treatment allocation at each site was performed using MATEX, a program developed for Statistical Analysis System (SAS®; Cary, NC, USA).

The study protocol was approved by the ethics review committee of each study site and by the National Agency for Food and Drug Administration and Control in Nigeria and Western Institutional Review Board in the USA. Overall, this study was conducted in accordance with Good Clinical Practice guidelines and all applicable regulatory requirements, including the Declaration of Helsinki. The trial was conducted in partnership with the PATH Malaria Vaccine Initiative. An Independent Data Monitoring Committee oversaw the study's progress and safety of the children, assisted by a local safety monitor (an experienced physician) at each site.

2.2. Study population

Healthy children aged 5–17 months at the time of first vaccination were eligible for enrolment. As phase II evaluation of RTS,S/AS01 indicated that previous hepatitis B immunization may influence RTS,S-induced antibody responses in children [10], to be eligible for participation, all participants must have received three doses of hepatitis B vaccine before the study start. Exclusion criteria included a history of an immunodeficient or neurological condition, acute disease or fever (axillary temperature $\geq 37.5^\circ\text{C}$) at the time of enrolment, and an acute or chronic, clinically significant pulmonary, cardiovascular, hepatic or renal functional abnormality. Chronic administration of immune-modifying drugs was not permitted. Unapproved use of a drug or vaccine within 30 days before the first study vaccine dose and administration of a licensed vaccine within 7 days of the first dose were also exclusion criteria.

Written informed consent was obtained from the children's parents or guardians. Illiterate parents indicated consent with a thumbprint and a signature was obtained from an independent literate witness.

2.3. Study vaccine

Each vaccine dose contained lyophilized RTS,S (25 μg) reconstituted with 500 μl of AS01_E (referred to elsewhere in this paper as AS01), a liposome-based Adjuvant System containing monophosphoryl lipid A (MPL) and *Quillaja saponaria* Molina, fraction 21 (QS21, Antigenics Inc., a wholly owned subsidiary of Agenus Inc., Lexington, Massachusetts, USA). The vaccines were administered intramuscularly to the deltoid muscle of the left arm and vaccine recipients were observed for at least 60 min following each vaccination with appropriate medical treatment available in case of anaphylactic shock.

2.4. Study objectives

The co-primary objectives of the study were to first demonstrate consistency of anti-CS antibody responses at one month post-dose 3 for three commercial-scale RTS,S/AS01 lots. If the first primary objective was met, then the second primary objective was to demonstrate non-inferiority of anti-CS antibody responses at one month post-dose 3 of the RTS,S/AS01 commercial-scale lots compared to the pilot-scale lot. The safety and reactogenicity of the vaccine lots were evaluated as secondary endpoints.

2.5. Immunogenicity assessment

Assessment of anti-CS and anti-hepatitis B surface antigen (anti-HBs) antibody titres were performed at the Centre for Vaccinology, Ghent University, Belgium, on serum samples taken before dose 1 and one month after dose 3. Antibodies against CS were measured by evaluating immunoglobulin (Ig) G responses to the CS-repeat region, using a validated enzyme-linked immunosorbent assay (ELISA) with R32LR as the capture antigen and a threshold for a positive titre of 0.5 EU/ml [11]. Anti-HBs antibodies were measured using an in-house sandwich ELISA. The cut-off for seroprotection was 10 mIU/ml [12].

2.6. Safety and reactogenicity assessments

Solicited local (injection site pain, redness and swelling) and general (drowsiness, irritability, loss of appetite and fever) adverse events (AEs) were recorded during the 7-day follow-up, and unsolicited AEs during the 30-day follow-up, after each vaccine dose. Serious AEs (SAEs) were reported throughout the study. Grade 3 (severe) solicited AEs were defined as follows: pain causing crying when limb is moved/spontaneously painful, swelling or redness >20 mm in diameter, drowsiness that prevented normal daily activity, irritability (crying that could not be comforted) that prevented normal activity, loss of appetite (not eating at all), fever with axillary temperature $>39.0^\circ\text{C}$, or any other AE that prevented normal daily activity. All solicited local reactions were considered causally related to vaccination; the relationship of other AEs was classified as possible or not causally related. Fever (temperature $>37.5^\circ\text{C}$) was evaluated for cause by study investigators.

2.7. Statistical analyses

Statistical analyses were performed using SAS version 9.2 on Windows and StatXact-8.1 procedure on SAS.

A sample size of 80 children per group was planned to have at least 70 evaluable children in each group (3 lots of commercial-scale and 1 pilot-scale lot). This sample size had $>90\%$ power to

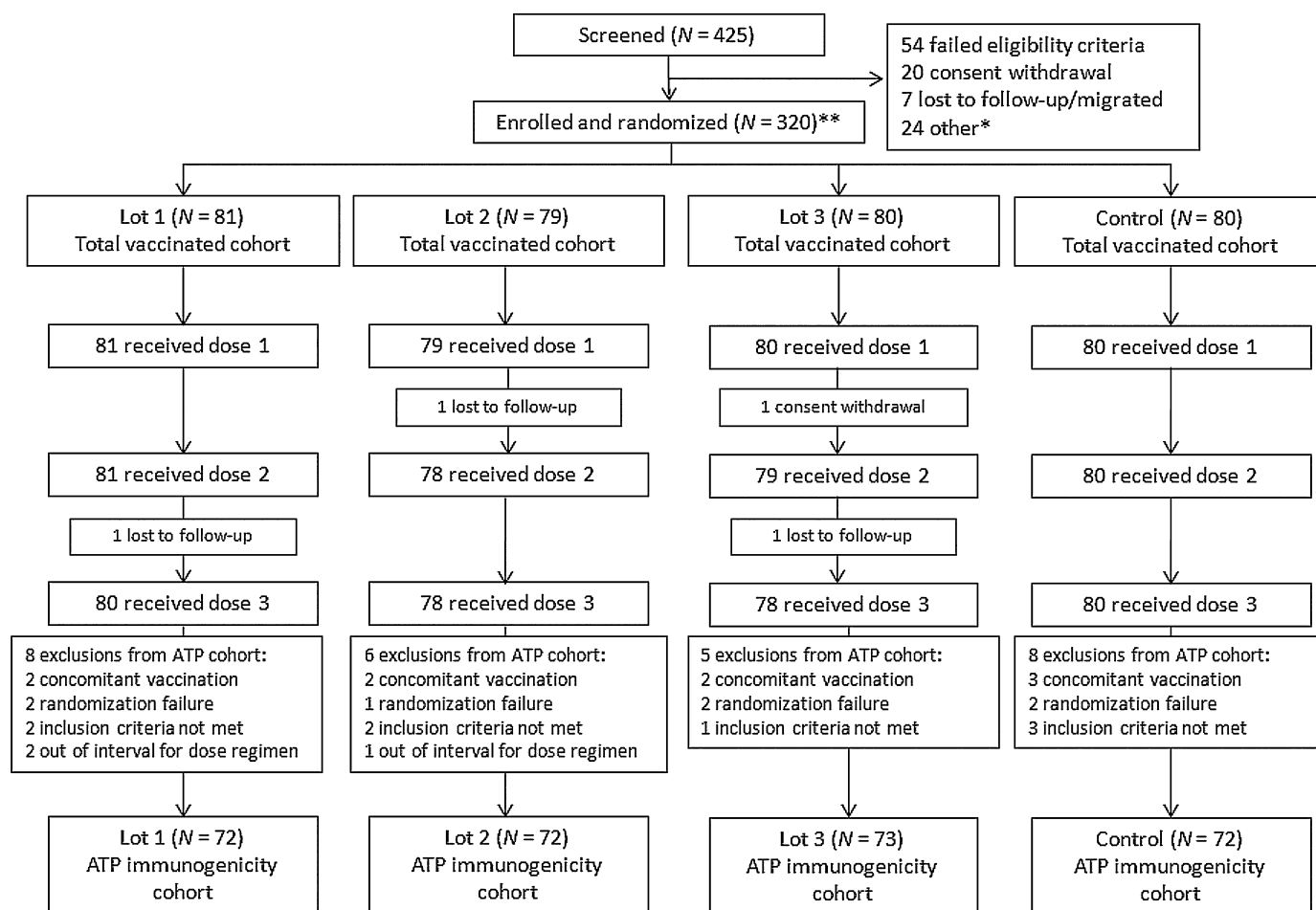


Fig. 1. Study profile. Footnote on the exclusion from ATP analyses: 7 subjects were excluded from the ATP analysis due to randomization failure: 5 subjects received 3 injections and were found at unblinding to have received vaccines from the wrong lot, and for 2 subjects one dose of the vaccine was switched due to an error in labelling at the investigator sites; 9 subjects received concomitant Hepatitis B vaccination in violation of protocol; 8 subjects were excluded for not meeting inclusion criteria: 7 subjects violated the weight-for-age Z-score criteria after recalculation by the GSK-statistician, and 1 subject was too old for inclusion after comparing the date-of-birth provided by parents with the date-of-birth provided on the vaccination card; 3 subjects were vaccinated outside the protocol-defined interval for vaccination.

reach the primary endpoint of equivalence of anti-CS antibody responses one month post-dose 3 between the three commercial-scale lots and, if reached, demonstrating non-inferiority of the pooled commercial-scale lots versus the pilot-scale lot in terms of anti-CS antibody response one month post-dose 3, using an alpha level of 5% (2-sided).

Immunogenicity analysis was performed on the according-to-protocol (ATP) cohort for immunogenicity, i.e. those meeting all eligibility criteria, complying with the procedures defined in the protocol. Anti-CS and anti-HBs antibody geometric mean titres (GMTs) were calculated with 95% confidence intervals (CIs). Percentages of subjects with seropositive levels of anti-CS antibodies (≥ 0.5 EU/ml) and seroprotective levels of anti-HBs antibodies (≥ 10 mIU/ml) were determined. Pairwise anti-CS antibody GMT ratios between the groups and their two-sided 95% CIs were computed using an ANOVA model on the $\log_{10}$ -transformed titre with the vaccine group as fixed effect. Lot-to-lot equivalence was concluded if all three 95% CIs on the GMT ratios were within the range 0.5–2, ruling out a 2-fold increase/decrease between each pair of lots. Non-inferiority of the pooled commercial-scale lots was demonstrated by evaluating the upper limit of the two-sided 95% CI of the GMT ratio of comparator pilot-scale lot and the pooled commercial-scale lots. If the upper limit of the two-sided 95% CI was below 2, non-inferiority was concluded. The co-primary endpoints were reached if the three equivalence criteria and the

non-inferiority criteria were reached, so no type 1 error rate adjustment was proposed; instead the type 2 error rate was adjusted to have sufficient overall power.

Safety analysis was conducted on the total vaccinated cohort. The percentage of doses followed by at least one solicited AE and percentage of children with an unsolicited AE were calculated with exact 95% CI.

3. Results

3.1. Study population

A total of 320 children (80 per group) were randomized 1:1:1:1 to 3 treatment groups receiving three doses of RTS,S/AS01 vaccine from one of three commercial-scale (1600L) lots or a comparator group, which received the RTS,S/AS01 vaccine pilot-scale (20L) lot. Despite best efforts to monitor the study as frequently as possible during a period of civil unrest in Nigeria, there were deviations which led to the exclusion of 27 of 316 subjects who received all 3 injections from the ATP analyses. Reasons for not receiving three vaccine doses and reasons for exclusion from the ATP cohort for immunogenicity are shown in Fig. 1.

Three children were withdrawn from the study because of migration from the study area, two because of consent withdrawal not due to an AE and three were lost to follow-up (Fig. 1). The

Table 1
Demographic characteristics (ATP cohort for immunogenicity).

	Commercial-scale lot 1 (N = 72)	Commercial-scale lot 2 (N = 72)	Commercial-scale lot 3 (N = 73)	Pilot-scale lot (comparator) (N = 72)
Mean age $\pm$ SD (months)	9.7 $\pm$ 3.2	10.2 $\pm$ 3.6	10.1 $\pm$ 3.2	10.2 $\pm$ 3.0
Mean weight-for-age Z-score $\pm$ SD	−0.7 $\pm$ 1.1	−0.8 $\pm$ 1.0	−0.6 $\pm$ 1.0	−0.8 $\pm$ 0.9
Gender (%), female/male	55.6/44.4	54.2/45.8	42.5/57.5	36.1/63.9

SD, standard deviation; N, number of children.

Table 2
Assessment of consistency among three commercial-scale vaccine lots in terms of anti-CS antibody geometric mean titre (GMT) ratios one month after the third RTS,S/AS01 vaccine dose (ATP cohort for immunogenicity).

Ratio order	N	Anti-CS antibody GMT ratio	
		Value	95% CI ^a
Commercial-scale Lot 1:Lot 2	72:72	1.32	1.06, 1.65
Commercial-scale Lot 1:Lot 3	72:73	1.06	0.85, 1.32
Commercial-scale Lot 2:Lot 3	72:73	0.80	0.64, 1.00

N, number of children with available results.

^a Equivalence was concluded if all three 95% CIs on GMT ratios were within the range 0.5–2, ruling out a 2-fold increase/decrease between each pair of lots.

demographic characteristics of the participants were consistent among groups in terms of mean age and mean weight-for-age Z-score; some variability in gender ratios was observed (Table 1).

3.2. Immunogenicity

Consistent immune responses were demonstrated for the three commercial-scale lots of RTS,S/AS01: one month after the third vaccine dose, the two-sided 95% CI of the anti-CS antibody GMT ratio between each pair of lots was within the range 0.5–2 (Table 2). Non-inferiority of the pooled commercial-scale lots to the pilot-scale lot was also demonstrated; the anti-CS antibody GMT ratio, pilot-scale lot: pooled commercial-scale lot, was 0.95 (95% CI: 0.79, 1.15). The anti-CS antibody GMT was 271.7 EU/ml (95% CI: 228.5, 323.1) for the pilot-scale lot and 285.8 EU/ml (95% CI: 260.7, 313.3) for the pooled commercial-scale lot (Table 3).

Before vaccination, anti-CS prevalence was below 3% in all groups, with low titres in those who were positive (Table 3). One month after the third vaccine dose, all vaccine recipients in each group were seropositive for anti-CS antibodies (Fig. 2a), with anti-CS antibody GMTs ranging from 241.4 EU/ml (95% CI: 207.6, 280.7) to 319.6 EU/ml (95% CI: 268.9, 379.8) (Table 3).

The majority of children in each group ($\geq 91.8\%$) had seroprotective anti-HBs antibody titres before vaccination reflecting prior hepatitis B vaccination (Table 3). One month after the third

vaccine dose, all children in each group had seroprotective anti-HBs antibody titres (Fig. 2b) and GMTs ranged from 46,384.7 to 74,105 (Table 3).

3.3. Reactogenicity and safety

Overall per dose incidences of each solicited local and general AE during the 7-day period after vaccination were comparable among groups (Fig. 3). In each group, pain was the most common solicited local AE and fever was the most common solicited general AE (Fig. 3). There were five reports of grade 3 fever ($>39.0^{\circ}\text{C}$); one following a commercial-scale lot 1 dose (incidence 0.4%; 95% CI: 0.0–2.3) and four following commercial-scale lot 3 doses (1.7%; 95% CI: 0.5–4.3). There were no other reports of grade 3 solicited local or general AEs.

During the 30-day period after vaccination, at least one unsolicited AE was reported in a similar proportion of children in each group (77.8%, 75.9%, 87.5% and 72.5% of children in commercial-scale lots 1, 2, 3 and the pilot-scale lot, respectively – Supplementary Table 1); none were of grade 3 intensity and none were considered causally related to vaccination. The most commonly reported unsolicited AEs were malaria (reported in 36, 35, 41 and 33 children in commercial-scale lots 1, 2, 3 and pilot-scale lot, respectively) and respiratory tract infection (27, 23, 27 and 23, respectively).

Thirteen SAEs were reported during the study in eight children (three children in commercial-scale lot 1, two in lot 2, one in lot 3 group and two in the pilot-scale lot), including four reports of severe/complicated malaria and three sepsis reports. None of the SAEs were considered related to vaccination and all events resolved during the study.

4. Discussion

In this phase III, randomized, double-blind study in young Nigerian children, consistency of anti-CS antibody responses was demonstrated for the three RTS,S/AS01 vaccine commercial-scale lots. Furthermore, the anti-CS antibody response to

Table 3
Anti-CS antibody seropositivity and anti-HBs antibody seroprotective rates, and geometric mean titres (GMTs) for commercial-scale lots 1, 2 and 3, and the comparator pilot-scale lot (ATP cohort for immunogenicity).

Group	Timing	N	Anti-CS titre, EU/ml (95% CI)		Anti-HBs titre, mIU/ml (95% CI)	
			≥ 0.5 , % children	GMT	≥ 10 , % children	GMT
Commercial-scale Lot 1	PRE	72	2.8 (0.3–9.7)	0.3 (0.2–0.3)	95.8 (88.3–99.1)	352.8 (217.1–573.4)
	Month 3	72	100 (95.0–100)	319.6 (268.9–379.8)	100 (95.0–100)	54,250.2 (43,293.6–67,979.7)
Commercial-scale Lot 2	PRE	72	0.0 (0.0–5.0)	0.3 (0.3–0.3)	94.4 (86.4–98.5)	202.3 (131.1–312.3)
	Month 3	72	100 (95.0–100)	241.4 (207.6–280.7)	100 (95.0–100)	46,067.3 (33,919.2–62,566.2)
Commercial-scale Lot 3	PRE	73	2.7 (0.3–9.5)	0.3 (0.2–0.3)	91.8 (83.0–96.9)	293.7 (170.5–506.1)
	Month 3	73	100 (95.1–100)	302.3 (259.4–352.3)	100 (95.1–100)	67,384.7 (52,271.4–86,867.7)
Pooled commercial-scale Lots 1, 2, 3	PRE	217	1.8 (0.5–4.7)	0.3 (0.2–0.3)	94.0 (90.0–96.8)	275.8 (208.4–365.1)
	Month 3	217	100 (98.3–100)	285.8 (260.7–313.3)	100 (98.3–100)	55,273.5 (47,508.3–64,308.0)
Pilot-scale (Comparator) Lot	PRE	72	1.4 (0.0–7.5)	0.3 (0.2–0.3)	95.8 (88.3–99.1)	313.7 (201.7–487.6)
	Month 3	72	100 (95.0–100)	271.7 (228.5–323.1)	100 (95.0–100)	74,105.0 (58,613.6–93,690.7)

N, number of children with available results; PRE, pre-vaccination; Month 3, one month after third vaccine dose.

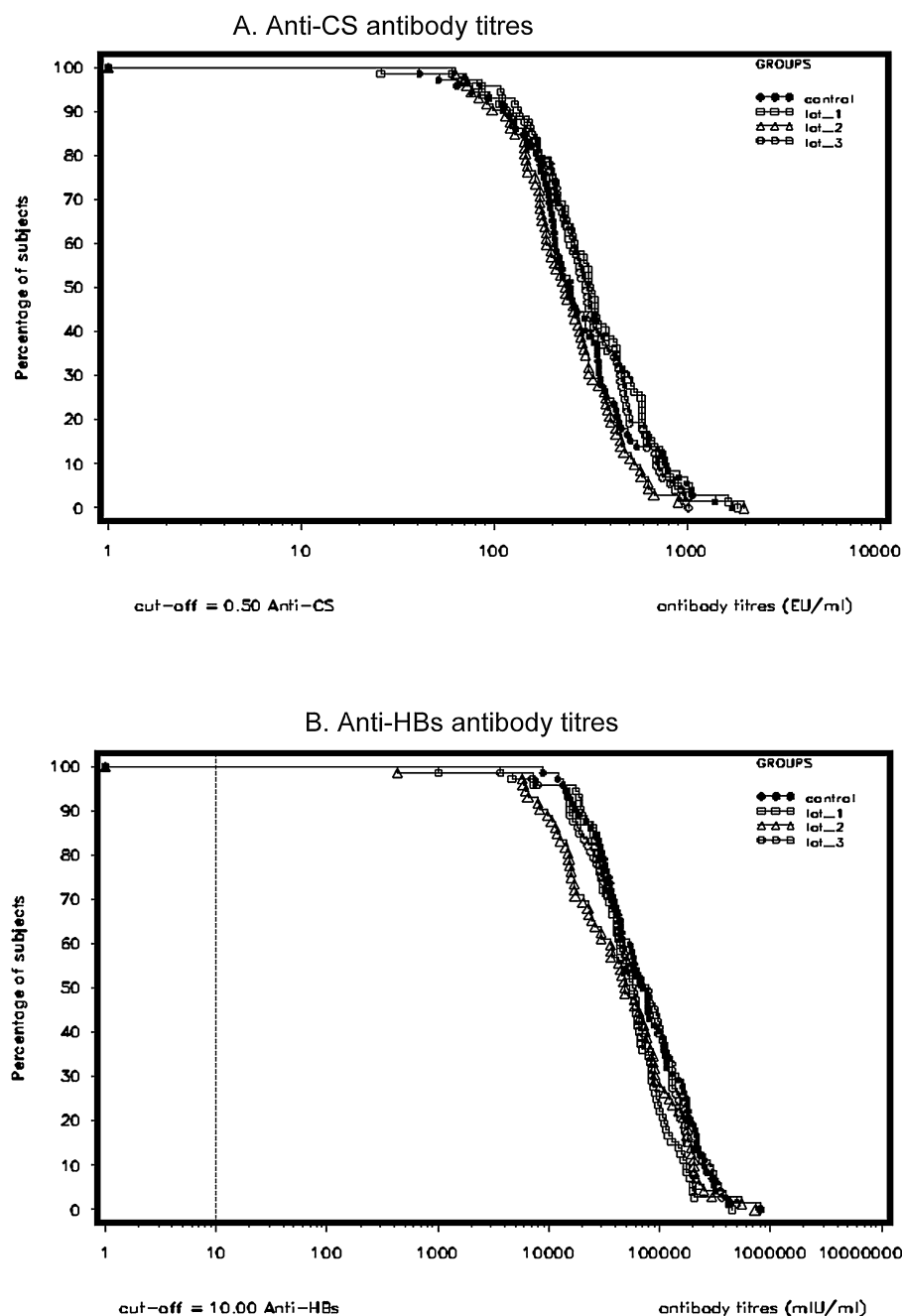


Fig. 2. Reverse cumulative distribution curve for anti-CS and anti-HBs antibody titres one month after the third vaccine dose (ATP cohort for immunogenicity). (A) Anti-CS antibody titres. (B) Anti-HBs antibody titres.

commercial-scale lots was non-inferior to the response to a RTS,S/AS01 pilot-scale lot.

The anti-CS antibody GMTs observed in this trial one month after the third dose were 286 EU/ml for the pooled commercial-scale lots and 272 EU/ml for the pilot-scale lot. This was lower than observed in other RTS,S/AS01 studies of children of the same age, using the same validated anti-CS assay [2,13]. The anti-CS antibody GMT in the phase 3 multicentre efficacy trial was 621 EU/ml (95% CI: 592–652) in 5–17 month old children, but this pooled value masked the substantial variation in anti-CS antibody GMTs by site which ranged from 348 to 787 EU/ml [14]. Despite this variation, vaccine efficacy was at least 40% for all sites in the phase 3 efficacy trial, and no association was seen at site-level between GMTs and vaccine efficacy. Further understanding of immunological correlates of protection is expected to be generated from the phase

3 multicentre RTS,S/AS01 efficacy trial that is ongoing [15]. Variation in immune responses has been described for other vaccine antigens [16] and is believed to have both host and environmental origins [17,18]. Because we did not assess vaccine efficacy, and in the absence of a control (placebo or non-RTS,S vaccine), the clinical relevance of this finding cannot be directly assessed in the current trial.

Each RTS,S/AS01 lot had an acceptable tolerability profile, which was in line with observations in the large RTS,S/AS01 phase III trial among children of a similar age [2]. In both studies, the most frequently reported solicited symptoms were pain and fever and grade 3 symptoms occurred infrequently. No safety signals were identified in the present study and none of the SAEs were considered related to vaccination. The most frequently reported unsolicited AEs were malaria, respiratory tract infections, diarrhoea, and

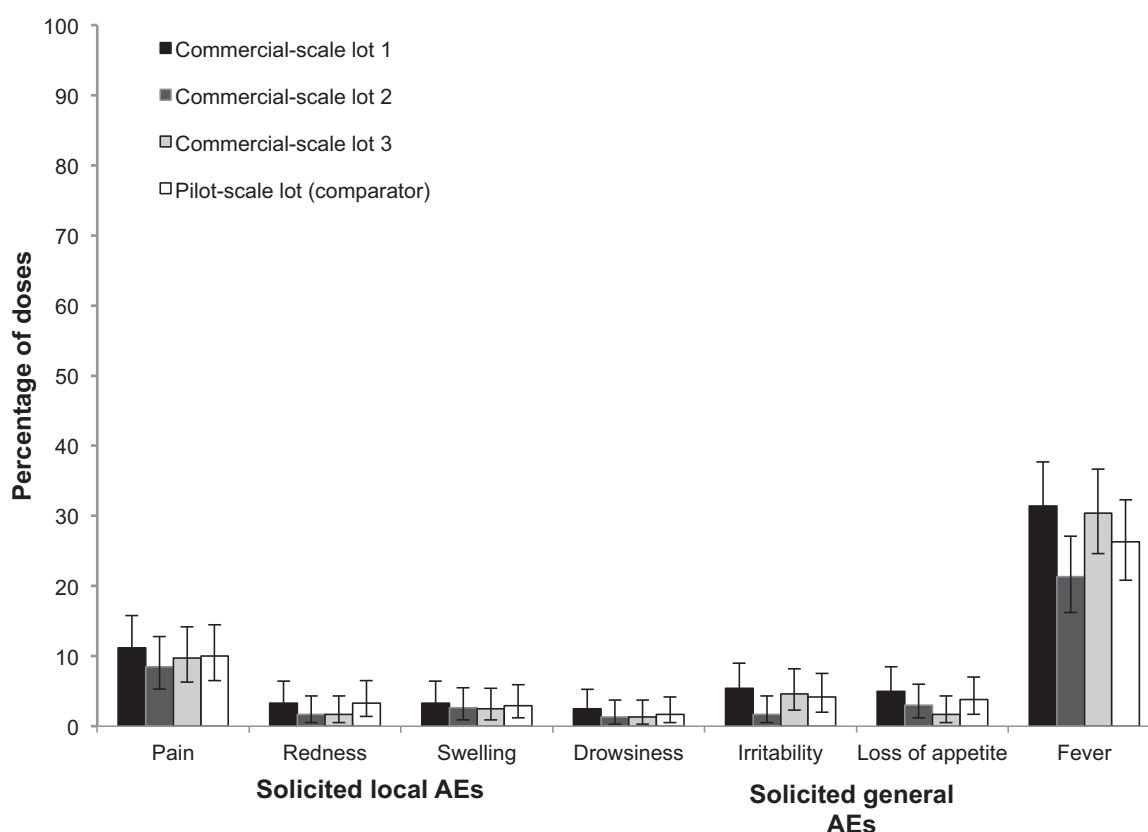


Fig. 3. Frequency of solicited local and general adverse events (overall per dose with 95% CIs) occurring within 7 days of vaccination (total vaccinated cohort).

gastroenteritis in all groups. These are common in children of the study age group (Malaria-055).

In conclusion, these results confirm that RTS,S/AS01 vaccines formulated from commercial-scale purified antigen bulk lots are produced consistently. Anti-CS antibody responses induced were non-inferior to those induced by the batch made from pilot-scale purified antigen bulk lot.

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Contributors: R.U., S.O., T.O., S.P., E.S., J.-T.O., C.A.D. and D.S. were investigators in this study and were responsible for the recruitment

of subjects, collection and assembly of data, and provided interpretation of the results. M.L. and G.C. were responsible for the statistical analyses. E.J. was responsible for lab analysis. M.L. and A.L. designed the study. A.A., E.J. M.L., G.C., O.O.A. and A.L. interpreted the results. All authors critically reviewed the manuscript drafts and approved the final manuscript. *Conflict of interest:* Tagbo Oguonu reports receiving a salary from PATH-MVI as an investigator on the study and speaker fees from GlaxoSmithKline outside the work submitted. At the time of study conduct, Abdullahi Ahmad was a WHO/TDR fellow at GlaxoSmithKline vaccines. Erik Jongert, Grégoire Catteau, Marc Lievens, Opokua Ofori-Anyinam and Amanda Leach are employed by the GlaxoSmithKline group of companies. E.J., M.L., O.O.A. and A.L. own GlaxoSmithKline stocks/stock options. *Funding:* This study was funded by GlaxoSmithKline Biologicals SA.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.vaccine.2014.07.067>.

References

- [1] Regules JA, Cummings JF, Ockenhouse CF. The RTS,S vaccine candidate for malaria. *Expert Rev Vaccines* 2011;10:589–99.
- [2] The RTS,S Clinical Trial Partnership. First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children. *N Engl J Med* 2011;365:1863–75.
- [3] The RTS,S Clinical Trial Partnership. A phase 3 trial of RTS,S/AS01 malaria vaccine in African infants. *N Engl J Med* 2012;367:2284–95.
- [4] Vekemans J, Guerra Y, Lievens M, Benns S, Lapierre D, Leach A, et al. Pooled analysis of safety data from pediatric Phase II RTS,S/AS malaria candidate vaccine trials. *Hum Vaccine* 2011;7:1309–16.
- [5] Dellepiane N, Griffiths E, Milstien JB. New challenges in assuring vaccine quality. *Bull World Health Organ* 2000;78:155–62.

- [6] Kanesa-athan N, Shaw A, Stoddard JJ, Vernon TM. Ensuring the optimal safety of licensed vaccines: a perspective of the vaccine research, development, and manufacturing companies. *Pediatrics* 2011;127(Suppl. 1):S16–22.
- [7] Marshall V, Baylor NW. Food and Drug Administration regulation and evaluation of vaccines. *Pediatrics* 2011;127(Suppl. 1):S23–30.
- [8] European Medicines Agency. Guideline on clinical Evaluation of New Vaccines. EMEA/CHMP/VMP/164653/2005, 2006. London: European Medicines Agency; 2006.
- [9] Guidelines on clinical evaluation of vaccines: regulatory expectations. In: WHO Expert Committee on Biological Standardization, fifty-second Report. Technical Report Series No. 924. Geneva: World Health Organization; 2004.
- [10] Lell B, Agnandji S, von Glasenapp I, Haertle S, Oyakhiromen S, Issifou S, et al. A randomized trial assessing the safety and immunogenicity of AS01 and AS02 adjuvanted RTS,S malaria vaccine candidates in children in Gabon. *PLoS ONE* 2009;4:e7611.
- [11] Clement F, Dewar V, Van Braeckel E, Desombere I, Dewerchin M, Swysen C, et al. Validation of an enzyme-linked immunosorbent assay for the quantification of human IgG directed against the repeat region of the circumsporozoite protein of the parasite *Plasmodium falciparum*. *Malar J* 2012;11:384.
- [12] Centers for Disease Control and Prevention. Hepatitis B virus: a comprehensive strategy for eliminating transmission in the United States through universal childhood vaccination. Recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR Recomm Rep* 1991;40:1–25.
- [13] Bejon P, Lusingu J, Olotu A, Leach A, Lievens M, Vekemans J, et al. Efficacy of RTS,S/AS01E vaccine against malaria in children 5 to 17 months of age. *N Engl J Med* 2008;359:2521–32.
- [14] Otieno L. Efficacy of RTS,S/AS01 vaccine candidate against malaria in African infants and children 18 months post-primary vaccination. In: Presented at MIM. 2013.
- [15] Leach A, Vekemans J, Lievens M, Ofori-Anyinam O, Cahill C, Owusu-Agyei S, et al. Design of a phase III multicenter trial to evaluate the efficacy of the RTS,S/AS01 malaria vaccine in children across diverse transmission settings in Africa. *Malar J* 2011;10:224.
- [16] Newport MJ, Goetghebuer T, Weiss HA, Whittle H, Siegrist CA, Marchant A, et al. Genetic regulation of immune responses to vaccines in early life. *Genes Immun* 2004;5:122–9.
- [17] Poland GA, Ovsyannikova IG, Jacobson RM, Smith DI. Heterogeneity in vaccine immune response: the role of immunogenetics and the emerging field of vaccinomics. *Clin Pharmacol Ther* 2007;82:653–64.
- [18] Posteraro B, Pastorino R, Di Giannantonio P, Ianuale C, Amore R, Ricciardi W, et al. The link between genetic variation and variability in vaccine responses: systematic review and meta-analyses. *Vaccine* 2014;15:1661–9.