Safety and efficacy of novel malaria vaccine regimens of RTS, S/AS01B alone, or with concomitant ChAd63-MVA vectored vaccines expressing ME-TRAP

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We assessed a combination multi-stage malaria vaccine schedule in which RTS,S/AS01B was given concomitantly with viral vectors expressing multiple-epitope thrombospondin-related adhesion protein (ME-TRAP) in a 0-month, 1-month, and 2-month schedule. RTS,S/AS01B was given as either three full doses or with a fractional (1/5th) third dose. Efficacy was assessed by controlled human malaria infection (CHMI). Safety and immunogenicity of the vaccine regimen was also assessed. Forty-one malaria-naive adults received RTS,S/AS01B at 0, 4 and 8 weeks, either alone (Groups 1 and 2) or with ChAd63 ME-TRAP at week 0, and modified vaccinia Ankara (MVA) ME-TRAP at weeks 4 and 8 (Groups 3 and 4). Groups 2 and 4 received a fractional (1/5th) dose of RTS,S/AS01B at week 8. CHMI was delivered by mosquito bite 11 weeks after first vaccination. Vaccine efficacy was 6/8 (75%), 8/9 (88.9%), 6/10 (60%), and 5/9 (55.6%) of subjects in Groups 1, 2, 3, and 4, respectively. Immunological analysis indicated significant reductions in anti-circumsporozoite protein antibodies and TRAP-specific T cells at CHMI in the combination vaccine groups. This reduced immunogenicity was only observed after concomitant administration of the third dose of RTS,S/AS01B with the second dose of MVA ME-TRAP. The second dose of the MVA vector with a four-week interval caused significantly higher anti-vector immunity than the first and may have been the cause of immunological interference. Co-administration of ChAd63/MVA ME-TRAP with RTS,S/AS01B led to reduced immunogenicity and efficacy, indicating the need for evaluation of alternative schedules or immunization sites in attempts to generate optimal efficacy.

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INTRODUCTION

Although the incidence of malaria has decreased globally since 2000, it remains a leading cause of mortality. An estimated 3.2 billion people remain at risk of disease, and approximately 445,000 deaths were attributed to malaria in 2016. No licensed malaria vaccine is available, although several candidates are in development, at stages ranging from demonstrated efficacy in controlled human malaria infection (CHMI) studies, to completion of phase 3 efficacy testing and positive European Medicines Agency scientific opinion. A strategy for increasing vaccine efficacy (VE) is combining antigenically distinct vaccines, targeting different stages of the parasite life cycle, into a single regimen. There are strong arguments that combining vaccines targeting different stages of the parasite life cycle into one regimen could increase VE. Different vaccine platforms exert efficacy against malaria through differing immune mechanisms, and an additional benefit of combining vaccine types is induction of both humoral and cellular immune responses to potentially increase efficacy. Based on supportive pre-clinical findings, we previously reported a study demonstrating high VE (as defined by sterile protection (SP) of subjects) against CHMI (14/17 subjects protected; VE 82.4% (95% confidence interval (CI): 64–100)) in healthy, malaria-naive adults with an estimated sustained sterile efficacy of 72% observed in a subset of subjects who underwent re-challenge at 6 months. Subjects received a vaccination schedule consisting of three standard doses of the sporozoite stage subunit vaccine RTS,S/AS01B, in addition to the heterologous prime-boost viral vector vaccine regimen of ChAd63-modified vaccinia Ankara (ChAd63-MVA) multiple-epitope thrombospondin-related adhesion protein (ME-TRAP), which targets the liver stage of infection. This study was notable, not just because it demonstrated high VE, but also in that it combined two distinct vaccine types: the first (RTS,S) induces high-titer antibodies to the circumsporozoite protein (CSP) and another inducing potent T cell
responses to TRAP using viral vectors (ChAd63-MVA ME-TRAP). Although the efficacy observed in the combination group was higher than in the comparator group that received three standard doses of RTS,S alone (12/16 subjects protected; VE 75% (95% CI: 54–96) estimated sustained VE at 6 months of 62.5%), the number of subjects in the study was small, and the difference in efficacy between the groups, or estimated sustained efficacy at re-challenge, was not statistically significant. The need for further evaluation of this approach was apparent. Furthermore, in this study, the RTS,S and viral vector vaccines were given separately at staggered time points, with a minimum interval of 2 weeks between each dose, resulting in a five-dose vaccination regimen,
over a course of 10 weeks. Cumulative number of doses is a significant cost and logistic consideration for a vaccine regimen to be deployable in malaria endemic countries. Ideally, a malaria vaccine would be deliverable concurrently within the Expanded Program of Immunizations (EPI) such as the three-dose diphtheria, pertussis, and tetanus–hepatitis B virus vaccine.\(^{16,17}\)

In 1997, during the first CHMI trial of RTS\(_S\), reactivity concerns after the second dose of vaccine led to a reduction in the third dose in two of the study groups. One group received a regimen consisting of two standard doses of RTS\(_S\)/AS02 at 0 and 1 month, and a third dose at month 7 which was 1/5th of the standard dose. Following CHMI, 6/7 subjects remained protected, resulting in a VE of 86% (95% CI: 0.02–0.88, P < 0.005).\(^{5}\) The beneficial effect of a fractional third dose on VE was further demonstrated in a recent CHMI trial, in which subjects received a 0-month, 1-month, and 7-month regimen consisting of two standard doses of RTS\(_S\)/AS01B followed by a fractional (1/5th) third dose.\(^{18}\) Following CHMI, 26/30 subjects were protected against malaria (VE 86.7% (95% CI: 66.8–94.6)) compared with 10/16 in the standard 0-month, 1-month, 2-month full-dose group (VE 62.5% (95% CI: 29.4–80.1)); log-rank P = 0.040.

In this phase I/IIa, open-label, CHMI study, we assessed whether the efficacy of a standard three-dose regimen RTS\(_S\)/AS01B could be improved by the concurrent, same site administration of ChAd63 and MVA viral vectors expressing ME-TRAP. Safety and immunogenicity of this novel schedule were also assessed. In addition, we assessed for the first time whether the efficacy of a standard three-dose regimen of RTS\(_S\)/AS01B could be improved by a regimen consisting of two standard doses followed by a fractional (1/5th) third dose given on a 0-month, 1-month, and 2-month schedule, either alone or given concurrently with viral vectors expressing ME-TRAP.

**RESULTS**

Study participants

Seventy-four subjects were screened for eligibility and 45 subjects were identified as eligible at enrolment (Supplementary Figure SF1). Ten subjects each were allocated to Groups 1, 2, and 3 to receive R-R-R, R-R-r, or RA-RM-RM, respectively. Eleven subjects were allocated to Group 4 to receive RA-RM-rM. Group allocation numbers were lower than the planned 12 per group at CHMI, as a result of consent withdrawals and ineligibility at the time of first vaccination. Four subjects were allocated to Group 5 as unvaccinated infectivity controls. Vaccinations took place between 5 January 2015 and 27 February 2015. CHMI was performed on 23 March 2015 and 24 March 2015.

Safety

The majority of adverse events (AEs) following vaccination in all vaccine groups were mild in severity and self-limiting. There were no serious AEs (SAEs) related to vaccination or suspected unexpected serious adverse reactions (SUSARs). Commonly reported AEs following vaccination were vaccine site pain, feverishness, fatigue, malaise, headache, and myalgia. There were no significant differences in the rates of occurrence of grade 3 (severe) solicited or unsolicited AEs, between the RTS\(_S\)/AS01B-alone groups (1 and 2) and the RTS\(_S\)/AS01B plus viral vectors groups (3 and 4) (Supplementary Table S4). Tabulations of AEs can be found in the Supplemental Tables S1–S5.

Protective efficacy against CHMI

Forty subjects underwent CHMI and completed follow-up. By day (D) 23 following CHMI, 6/8 subjects in Group 1 were protected (VE 75% (95% CI: 31.5–93.1)); in Group 2, 8/9 subjects were protected (VE 88.9% (95% CI: 43.3–98.4)); in Group 3, 6/10 subjects were protected (VE 60% (95% CI: 25.3–82.7)); in Group 4, 5/9 subjects were protected (VE 55.6% (95% CI: 20.4–80.5)) (Fig. 1a). All four unvaccinated controls in Group 5 were diagnosed with malaria. Pooling the outcome of subjects in Groups 1 and 2, who had received RTS\(_S\)/AS01B alone, 14/17 subjects were protected (82.4% (95% CI: 54.7–93.9)) compared with 11/19 subjects who had received RTS\(_S\)/AS01B with viral vectors in Groups 3 and 4 (VE 57.9% (95% CI: 33.2–76.3); P = 0.074) (Fig. 1b). Mean time to diagnosis was 11.6 days (range 11.5–12, SD = 0.22 days) in Group 5, while mean time to diagnosis was 15.5, 16.5, 14.1, and 14 days in Groups 1, 2, 3, and 4, respectively. Analysis of time to parasitemia measured by quantitative polymerase chain reaction (qPCR) showed significant difference in time to parasitemia for all vaccine groups compared with controls, using thresholds of either 20 or 500 parasites per milliliter of blood (Fig. 1c, d and Supplementary Table S6).

T cell immunogenicity

T cell responses to vaccine antigens were measured by interferon γ (IFNγ) enzyme-linked immunosorbent spot (ELISPOT) assay (Supplementary Figure SF2). Responses at baseline and after vaccination were assessed to CSP in all groups and to ME-TRAP in Groups 3 and 4 only. CSP-specific T cell frequencies are described in the supplementary information (Supplementary Figure SF2A). T cell responses to ME-TRAP peaked 1 week after the first dose of MVA at D35 (Supplementary Figure SF2B) at a median of 2531 SFC (interquartile range (IQR): 1949–4042). This high level of cellular immunogenicity is comparable to what we previously observed after an 8-week prime-boost interval with ChAd63 and MVA ME-
Humoral immunogenicity of RTS,S/AS01B co-administered with ChAd63-MVA ME-TRAP

Antibody (Ab) responses induced by RTS,S vaccination were measured by enzyme-linked immunosorbent assay (ELISA) against the NANP repeats, C-terminal regions of CSP and the full-length CSP protein (Fig. 2a–d). NANNP IgG responses peaked at D42 in all groups, declined by D56 and increased again following the third vaccination in the groups receiving RTS,S alone (Groups 1 and 2) but not in the RTS,S with viral vectors groups (Groups 3 and 4) (Fig. 2a). There was no significant difference in NANNP IgG titers between Groups 1 and 2 pooled compared with Groups 3 and 4 pooled after first vaccination (Fig. 2b), a trend to higher titers in Groups 1 and 2 pooled after second and third vaccinations (Fig. 2d), and significantly higher titers in this group after third vaccination (* P = 0.007) and 0.0005 (**), respectively; Bars represent medians with interquartile range (IQR); n.s., not statistically significant.

Humoral Immunogenicity of RTS,S/AS01B co-administered with ChAd63-MVA ME-TRAP

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Flow cytometry with intracellular cytokine staining (ICS) demonstrated that cytokine responses to CSP were predominantly from CD4+ T cells and were not significantly different between groups either at D42 or C-1 (Supplementary Figure SF3A). ICS responses to TRAP were assessed in Groups 3 and 4 only and comprised expression of cytokines from similar proportions of CD4+ and CD8+ T cells (Supplementary Figure SF3B). CD8+ T cell responses to TRAP were dominated by cells expressing IFNγ either alone or in combination with other cytokines. Responses did not change substantially between post Ad and post first MVA, but the second dose of MVA substantially reduced the proportion of monofunctional cells expressing IFNγ (D63 and C-1, P = 0.007 two-tailed t test), (Supplementary Figure SF4), a population that we have previously shown to be associated with vaccine-induced protection against malaria.2

TRAP (2436 SFC, IQR: 1064–3862, Supplementary Figure 2C).2 Administration of a second dose of MVA induced a re-boosting of T cell responses but to a significantly lower median magnitude than after the first MVA dose (2531 SFC, IQR: 1949–4042 after first MVA compared with 901 SFC, IQR: 509–1910, P = 0.04, one-way analysis of variance).

Fig. 2 Humoral immunogenicity to CSP. a Median NANNP IgG time courses. b NANNP IgG responses after first RTS,S vaccination (day 28, D28), after second (day 56, D56), and third (C-1) RTS,S vaccinations, for volunteers receiving RTS,S/AS01B alone or RTS,S/AS01B and viral vectors co-administered. Two-tailed Mann–Whitney analyses. A trend to higher NANNP IgG in RTS,S-alone group was observed after second vaccination P = 0.06 and significantly higher titers after third vaccination P = 0.0007 (**). C-terminal IgG responses after each RTS,S vaccination. Significantly higher titers in RTS,S-only group after second and third vaccinations, two-tailed Mann–Whitney, 0.007 (**) and 0.0005 (**), respectively. D, IgG responses against full-length CSP. Significantly higher titers in RTS,S-only group after second and third vaccinations, two-tailed Mann–Whitney P = 0.03 (*) and 0.001 (**), respectively; Bars represent medians with interquartile range (IQR); n.s., not statistically significant.
Fig. 3  Avidity of IgG responses to CSP and total IgG responses to TRAP. a Avidity of total IgG against full-length CSP in each group at D28, D56, and C-1. Friedman test with Dunn’s correction for comparison of time points within each group P value = 0.0009, <0.0001, <0.0001, and 0.003 for G1, 2, 3, and 4, respectively. No significant difference between groups at any time point, Kruskal–Wallis analysis with Dunn’s correction. b Avidity of total IgG, IgG1, and IgG3 against NANP for G1, 2, 3, and 4. No significant differences between groups for total IgG or IgG2 avidity. Significantly higher IgG1 avidity in G2 compared with G1, two-tailed Mann–Whitney, P value = 0.01 (*), no significant differences between other groups or isotypes. c TRAP-specific IgG responses. RTS,S co-administered with vectors (VAC59 G3/4, black circles), and RTS,S and vectors administered at a 2-week interval (VAC55 G1, gray squares). No significant difference between trials at peak post ChAd vaccination (21 days after ChAd), 35 days post challenge (C + 35), or 90 days post challenge (C + 90) and peak post MVA vaccinations compared between trials, two-tailed Mann–Whitney. No significant difference between peak post MVA in VAC55 (C-1) and peak post first MVA in VAC59 (D42), significantly lower TRAP IgG titers at peak post second MVA in VAC59 (C-1), two-tailed Mann–Whitney P = 0.02 (*), respectively. d Median TRAP IgG time courses in VAC55 (gray squares) and VAC59 (black circles). R, full-dose RTS,S/AS01B; A, ChAd63 ME-TRAP; M, MVA ME-TRAP; r rational dose RTS,S/AS01B; CHMI, controlled human malaria infection; C-1, day before CHMI; SFCs, spot-forming cells per million PBMC; n.s., not statistically significant; Bars represent medians with IQR. *= P ≤ 0.05; ** = P ≤ 0.01; *** = P ≤ 0.001

Comparing the groups that received the fractional third dose of RTS,S with those that received the full dose (P = 0.905, P = 0.842 in comparing Groups 1 with Group 2, and Groups 3 with 4, respectively)

Avidity of IgG responses to CSP and total IgG responses to TRAP Differences in the quality of the Ab response in each group were compared using a sodium thiocyanate displacement ELISA to measure avidity of total IgG against full-length CSP at D28, D56, and C-1 (Fig. 3a), and of total IgG, IgG1, and IgG2 against NANP at C-1 (Fig. 3b). Avidity of CSP-specific IgG increased significantly after the second vaccination and was not further increased by a third dose in any group. There were no significant differences in CSP IgG avidity between groups at any time point. There were no significant differences in the avidity of NANP-specific total IgG, or IgG2 between groups at C-1. The avidity of NANP-specific IgG1 was significantly higher in Group 2 compared with Group 1 directly (Mann–Whitney analysis, P = 0.01), but there were no significant differences in a comparison of all groups (Kruskal–Wallis with Dunn’s correction P = 0.0837). Total IgG responses to TRAP are described in Supplementary Information (Fig. 3c, d).

Anti-vector Ab responses Antibody responses to MVA were measured using the WR113/D8L protein from MVA19 in Groups 3 and 4 by total IgG ELISA at baseline, and after each MVA vaccination (D42, D76/C-1) (Fig. 4a). At baseline, two volunteers were borderline positive and one volunteer was strongly positive for anti-MVA antibodies, although none had received smallpox vaccination. Anti-MVA IgG titers significantly increased after the first MVA vaccination and again after the second vaccination, after which all volunteers were seropositive (Kruskal–Wallis analysis with Dunn’s correction P < 0.0001). The increase in anti-vector antibodies after initial MVA vaccination at 4 weeks was comparable to that induced by a single MVA given at 8 weeks in a previous trial (Fig. 4b). However, the fold change in anti-MVA titers was significantly higher after two MVA doses (Kruskal–Wallis analysis with Dunn’s correction P = 0.0002). Anti-MVA titers at baseline and C-1 were comparable between protected and non-protected volunteers, but titers were significantly higher in non-protected volunteers after the initial MVA vaccination (Fig. 4c, P = 0.02). There was a trend towards a negative association between anti-MVA IgG after the initial MVA (D42) and TRAP-specific T cell responses after the second MVA (C-1) (Fig. 4d, r = −0.6333, P = 0.08), but no association between anti-MVA antibodies at D42 and TRAP-specific T cell responses at D42 (Supplementary Figure SF5A) or between anti-MVA antibodies at
C-1 and TRAP-specific T cells at C-1 after a single MVA administered at 8 weeks (Supplementary Figure SFSB). However, some of the lowest C-1 TRAP T cell responses were observed in individuals with high titers of anti-MVA IgG at D42.

Potential additive effect of TRAP-specific T cells to efficacy in co-administration groups

In the groups that received RTS,S alone (Groups 1 and 2), SP was achieved with CSP titers as low as 451 ELISA units (EU), with 100% of volunteers protected above 1600 EU. A statistically significant reduction in CSP IgG titer was observed between groups that received RTS,S in combination with viral vectors as compared to groups that received RTS,S alone ($p = 0.009$, two-tailed Mann–Whitney test, Fig. 5a, left-hand axis). Median NAMP-specific IgG titers at C-1 in the combination group with SP (659 EU, IQR: 374–1520) were comparable to non-protected (NP) volunteers in the RTS,S-alone group (667, IQR: 394–968) and were not significantly different to median CSP IgG titers in non-protected volunteers receiving RTS,S with viral vectors (462 EU, IQR: 259–595, $p = 0.15$, Mann–Whitney test). Interestsingly, median ELISPOT responses were twice as high in SP volunteers in the combination group (median 1505 SFC, IQR: 898–2167, Fig. 5, right-hand axis) compared with NP (median 738, IQR 382–1827), although differences were not statistically significant ($p = 0.09$, Mann–Whitney test). Individual ELISPOT and Ab titers with protection status indicated are presented in Fig. 5b.

**DISCUSSION**

This is the second study to combine RTS,S and the viral vectors ChAd63 and MVA encoding ME-TRAP in the same regimen, but the first in which viral vectors have been concomitantly administered with RTS,S. It is the third study to evaluate an RTS,S/Sylvitec regimen that incorporates a fractional third dose, but the first with it administered in a 0-month, 1-month, and 2-month schedule. We have shown that administering these vaccines concomitantly is safe, with no SUSARs, and no vaccine-related SAEs. As expected, a higher frequency of AEs was observed in the groups that received the viral vectors with RTS,S, but the majority of AEs were mild, and all were self-limiting.

In this study, we have again observed a high level of VE in the groups that received only RTS,S either at three full standard doses or with a reduced third dose. However, in the groups that received viral vectors and RTS,S, observed VE was lower than in the RTS,S-alone groups. Although no comparisons of VE between vaccinated groups are statistically significant, they suggest that concomitant administration of these vaccines, according to the schedules and
immunization routes used in this trial, may reduce Ab responses to CSP and negatively impact VE.

As with previous CHMI studies of RTS,S/AS01B, anti-CSP Ab titers in this study were significantly higher in protected subjects immediately prior to CHMI than in non-protected subjects. In this study, the third dose of RTS,S appears to be ineffective at boosting to levels greater than those after the second dose, and in the subjects receiving RTS,S with viral vectors the third dose of RTS,S was ineffective at even maintaining anti-CSP Ab titers. As a result, anti-CSP Ab titers were significantly lower on the day preceding CHMI than in non-protected subjects. In this study, the third dose of RTS,S appears to be ineffective at boosting into CHMI than in non-protected subjects. In this study, the third dose of RTS,S appears to be ineffective at boosting.

Concomitant administration can be overcome and to identify whether vaccines inducing TRAP-specific T cells could contribute to efficacy in combination with anti-CSP Ab-inducing vaccines when administered simultaneously. Alternative solutions include concomitant administration but at different vaccination sites, administering only a single dose of MVA ME-TRAP, or adjusting the vaccine dose or formulation. Other options include separation of the administration time points of the anti-sporozoite-stage and anti-liver-stage vectors, based on the durable high efficacy of this approach which we reported previously. The RTS,S phase 3 trial showed highest immunogenicity and efficacy of this vaccine in children aged 5–17 months compared to 6–12 week olds, leading to the WHO recommendation for pilot implementation in the younger age group. In addition, anti-CSP IgG titers are a surrogate of protection, with reduced efficacy and durability also observed in the younger age group. In contrast, recent phase Ib data on ME-TRAP vector administration in Gambian and Burkinafaso infants showed optimal T cell immunogenicity in 2–4-month-old infants even when the vectored vaccines were co-administered with standard EPI vaccines. Hence, vaccination strategies aimed at exploiting the differences in immune responses in this younger age group should be explored.

In conclusion, no safety concerns arose from concomitant administration of ChAd63 and MVA viral vectors encoding ME-TRAP with RTS,S. Further work is required to evaluate the impact of concomitant administration, and the use of a fractional third dose of RTS,S on VE.

**METHODS**

**Participants**

Recruitment and vaccination was conducted at four UK sites: Oxford, Southampton, London, and Guildford. The CHMI procedure was performed as previously described using five infectious bites from *P. falciparum* 3D7-strain-infected *Anopheles stephensi* mosquitoes at Imperial College,
Table 1. Study design

| Week | Group 1 (n = 13) | Group 2 (n = 13) | Group 3 (n = 13) | Group 4 (n = 13) | Group 5 (n = 4) |
|------|-----------------|-----------------|-----------------|-----------------|----------------|
| 0    | RTS,S/AS01b     | RTS,S/AS01b     | RTS,S/AS01b     | RTS,S/AS01b     | Control        |
|      | (Standard dose) | (Standard dose) | (Standard dose) | (Standard dose) |
| 4    | RTS,S/AS01b     | RTS,S/AS01b     | RTS,S/AS01b     | RTS,S/AS01b     |                |
|      | (Standard dose) | (Standard dose) | (Standard dose) | (Standard dose) |
| 8    | RTS,S/AS01b     | RTS,S/AS01b     | RTS,S/AS01b     |               |
|      | (Standard dose) | (Standard dose) | (Standard dose) |               |
| 11   | CHMI (n = 12)   | CHMI (n = 12)   | CHMI (n = 12)   | CHMI (n = 12)   |               |

Group 1 received 3 standard doses (50 µg) of RTS,S/AS01B (R-R-R); Group 2 received two standard doses of RTS,S/AS01B followed by a third fractional dose of RTS,S/AS01B at 1/5th of the standard dose (R-R-r); Group 3 received three standard doses of RTS,S/AS01B in addition to ChAd63 ME-TRAP 5 × 1010 virus particles (vp) at week 0 and MVA ME-TRAP 2 × 108 plaque-forming units (PFU) at weeks 4 and 8 (RA-RM-RM); Group 4 received two standard doses of RTS,S/AS01B followed by a third fractional dose of RTS,S/AS01B at 1/5th of the standard dose in addition to ChAd63 ME-TRAP 5 × 1010 vp at week 0 and MVA ME-TRAP 2 × 108 PFU at weeks 4 and 8 (RA-RM+M); Group 5 (n = 4) received no vaccinations.

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Author contributions
T.R., K.J.E., and A.V.S.H. designed and supervised the trial. T.R. wrote the first draft of the manuscript. G.B., N.J.E., D.W., J.P., C.B., A.G., H.D., S.G., and S.A. contributed to the immunology and molecular experiments, S.S., R.P., N.V., L.D.P., H.D.G., D.G., R.R., B.A., R.E.S., A.M.L., D.J.M.L., G.S.C., and S.N.F. provided clinical support for the trial; K.I., R.W., B.-Y.R., U.W.-R., C.L., C.O., J.V., D.M., M.L., and R.W.B. contributed to study design and provided support and input throughout. T.R. and K.J.E. contributed equally to this manuscript. All the authors have read and commented on the final manuscript and have agreed to its submission.

Additional information
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Competing interests: A.V.S.H. and S.C.G. are named inventors on patent applications and patents relating to malaria vectored vaccines and immunization regimens. D.M., M.L., and R.W.B are employees of GSK, which is developing vaccines for malaria and other diseases. S.N.F. acts on behalf of the University of Southampton/University Hospital Southampton NHS Foundation Trust as chief and principal investigator for clinical trials Sponsoring vaccine manufacturers including GSK, but receives no personal payments for the work. The other authors declare no competing interests.

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