The Pediatric Infectious Disease Journal Publish Ahead of Print

DOI: 10.1097/INF.0000000000001937

Safety and Immunogenicity of Seven Dosing Regimens of the Candidate RTS,S/AS01$_E$ Malaria Vaccine Integrated within an Expanded Program on Immunization Regimen: A Phase II, Single-Center, Open, Controlled Trial in Infants in Malawi

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Funding: GlaxoSmithKline Biologicals SA

Abbreviated title: Phase II Study of RTS,S/AS01 Malaria Vaccine Schedules in Infants

Running title: RTS,S/AS01 Malaria Vaccine Schedules in Infants

Conflicts of Interest and Source of Funding

The trial was sponsored and funded by GlaxoSmithKline Biologicals SA, the vaccine developer and manufacturer. YGM, AL, ML, OOA are employees, and JV a former employee, of the GSK group of companies. AL, ML, OOA hold shares in the GSK group of companies as part of their remuneration. NC declares that his institution received a grant from the GSK group of companies to conduct studies related to rotavirus vaccine in the UK and Malawi. NC declares that he received support from the GSK group of companies for participation in rotavirus vaccine advisory board meetings. PC, AT, DW, EN, TW, JN and JV declare no conflict of interest. GlaxoSmithKline Biologicals SA was the sponsor, funded the trial and was involved in the study design, data collection, analysis and interpretation. The authors had access to data from the study and took responsibility for the decision to submit for publication.

Author contributions

NC, AL, ML, EN, JN, OO-A, JV and DW were involved in the study conception and design. PC, NC, YMG, EN, JN, OOA, AT, TW and DW participated in the data collection. NC, YMG, AL, ML, OOA, JV and DW were involved in the data analysis and/or interpretation.

Acknowledgements

The authors would like to thank all the participants and their parents, as well as the Bangwe Health Centre Ministry of Health and Council staff for their co-operation and assistance; the Study Teams at the Bangwe Clinic, Kachere Office and Queen Elizabeth Central Hospital for their support and care for the participants; and the Laboratory teams at the Johns Hopkins Research Project and the Malawi-Liverpool- Wellcome Trust Clinical Research Programme, for their local safety testing and analyses.

The authors are grateful to the Independent Data Monitoring Committee and the Local Safety Monitors, Prof E.M. Molyneux and Prof R.L. Broadhead.

The authors wish to thank the Global and Regional clinical teams of GlaxoSmithKline Vaccines for their contribution to the study, the Research & Development group for analyses of laboratory work, the scientific writers for the clinical protocol and clinical report writing, and the Statistician team for their input on the statistical analyses.

The authors would like to thank Olivier Godeaux for his contribution to the development of the clinical study protocol and Florence Richard and Lynette Wane for their help in study coordination.

Abstract

Background: In a phase III trial, the RTS,S/AS01 malaria vaccine produced lower anti-circumsporozoite (CS) antibody titres when co-administered with Expanded Programme on Immunisation (EPI) vaccines (0,1,2-month schedule) at 6-12 weeks compared to 5-17 months at first vaccination. Alternative infant immunisation schedules within the EPI were investigated.

Methods: This phase II, open, single site (Blantyre, Malawi) trial was conducted in infants aged 1-7 days. Subjects were equally randomised across seven groups to receive three doses of RTS,S/AS01$_E$ at time points that included ≤7 days, 6, 10, 14, 26 weeks, and 9 months. All RTS,S/AS01$_E$ groups plus a control group (without RTS,S/AS01$_E$) received BCG+OPV at ≤7 days, DTPwHepB/Hib+OPV at 6,10,14 weeks and measles vaccine at 9 months; one RTS,S/AS01$_E$ group and the control additionally received hepatitis B vaccination at ≤7 days. Serum anti-CS antibody geometric mean concentration (GMC; ELISA) and safety were assessed up to age 18 months.

Results: Of the 480 infants enrolled, 391 completed the study. No causally related serious adverse event was reported. A higher frequency of fever within 7 days of RTS,S/AS01$_E$ vaccination compared to control was observed. Compared to the standard 6,10,14 week schedule, anti-CS antibody GMC ratios post-Dose 3 were significantly higher in the 10,14,26 week group only (ratio 1.80; 95%CI:1.24, 2.60); RTS,S/AS01$_E$ vaccination at ≤7 days, 10,14 weeks produced significantly lower anti-CS GMCs (ratio 0.59; 95%CI:0.38, 0.92).

Conclusions: Initiation of RTS,S/AS01$_E$ vaccination above six weeks of age tended to improve anti-CS antibody responses. Neonatal vaccination was well tolerated, but produced a comparatively lower immune response.

REGISTRATION

Clinical Trials.gov identifier: NCT01231503

GlaxoSmithKline Study ID number: 111315 (Malaria-057)

KEYWORDS: RTS,S/AS01; *falciparum*; malaria; immunogenicity; neonates

INTRODUCTION

Malaria causes devastating morbidity and mortality, with an estimated 214 million malaria cases and 438,000 malaria deaths occurring worldwide in 2015.[1] Malaria mortality occurs predominantly in children, with an estimated 306,000 deaths in children younger than five years of age each year, of which 95% occur in Africa.

The development of efficacious malaria vaccines has been identified as a key component of a sustainable malaria control program with consequent benefits for health and the economy.[2] GSK Vaccines' pre-erythrocytic *Plasmodium falciparum* malaria vaccine, RTS,S/AS01$_E$, consists of sequences of the circumsporozoite (CS) protein fused to hepatitis B surface antigen (HBsAg) adjuvanted with AS01 (liposome formulation with MPL and QS-21 immunostimulants).[3]

The Expanded Programme on Immunisation (EPI) is an established program that uses immunization regimens aimed at maximizing immunization coverage in order to address disease burden early in life.

During phase II development, lower anti-CS antibody titers were produced when an earlier formulation of the vaccine (RTS,S/AS02) as well as the RTS,S/AS01$_E$ formulation were co-administered with EPI vaccines on a 0, 1, 2-month schedule to infants aged 6-12 weeks at first vaccination, compared to children aged 1-4 years[4-8]; this difference in immunogenicity did not appear to result in a difference in protective efficacy. Although an immature immune system and/or an inhibitory effect of maternally derived anti-CS antibodies may have played a role, interference of co-administered routine EPI vaccines may also have contributed to the lower immunogenicity observed in young infants.[9]

The current study was designed to investigate alternative vaccination schedules in order to improve anti-CS immune responses of RTS,S/AS01$_E$ in infants when integrated within an EPI

regimen in Blantyre, Malawi. Although no correlate of protection has been established, a link between higher anti-CS titers and decreased risk of infection has been shown in some trials.[4,6] The exploratory schedules comprised administration of three doses of RTS,S/AS01$_E$ between birth (neonatal dose) and nine months of age, using different existing contacts with the health service. In previous trials with GSK Vaccines' hepatitis B vaccine, it was observed that increasing the interval between the second and third dose enhances humoral response.[10,11] In this study, administration of the third dose of RTS,S/AS01$_E$ at 26 weeks of age was investigated to assess if increased spacing of RTS,S/AS01$_E$ doses improves anti-CS antibody responses. Starting hepatitis B immunization at birth or in the neonatal period, as recommended by WHO,[12] is desirable to control mother to infant transmission of hepatitis B infection and the subsequent development of chronic carriage. Furthermore, previous phase II trials indicated that prior hepatitis B vaccination may 'prime' for anti-CS antibody response.[13,14] Thus, a schedule including a neonatal dose of a hepatitis B vaccine (*Engerix-B**) was investigated.

Potential inhibition of RTS,S/AS01$_E$ specific immune responses following co-administration with DTPw-HepB/Hib was evaluated by the inclusion of schedules in which doses of RTS,S/AS01$_E$ were not co-administered with DTPw-HepB/Hib (*Tritanrix HepB/Hib**). These schedules also investigated any effect on anti-CS antibody response of age at first dose of RTS,S/AS01$_E$.

Although there is currently no scheduled visit at six months of age in the EPI, at this time point parents of children in Africa are requested to bring their child to their local health facility for administration of Vitamin A. An exploratory vaccination schedule within existing EPI visits, but outside the EPI DTPw-HepB/Hib vaccination schedule, was also investigated given the substantial number of antigens currently administered at these time points and possible future

increase in co-administered antigens. In this schedule, the first dose of RTS,S/AS01$_E$ was co-administered with the 3rd dose of DTPw-HepB/Hib, the second dose without co-administration but coinciding with a Vitamin A EPI visit and the third dose in co-administration with the measles vaccine (*Rouvax**).

The primary aims of the study were to compare CS antigen immunogenicity of RTS,S/AS01$_E$ between exploratory schedules and the reference schedule at 6, 10, 14 weeks of age, and to evaluate safety over an 18 month period.

**Engerix-B*, *Tritanrix* and *Hiberix* are trademarks of the GSK group of companies. *Rouvax* is a trademark of Sanofi Pasteur.

MATERIALS AND METHODS

A summary of the protocol is available at www.gsk-clinicalstudyregister.com (GSK study 111315).

Ethics

The study was registered with the Pharmacy, Medicines and Poisons Board, Malawi and at ClinicalTrials.gov (ClinicalTrials.gov number: NCT01231503). Approval was obtained from the National Health Sciences Research Committee, Malawi, the College of Medicine Research Ethics Committee, University of Malawi, and the Research Ethics Sub-committee for Physical Interventions, University of Liverpool, UK. The trial was conducted in accordance with the provisions of the International Conference on Harmonisation and Good Clinical Practice guidelines.

Recruitment of study subjects

Prior to study start, activities were carried out to raise awareness amongst health professionals in Blantyre district, as well as at different levels of the community.

Pregnant women who were interested in participating in the study were identified at the antenatal clinic at the Bangwe Health Centre, Blantyre, Malawi. Potential recruits were required to be resident in the Bangwe study clinic catchment area for the foreseeable future, a minimum age of 18 years at the time of delivery, in the third trimester of pregnancy (27-42 weeks) and to provide informed consent for antenatal screening for hepatitis B and HIV infection. Written informed consent for subject participation was requested from mothers antenatally, as ideally cord blood was taken for screening purposes. If consent was given >7 days prior to procedures being carried out on the child, a brief re-consenting process was performed.

Only infants whose mothers were negative for carriage of the hepatitis B virus and HIV infection were eligible to be screened for the trial. Eligibility criteria included male or female infants aged between one and seven days (inclusive), born at full term (gestation period between 37 and 42 weeks) confirmed by Dubowitz score,[15] and with a minimum weight of 2.5 kg (see Supplementary Appendix, Section 1, http://links.lww.com/INF/C993). Subjects had to be healthy as determined by physical examination, medical history records and laboratory screening tests of hematology and renal and hepatic function. For participation in the study, no previous vaccinations with diphtheria, tetanus, pertussis (whole-cell or acellular), *Haemophilus influenzae type b*, hepatitis B, Bacillus Calmette-Guérin (BCG) tuberculosis, measles or oral polio vaccines were allowed.

Study design

The study was a phase II, open, randomized, controlled trial with eight groups. All groups received standard EPI vaccines. Seven groups additionally received RTS,S/AS01$_E$ as a 3-dose schedule with or without neonatal hepatitis B vaccine. The control group additionally received a

neonatal dose of hepatitis B vaccine (Fig. 1). The study duration was approximately 18 months per child.

Study vaccines, vaccination, randomization

RTS,S/AS01 is manufactured by GSK Vaccines (Rixensart, Belgium). Each 0.5 mL RTS,S/AS01 dose as tested in this study contained RTS,S (25 µg) and $AS01_E$, an Adjuvant System containing 25 µg MPL and 25 µg QS-21 (*Quillaja saponaria* Molina, fraction 21) (Licensed by GSK from Antigenics LLC, a wholly owned subsidiary of Agenus Inc., a Delaware, USA corporation).[3] RTS,S/AS01 was administered intramuscularly in the left antero-lateral thigh.

EPI vaccines comprised a diphtheria, tetanus, whole-cell pertussis, hepatitis B and *Haemophilus influenzae* type b pentavalent vaccine (*Tritanrix HepB/Hib*, GSK Vaccines), an oral poliovirus vaccine containing serotypes 1, 2 and 3 (*Polio Sabin**, GSK Vaccines), a hepatitis B vaccine (HepB; *Engerix-B*, GSK Vaccines), a BCG vaccine (Statens Serum Institute) and an attenuated measles vaccine (*Rouvax*, Sanofi Pasteur). Details for the EPI vaccines and administration are provided in Supplementary Appendix, Section 2, http://links.lww.com/INF/C993.

Treatment allocation was performed at the investigator site using a standard Statistical Analysis System (SAS) programmed randomization list generated at GSK Vaccines, Belgium. Randomization was not stratified, but ensured an equal number distribution of subjects across treatment groups. The sample size of this descriptive phase II trial was powered to detect at least a 2-fold difference in GMT between any two groups.

**Polio Sabin* is a trademark of the GSK group of companies.

Immunogenicity assessments

Immunology assessments are described in detail in Supplementary Appendix Section 3, http://links.lww.com/INF/C993. Briefly, anti-CS repeat region IgG antibodies were measured by standard ELISA methodology using plate-adsorbed R32LR antigen $[NVDP(NANP)_{15}]_2LR$, as previously described.[16] Antibodies against HBsAg were measured by chemiluminometric immunoassay (as measured by the Siemens Centaur XP CLIA).

Safety assessments

As this was the first trial of the RTS,S/$AS01_E$ candidate malaria vaccine in neonates, a rigorous safety monitoring plan was put in place (see Supplementary Appendix, Section 4, http://links.lww.com/INF/C993). The study was overseen by an Independent Data Monitoring Committee (IDMC) operating under a charter and assisted by two Local Safety Monitors.

The local injection site and general solicited adverse events (AEs) were monitored over 7 days after each vaccination (Days 0-6) and were graded as mild, moderate or severe (Grade 1, 2, 3, respectively). All other AEs (unsolicited) were recorded over a 30-day (Days 0-29) period after each vaccination. Serious adverse events (SAEs) were captured throughout the study. All injection site AEs were considered causally related to vaccination; the causality of all other AEs was assessed by the investigator. Hematologic and biochemical tests for safety assessment were conducted at various time points during the study.

At the time of protocol development, two SAEs of febrile seizure within 7 days of vaccination in two previous phase II trials, were considered to be causally related to vaccination with RTS,S/$AS01_E$.[8,14] In the large phase III trial,[9,17,18] an imbalance of meningitis cases of any etiology (i.e. including cases with and without confirmed etiology) was observed in children 5-17 months of age at first dose. Consequently, any febrile seizure occurring within 30 days of

vaccination and any case of meningitis occurring during the study were to be recorded as SAEs. See Supplementary Appendix, Sections 4 and 5.2, http://links.lww.com/INF/C993 for additional details on safety assessments, including assessment of AEs of specific interest.

Statistical analysis

All analyses were conducted according to a pre-defined analysis plan. The co-primary endpoints of the study were: anti-CS antibody concentrations at 1 month post-Dose 3 of RTS,S/AS01$_E$ and occurrence of SAEs from study start until Month 10. A second analysis included occurrence of SAEs from study start until study end (Month 18; tertiary objective).

The Total Vaccinated Cohort (TVC) included all subjects who were randomized and received a dose of BCG vaccine. All safety analyses were performed on the TVC population. The According To Protocol (ATP) cohort for immunogenicity included all subjects in the TVC who received all vaccinations according to protocol procedures within specified intervals and did not take any immune modifying medication or have blood transfusions.

Seropositivity rates for CS (≥0.5 EU/mL), pertussis (≥15 EL.U/mL) and measles (≥150 mIU/mL) and seroprotective rates for HBs (≥10 mIU/mL), diphtheria (≥0.1 IU/mL), PRP (purified capsular polyoside - polyribosylribitol phosphate - of *Haemophilus influenzae* type b) (≥0.15 µg/mL), polio (≥8 ED50 [endpoint dilution 50%]) and tetanus (≥0.1 IU/mL), together with geometric mean concentrations (GMCs)/titers (GMTs) plus exact 95% confidence intervals (CI) were evaluated. GMC ratios relative to the 6, 10, 14 week regimen were assessed. All safety analyses were performed by group and pooled neonatal groups in which safety was evaluated after neonatal Dose 1 (pooled RTS,S: ≤7 days, 10, 14 weeks and ≤7 days, 10, 26 weeks groups; pooled HepB: ≤7 days HepB, 6, 10, 26 weeks and control groups). Analyses of safety following three doses of RTS,S/AS01$_E$ or DTPw-HepB/Hib alone enabled comparison of equal follow up

and number of vaccination visits; age at vaccination and number of doses of RTS,S/AS01$_E$ co-administered with DTPw-HepB/Hib differed. Statistical analyses were conducted using SAS version 8 (SAS, Cary, NC, USA) and further details are available in Supplementary Appendix, Section 5, http://links.lww.com/INF/C993.

RESULTS

Study population

Of the 480 subjects enrolled at birth, 479 were vaccinated, of whom 391 completed the study. The main reasons for withdrawal of the 88 subjects were consent withdrawal and migration/lost to follow up (Fig. 2).

All infants were less than 7 days old at the time of neonatal vaccination with BCG (Screening visit). An equal proportion of males and females were enrolled (Supplementary Table 1, http://links.lww.com/INF/C993).

Immunogenicity outcomes

Post-Dose 3 anti-CS GMCs ranged from 128.2 EU/mL to 392.6 EU/mL across all RTS,S/AS01$_E$ co-administration schedules (Fig. 3). Anti-CS antibody responses persisted to Month 18 in all seven RTS,S/AS01$_E$ schedules.

In subjects vaccinated at 6, 10, 14 weeks of age (i.e. the reference group for evaluation of anti-CS antibody response) the anti-CS antibody GMC post-Dose 3 was 218.3 EU/mL (95% CI: 160, 298). A higher anti-CS antibody GMC ratio was observed post-Dose 3 only in the two groups where vaccination with RTS,S/AS01$_E$ started after 6 weeks of age, i.e. in the 10, 14, 26 week and 14, 26 week, 9 month groups, with a statistically significant increase in the 10, 14, 26 week schedule only (GMC ratio 1.80; 95% CI: 1.24, 2.60) as compared to the reference schedule (6, 10, 14 weeks in co-administration with DTPw-HepB/Hib + OPV) (Table 1).

Relatively lower anti-CS responses post-Dose 3 were observed in groups receiving the first dose of RTS,S/AS01$_E$ within 7 days of birth (≤7 days, 10, 14 week group: 128 EU/mL [95% CI: 92, 178]; ≤7 days, 10, 26 week group: 137 EU/mL [95% CI: 93, 201]), which was statistically significant compared to vaccination at 6, 10, 14 weeks of age in the ≤7 days, 10, 14 week group (GMC ratio 0.59; 95% CI: 0.38, 0.92).

Increasing the interval from 1 month to 3 months between Doses 2 and 3 of RTS,S/AS01$_E$ did not increase anti-CS response (post-Dose 3 anti-CS antibody GMC ratios: ≤7 days, 10, 14 week group vs ≤7 days, 10, 26 week group, 0.94 [95% CI: 0.57, 1.54]; 6, 10, 14 week group vs 6, 10, 26 week group, 1.39 [95% CI: 0.82, 2.38]).

A neonatal dose of a hepatitis B vaccine did not increase the anti-CS response (post-Dose 3 anti-CS antibody GMT ratio: 6, 10, 26 week group vs HepB ≤7 days, 6, 10, 26 week group, 0.92 [95% CI: 0.51, 1.66]).

One month after a single dose of measles vaccine at Month 9, there was a trend for lower anti-measles antibody GMCs with RTS,S/AS01$_E$ co-administration (14, 26 week, 9 month group), though similar seropositivity rates were observed with or without (control group) co-administration of RTS,S/AS01$_E$ (14, 26 week, 9 month group: 92.3% [95% CI: 81.5, 97.9] seropositive, GMC 1018 mIU/mL [95% CI: 752, 1377]; control group: 89.1% [95% CI: 76.4, 96.4] seropositive, GMC 1431 mIU/mL [95% CI: 997, 2053]). Antibody responses to pertussis, diphtheria, PRP, tetanus, OPV and measles antigens are summarized in Supplementary Table 10, http://links.lww.com/INF/C993.

All RTS,S/AS01$_E$ regimens induced high anti-HBs antibody responses (Supplementary Figure 1, http://links.lww.com/INF/C993); 100% of subjects were seroprotected for anti-HBs antibodies at all time points assessed up to Month 18. In the control group receiving a hepatitis B vaccine as a

neonatal dose followed by three doses of DTPw-HepB/Hib at 6, 10, 14 weeks of age (current standard of care), 100%, 97.1% and 88.1% of subjects were seroprotected for anti-HBs antibodies at Month 5, Month 10 and Month 18, respectively.

Safety outcomes

Serious AEs

The proportion of subjects who experienced at least one SAE from study start until Month 10 (primary safety endpoint) ranged across study groups from 5.0% to 16.7% (Table 2; Supplementary Table 3, http://links.lww.com/INF/C993). Two subjects (1.7%) reported SAEs following a neonatal dose of RTS,S/AS01$_E$ and 3 subjects (2.5%) following a neonatal dose of a hepatitis B vaccine. Over 18 months' surveillance, no safety concern was apparent across the different groups (Supplementary Table 4, http://links.lww.com/INF/C993). Three fatal SAEs were reported: respiratory distress (6, 10, 14 week group), acute respiratory distress syndrome (10, 14, 26 week group) and drowning (6, 10, 26 week group). No SAE was considered by the investigator to be causally related to vaccination.

Unsolicited AEs

The unsolicited AE profile was generally similar across groups (Supplementary Table 5, http://links.lww.com/INF/C993). Grade 3 (severe) unsolicited AEs were reported in 0.0% to 5.0% of subjects in any RTS,S/AS01$_E$ group and in 0.0% of subjects in the control group (Supplementary Table 6, http://links.lww.com/INF/C993). Two subjects reported unsolicited AEs considered to be causally related to vaccination: injection site swelling (6, 10, 26 week group) and gastroenteritis (≤7 day, 10, 26 week group). No subject was withdrawn due to an AE post RTS,S/AS01$_E$ vaccination. Unsolicited AEs within 30 days post-neonatal vaccination were reported in 21.8% and 27.5% of infants receiving RTS,S/AS01$_E$ or a hepatitis B vaccine,

respectively (Supplementary Table 7, http://links.lww.com/INF/C993); none was considered to be causally related to vaccination.

Meningitis occurred in two infants: neonatal meningitis with no etiologic diagnosis in one subject in the ≤7 days, 10, 14 week group which was reported 7 days post-Dose 1 of RTS,S/AS01$_E$ and pneumococcal meningitis in one subject in the 10, 14, 26 week group reported 8 days post-Dose 1 of DTPw-HepB/Hib + OPV administered at 6 weeks of age without RTS,S/AS01$_E$ co-administration. Neither event was considered to be related to vaccination and both resolved without sequelae. No case of generalized convulsive seizure within 30 days of vaccination was reported in any study group.

Reactogenicity

The incidence of solicited local AEs (pain, redness, swelling) and solicited general AEs of drowsiness, irritability and loss of appetite was generally similar in the RTS,S/AS01$_E$ groups and the control group and similar following a neonatal dose of RTS,S/AS01$_E$ or a hepatitis B vaccine (Supplementary Tables 8, 9, http://links.lww.com/INF/C993; Supplementary Figures 2, 3, http://links.lww.com/INF/C993). There was a higher frequency of fever (temperature ≥37.5°C) in most of the RTS,S/AS01$_E$ groups compared to the control group and following a neonatal dose of RTS,S/AS01$_E$ compared to a neonatal dose of a hepatitis B vaccine (Fig. 4, Supplementary Table 9, http://links.lww.com/INF/C993). Grade 3 fever (temperature >39.0°C) was reported in three subjects after Dose 1 of RTS,S/AS01$_E$ and in a further two subjects after Dose 3 of RTS,S/AS01$_E$. No Grade 3 fever was reported after a neonatal dose of RTS,S/AS01$_E$. No Grade 3 fever was reported in the control group.

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Clinical laboratory evaluations

No Grade 4 abnormalities (potentially life-threatening) in biochemistry values were reported. Low hemoglobin and/or platelet values were graded 4 in five subjects in the RTS,S/AS01$_E$ groups, two following a neonatal dose of RTS,S/AS01$_E$, and one subject in the control group, following a neonatal dose of a hepatitis B vaccine; none of the subjects had any relevant general medical history nor associated AE and none of the Grade 4 values was considered related to vaccination by the Investigator or by the IDMC. These subjects all continued in the trial and completed the study procedures in good health.

DISCUSSION

This study has shown that superior anti-CS antibody levels were achieved following administration of the first dose of the RTS,S/AS01$_E$ malaria vaccine to Malawian infants at 10 weeks of age on a 10, 14, 26 week schedule, compared to at 6 weeks of age on a 6, 10, 14 week schedule. This first study to include a neonatal dose of RTS,S/AS01$_E$, demonstrated an acceptable safety profile, but a reduced immune response.

In the pivotal phase III clinical trial of RTS,S/AS01$_E$, approximately 15,000 infants aged 6-12 weeks and children aged 5-17 months at first vaccination were enrolled across 11 sub-Saharan African sites. Administration of three doses of RTS,S/AS01$_E$ one month apart resulted in protection against clinical malaria of 45% in the 5-17 month age group and 27% in the 6-12 week age group during 18 months' follow up after the third dose.[9] Anti-CS antibody titres were lower in the younger age category in which the mean age at first vaccination with RTS,S/AS01$_E$ was 7.1 (standard deviation 1.4) weeks compared to 10.6 (standard deviation 3.8) months in the older age group.[17]

In the current study, all seven RTS,S/AS01$_E$ co-administration schedules yielded anti-CS antibody responses, which persisted to Month 18.

Of note, infants who received RTS,S/AS01$_E$ above six weeks of age as opposed to at 6, 10 and 14 weeks of age had an increase in post-Dose 3 anti-CS antibody GMC, which was statistically significant for the 10, 14 and 26 week group.

Lower anti-CS antibody responses were observed in infants receiving their first dose within a week of birth (≤7 days, 10, 14 week and ≤7 days, 10, 26 week schedules), with significantly lower titers compared to vaccination at 6, 10 and 14 weeks of age for the ≤7 days, 10, 14 week group. In the neonatal vaccine schedules, RTS,S/AS01$_E$ was co-administered with only one or two doses of EPI vaccines compared to all three doses in the 6, 10, 14 week reference schedule, possibly indicating that the maturity of the immune system and/or inhibition due to maternally acquired antibodies, rather than an inhibitory co-administration response, could be implicated in the observed reduction in anti-CS titers compared to older children.[18]

Consistent with previous findings, there was no indication that increasing the spacing of the third dose of RTS,S/AS01$_E$ was associated with higher anti-CS antibody responses,[19] contrary to responses to hepatitis B vaccination in which improved anti-HBs antibody responses have been observed using this approach.[10,11] Earlier studies reported enhanced anti-CS antibody responses post RTS,S/AS01$_E$ vaccination in subjects who had previously received a dose of hepatitis B vaccine as part of EPI prior to study participation.[13,14] However, in this study anti-CS responses were similar when RTS,S/AS01$_E$ was administered at 6, 10 and 26 weeks of age, with or without neonatal hepatitis B vaccination.

An exploratory schedule of RTS,S/AS01$_E$ outside the EPI DTPw-HepB/Hib vaccination visits, in which the first dose was given at 14 weeks of age in co-administration with the third dose of

DTPw-HepB/Hib, the second dose at 6 months of age without co-administration but correlating with a Vitamin A EPI visit and the third dose at 9 months of age in co-administration with measles vaccination, produced high post-Dose 3 anti-CS antibody responses. Although GMCs for anti-measles antibody responses tended to be lower in the RTS,S/AS01$_E$ co-administration group, seropositivity rates were similar with or without RTS,S/AS01$_E$ co-administration. Non-inferiority of anti-measles seroconversion rates has previously been demonstrated for RTS,S/AS01$_E$ co-administered with measles vaccine compared with measles vaccine given alone.[7]

High anti-HBs antibody titers following RTS,S/AS01$_E$ vaccination compared to standard hepatitis B containing EPI vaccines were demonstrated for all seven schedules investigated. All subjects receiving three doses of RTS,S/AS01$_E$ maintained seroprotective levels of anti-HBs antibodies over an 18 month duration. In contrast, anti-HBs seroprotection rates in subjects receiving a hepatitis B vaccine as a neonatal dose followed by three doses of DTPw-HepB/Hib decreased to 88.1% at Month 18.

RTS,S/AS01$_E$ was well tolerated in infants aged 1-7 days at first vaccination, with no SAE assessed as being causally related to vaccination. No subject was withdrawn due to an AE post RTS,S/AS01$_E$ vaccination over the 18 month surveillance period of the study. In this first study of neonatal administration of RTS,S/AS01$_E$, the reactogenicity profile of a neonatal dose was comparable to that following vaccination of infants six weeks of age or older. As has been previously observed with RTS,S/AS01$_E$ vaccination, transient mild to moderate fever occurs more frequently in RTS,S/AS01$_E$ groups than comparator groups.[18,20]

Since initiation of enrolment to the current study in January 2011, the candidate RTS,S/AS01$_E$ malaria vaccine has progressed to regulatory review. In 2015 the European Medicines Agency

adopted a positive scientific opinion on the benefit-risk balance of RTS,S/AS01$_E$.[21,22] Subsequently, the WHO recommended pilot implementation of RTS,S/AS01$_E$ vaccine in children as a four dose schedule in sub-Saharan Africa, covering moderate-to-high transmission settings; the first dose to be administered as soon as possible after reaching 5 months of age, the subsequent two doses after a minimal interval of 4 weeks and a fourth dose at 15 to 18 months after the third dose.[23] However, depending on malaria transmission, a substantial amount of malaria episodes can occur before the age of 5 months and an effective malaria vaccine that could be given together with some of the well-established EPI vaccination visits would be of added value to malaria control efforts.

The results of the present study indicate that first administration of RTS,S/AS01$_E$ in co-administration with EPI vaccines at a slightly older age improves anti-CS immune responses. We note that this was a phase II study, with a small sample size, and as a consequence results are descriptive rather than confirmatory since we have relatively low power to differentiate statistically between the groups for immunology and safety endpoints. Although no correlate of protection has been established, a link between higher anti-CS titers and decreased risk of infection has been shown in the challenge model and Active Detection of Infection trials,[4-6,24-28] but not in Active or Passive Case Detection trials.[5,8] The trial did not have an efficacy endpoint and in the absence of a correlate of protection, the potential effect of the assessed schedules on malaria reduction could not be measured. Nevertheless, this study shows the potential to optimize immune responses to RTS,S/AS01$_E$ by changing the immunization schedules, thereby potentially allowing for flexibility and alignment with health care delivery programs. More evidence is required on the feasibility of co-administration, and potential benefit in terms of efficacy, of RTS,S/AS01$_E$ vaccination at a later age within the EPI schedule.

Lastly, the authors wish to thank Myriam Wilbaux and Sophie Timmery (XPE Pharma & Science, Belgium c/o GlaxoSmithKline Vaccines) for publication management and Sarah Benns (professional writer) for drafting the manuscript.

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Figure legends

Figure 1. Study design overview: study groups, vaccinations

RTS,S = RTS,S/AS01$_E$

HepB = Hepatitis B vaccine (*Engerix-B*)

≤7D, 10, 14W group = RTS,S/AS01$_E$ at ≤7 days, 10 and 14 weeks

≤7D, 10, 26W group = RTS,S/AS01$_E$ at ≤7 days, 10 and 26 weeks

6, 10, 14W group = RTS,S/AS01$_E$ at 6, 10 and 14 weeks

6, 10, 26W group = RTS,S/AS01$_E$ at 6, 10 and 26 weeks

≤7D HepB, 6, 10, 26W = HepB vaccine at ≤7 days, and RTS,S/AS01$_E$ at 6, 10 and 26 week s

10, 14, 26W group = RTS,S/AS01$_E$ at 10, 14 and 26 weeks

14, 26W, 9M = RTS,S/AS01$_E$ at 14, 26 weeks and 9 months

Control = HepB vaccine at ≤7 days

BCG = *Bacillus Calmette-Guérin* (BCG) (tuberculosis) vaccine (Statens Serum Institute and Biovac Institute Commercial BCG)

OPV = oral poliovirus vaccine (*Polio Sabin*, GSK Vaccines)

DTPwHepB/Hib = diphtheria, tetanus, whole-cell pertussis, hepatitis B and *Haemophilus influenzae type b* vaccine (*Tritanrix HepB/Hib*, GSK Vaccines)

Measles vaccine = attenuated measles vaccine (*Rouvax*, Sanofi)

Figure 2. CONSORT diagram for study participants

≤7D, 10, 14W = RTS,S/AS01$_E$ at ≤7 days, 10 and 14 weeks

≤7D, 10, 26W = RTS,S/AS01$_E$ at ≤7 days, 10 and 26 weeks

6, 10, 14W = RTS,S/AS01$_E$ at 6, 10 and 14 weeks

6, 10, 26W = RTS,S/AS01$_E$ at 6, 10 and 26 weeks

≤7D HepB, 6, 10, 26W = HepB vaccine at ≤7 days, and RTS,S/AS01$_E$ at 6, 10 and 26 weeks

10, 14, 26W = RTS,S/AS01$_E$ at 10, 14 and 26 weeks

14, 26W, 9M = RTS,S/AS01$_E$ at 14, 26 weeks and 9 months

Control = HepB vaccine at ≤7 days

Enrolled = number of subjects who were enrolled in the study

Completed = number of subjects who completed last study visit

Withdrawn = number of subjects who did not come back for the last visit

CW = consent withdrawal (not due to an AE) or parent withdrew child

AE = adverse event

LFU = lost to follow up (subjects with complete/incomplete vaccination course) or migrated

SAE = serious adverse event

PV = protocol violation

Figure 3. GMCs for anti-CS antibody concentration at each time point by group (ATP cohort for immunogenicity)

6, 10, 14W = RTS,S/AS01$_E$ at 6, 10 and 14 weeks

≤7D, 10, 14W = RTS,S/AS01$_E$ at ≤7 days, 10 and 14 weeks

≤7D, 10, 26W = RTS,S/AS01$_E$ at ≤7 days, 10 and 26 weeks

6, 10, 26W = RTS,S/AS01$_E$ at 6, 10 and 26 weeks

≤7D HepB, 6, 10, 26W = HepB vaccine at ≤7 days, and RTS,S/AS01$_E$ at 6, 10 and 26 weeks

10, 14, 26W = RTS,S/AS01$_E$ at 10, 14 and 26 weeks

14, 26W, 9M = RTS,S/AS01$_E$ at 14, 26 weeks and 9 months

Control = HepB vaccine at ≤7 days

GMC = geometric mean concentration

Figure 4. Bar graph of solicited local and general symptoms reported during the 7-day (Days 0-6) post vaccination period following neonatal dose of RTS,S/AS01$_E$ or a hepatitis B vaccine; pooled neonatal groups (Total Vaccinated Cohort)

RTS,S = Pooled neonatal RTS,S groups (≤7 days, 10, 14 weeks and ≤7 days, 10, 26 weeks groups)

HepB = Pooled neonatal HepB vaccine groups (HepB vaccine at ≤7 days, RTS,S/AS01$_E$ at 6, 10 and 26 weeks and Control [HepB vaccine at ≤7 days] groups)

*Combined local reactogenicity of RTS,S/AS01$_E$ or HepB vaccine and BCG vaccination

**Combined general reactogenicity of RTS,S/AS01$_E$ or HepB vaccine and BCG vaccination

Table 1 Anti-CS antibody GMC ratio post-Dose 3 in each study group compared to 6, 10, 14 week group (ATP cohort for immunogenicity)

RTS,S/AS01 group*	N	Anti-CS antibody GMC			Anti-CS antibody GMC ratio vs 6, 10, 14W		
		GMC	95% CI		GMC ratio	95% CI**	
6, 10, 14W	45	218.3	160.1	297.6	-		
6, 10, 26W	46	156.5	100.4	244.0	0.72	0.42	1.22
≤7 days, 10, 14W	47	128.2	92.2	178.2	0.59	0.38	0.92
≤7 days, 10, 26W	43	136.6	93.0	200.7	0.63	0.39	1.02
≤7 days HepB, 6, 10, 26W	43	170.6	114.6	254.1	0.78	0.48	1.28
10, 14, 26W	41	392.6	323.3	476.7	1.80	1.24	2.60
14, 26W, 9M	47	269.9	183.3	397.5	1.24	0.76	2.02

GMC = geometric mean antibody concentration

N = number of subjects with post-vaccination results available post-Dose 3; numbers will differ to ATP cohort for immunogenicity

95% CI = 95% confidence interval

*See Figure 1 for study groups and vaccinations

**95% CI for the GMC ratio (Anova model - pooled variance)

Table 2 Safety outcomes following 18 months' surveillance

			Number (%) infants reporting symptoms									
Study groups[†]			**≤7D, 10, 14W N[1] = 60**	**≤7D, 10, 26W N[1] = 59**	**6, 10, 14W[‡‡] N[1] = 54**	**6, 10, 26W N[1] = 57**	**≤7D HepB, 6, 10, 26W N[1] = 57**	**10, 14, 26W N[1] = 52**	**14, 26W, 9M N[1] = 57**	**Control N[1] = 52**	**Pooled neonatal RTS,S N[2] = 119**	**HepB N[2] = 120**
Solicited injection site symptoms during 7-day post-vaccination period[††]												
Pain	RTS,S/AS01$_E$	All	2 (1.2)	4 (2.5)	8 (5.1)	4 (2.4)	2 (1.2)	1 (0.7)	0	-	5 (4.2)	-
		Grade 3[‡]	0	0	0	0	0	0	0	-	0	-
	Control	All	7 (6.8)	2 (3.7)	7 (4.5)	11 (9.9)	3 (2.7)	4 (3.9)	1 (1.8)	12 (7.8)	-	3 (2.5)
		Grade 3[‡†]	0	0	1 (0.6)	0	0	0	0	0	-	0
Redness	RTS,S/ AS01$_E$	All	2 (1.2)	4 (2.5)	7 (4.5)	5 (3.0)	2 (1.2)	1 (0.7)	0	-	5 (4.2)	-
		Grade 3[‡]	0	0	0	0	0	0	0	-	0	-
	Control	All	6 (5.8)	1 (1.9)	6 (3.8)	5 (4.5)	5 (4.4)	4 (3.9)	2 (3.5)	10 (6.5)	-	7 (5.9)
		Grade 3[‡]	0	0	0	0	0	0	0	0	-	0
Swelling	RTS,S/AS01$_E$	All	5 (3.1)	4 (2.5)	8 (5.1)	5 (3.0)	3 (1.8)	1 (0.7)	0	-	5 (4.2)	-
		Grade 3[‡]	0	0	0	0	0	0	0	-	0	-
	Control	All	10 (9.7)	1 (1.9)	9 (5.8)	12 (10.8)	8 (7.1)	6 (5.9)	2 (3.5)	12 (7.8)	-	6 (5.0)
		Grade 3[‡]	0	0	0	0	0	0	0	0	-	0
Solicited general symptoms during 7-day post-vaccination period												
Drowsiness		All	0	0	0	0	0	0	1 (0.6)	0	0	1 (0.8)
		Grade 3[‡]	0	0	0	0	0	0	0	0	0	0
Irritability		All	7 (4.3)	3 (1.9)	10 (6.4)	2 (1.2)	3 (1.8)	5 (3.4)	5 (3.0)	5 (3.2)	1 (0.8)	0
		Grade 3[‡]	0	0	1 (0.6)	0	0	0	0	1 (0.6)	0	0
Loss of appetite		All	0	0	1 (0.6)	0	1 (0.6)	0	2 (1.2)	1 (0.6)	0	0

	Grade 3‡	0	0	0	0	0	0	0	0	0	0
Fever	≥38.0°C	28 (17.2)	24 (14.8)	26 (16.7)	17 (10.3)	16 (9.6)	24 (16.3)	23 (13.6)	15 (9.7)	17 (14.3)	8 (6.7)
	>39.0°C‡	0	1 (0.6)	1 (0.6)	0	0	1 (0.7)	2 (1.2)	0	0	0
Unsolicited (spontaneously reported) symptoms during 30-day post-vaccination period											
	All	36 (60.0)	35 (59.3)	28 (51.9)	29 (50.9)	35 (61.4)	36 (69.2)	47 (82.5)	31 (59.6)	26 (21.8)	33 (27.5)
	Grade 3‡	1 (1.7)	0	1 (1.9)	1 (1.8)	2 (3.5)	2 (3.8)	1 (1.8)	0		
	Related	0	0	0	1 (1.8)	0	0	0	0		
		Number (%) infants reporting serious adverse events following all doses of study vaccine									
Study groups		**≤7D, 10, 14W N² = 60**	**≤7D, 10, 26W N² = 59**	**6, 10, 14W N² = 60**	**6, 10, 26W N² = 60**	**≤7D HepB, 6, 10, 26W N² = 60**	**10, 14, 26W N² = 60**	**14, 26W, 9M N² = 60**	**Control**	**≤7D RTS N² = 119**	**≤7D HepB N² = 120**
SAEs (Month 0-18)	All	7 (11.7)	5 (8.5)	6 (10.0)	9 (15.0)	8 (13.3)	12 (20.0)	5 (8.3)	4 (6.7)	2 (1.7)	3 (2.5)

N[1] = Number of subjects receiving 3 doses of RTS,S/AS01$_E$ or DTPw-HepB/Hib alone for control; analyses of safety following 3 doses of RTS,S/AS01$_E$ or DTPw-HepB/Hib alone enabled comparison of equal follow up and number of vaccination visits (age at vaccination and number of doses of RTS,S/AS01$_E$ co-administered with DTPw-HepB/Hib differed)

N² = Total Vaccinated Cohort

Pooled neonatal RTS,S groups (≤7 days, 10, 14 weeks and ≤7 days, 10, 26 weeks groups)

Pooled neonatal HepB vaccine groups (HepB vaccine at ≤7 days, RTS,S/AS01$_E$ at 6, 10 and 26 weeks and Control [HepB vaccine at ≤7 days] groups)

†See Figure 1 for study groups and vaccinations

††All solicited injection site symptoms were considered related to vaccination

‡Grade 3 AEs were defined as: pain - cries when limb is moved/spontaneously painful; redness/ swelling - diameter >20 mm; drowsiness/ irritability/ unsolicited AEs - events preventing normal activity; loss of appetite- not eating at all; fever - temperature >39°C

⌗**Standard 6,10,14 week reference schedule**

Figure 1 Study design overview: study groups, vaccinations

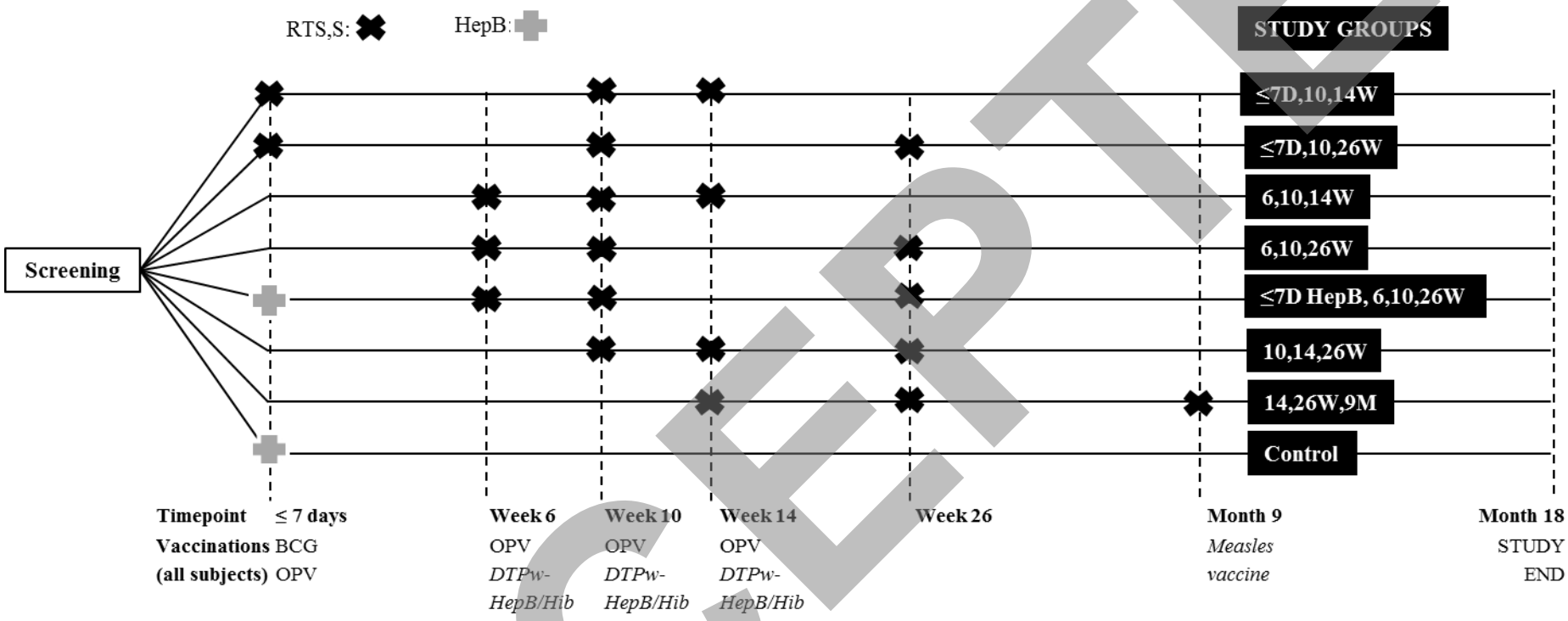

Figure 2 CONSORT diagram for study participants

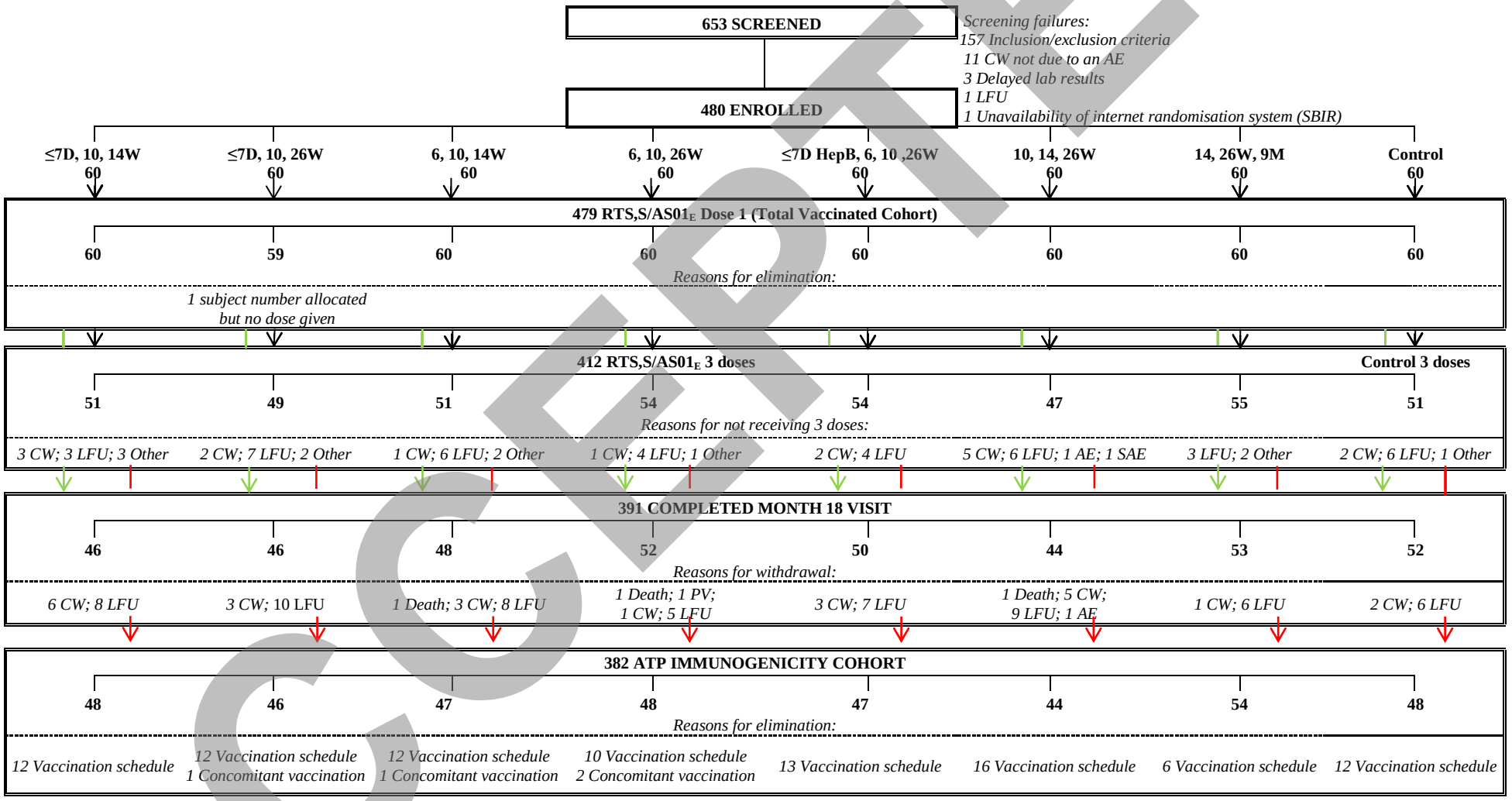

Figure 3 GMCs for anti-CS antibody concentration at each time point by group (ATP cohort for immunogenicity)

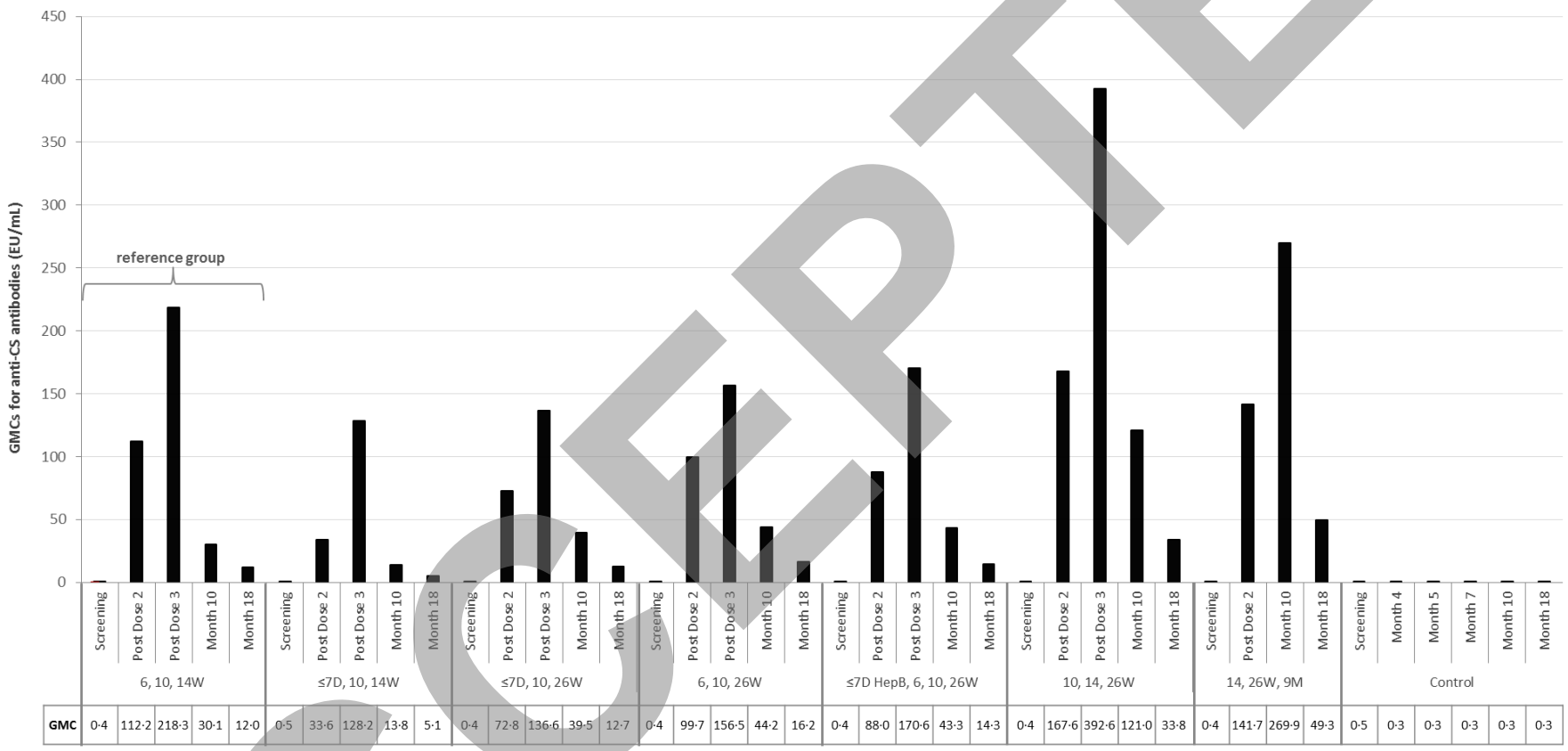

Figure 4 Bar graphs of solicited local and general symptoms reported during the 7-day (Days 0-6) post vaccination period following a neonatal dose of RTS,S/AS01$_E$ or a hepatitis B vaccine; pooled neonatal groups (Total Vaccinated Cohort)