# Evaluation of a novel malaria anti-sporozoite vaccine candidate, R21 in Matrix-M adjuvant, in the UK and Burkina Faso: two phase 1, first-in-human trials

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## Summary

**Background** Malaria remains a substantial public health burden among young children in sub-Saharan Africa and a highly efficacious vaccine eliciting a durable immune response would be a useful tool for controlling malaria. R21 is a malaria vaccine comprising nanoparticles, formed from a circumsporozoite protein and hepatitis B surface antigen (HBsAg) fusion protein, without any unfused HBsAg, and is administered with the saponin-based Matrix-M adjuvant. This study aimed to assess the safety and immunogenicity of the malaria vaccine candidate, R21, administered with or without adjuvant Matrix-M in adults naïve to malaria infection and in healthy adults from malaria endemic areas.

**Methods** In this Article we report two phase 1, first-in-human trials. The first trial was a phase 1a open-label study in the UK evaluating the safety and immunogenicity of R21 administered either alone, or with 50 µg of Matrix-M. The second trial was a phase 1b randomised controlled trial in Burkina Faso. Adults had to be aged 18–50 years for enrolment in the phase 1a trial, and 18–45 years in the phase 1b trial. The phase 1a trial doses were 2 µg, 10 µg, and 50 µg R21/Matrix-M, and 50 µg R21 only. The phase 1b trial doses were 10 µg R21/Matrix-M and saline placebo. Matrix-M was always dosed at 50 µg. Phase 1b implemented block randomisation by randomisation into study groups by an independent statistician based at the University of Oxford using a randomisation code list with allocation concealment using opaque sealed envelopes. The primary objective of the phase 1a trial was to assess the safety and tolerability of R21 with and without Matrix-M. The primary objective of the phase 1b trial was to assess the safety and tolerability of R21 with Matrix-M. Both trials are registered with ClinicalTrials.gov, NCT02572388 for phase 1a and NCT02925403 for phase 1b, and are completed.

**Findings** Between Oct 1, 2015, and Jan 3, 2017, 31 individuals were enrolled in the phase 1a study. Six individuals were assigned to receive 2 µg R21/Matrix-M, 11 to 10 µg R21/Matrix-M, ten to 50 µg R21/Matrix-M, and four to 50 µg R21 only. Between Aug 26, 2016, and Sept 28, 2017, 13 individuals were enrolled in the phase 1b study. Eight individuals were assigned to receive 10 µg R21/Matrix-M, and five to placebo. Vaccinations were well tolerated, and most local and systemic adverse events were mild. There were no serious adverse events deemed related to vaccination. Two serious adverse events occurred. The first in the 10 µg R21/Matrix-M group was worsening of previously undisclosed or undiagnosed palindromic rheumatism and was deemed unlikely to be related to vaccination and the second in the 2 µg R21/Matrix-M was hospital admission for an unplanned excision of a pre-existing Bartholin's cyst, also unrelated to vaccination. In the phase 1a study, a total of 21 adverse events were recorded in the 2 µg R21/Matrix-M group, 103 in the 10 µg R21/Matrix-M group, 94 in the 50 µg R21/Matrix-M group, and 21 in the 50 µg R21 alone group. In the phase 1b study, twelve adverse events were recorded in the 10 µg R21/Matrix-M group and 0 in the placebo group.

**Interpretation** R21 with Matrix-M adjuvant has an acceptable safety profile. These data have formed the basis for efficacy testing of this vaccine.

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## Introduction

Malaria is one of the leading infectious causes of mortality worldwide and, in 2022, there were an estimated 249 million cases of malaria and 608 000 deaths, with three-quarters of deaths among children in sub-Saharan Africa.[1] Increased distribution of long-lasting insecticidal nets, widespread

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## Research in context

### Evidence before this study

In 2021, malaria caused 247 million clinical cases and 619 000 deaths. There is currently only one licensed vaccine against malaria and therefore additional vaccines are needed to help further reduce morbidity and mortality from malaria. We searched PubMed from database inception to July 4, 2023, for published articles using the search terms "((malaria vaccine [All Fields]) AND (phase 3[All Fields]) AND (efficacy[All Fields]) and (clinical trial[All Fields])". No language or date restrictions were applied. RTS,S/AS01B (RTS,S) was the first malaria vaccine to show a protective effect against clinical disease episodes in young children in a phase 3 clinical trial. Coordinated by WHO, pilot implementation studies for the vaccine began in three African countries in 2019 for a planned duration of 4 years. The studies included over 900 000 children, and aimed to address safety concerns and assess the logistic feasibility of the deployment of a four-dose schedule. In October, 2021, WHO approved RTS,S for the prevention of *Plasmodium falciparum* malaria for children in regions with moderate to high malaria transmission, with roll-out starting in 2024. Additional questions relate to the future costs of RTS,S and whether the low current supply of 6 million doses annually of RTS,S is sufficient to meet the demand for hundreds of millions of doses per annum. The relatively high cost of RTS,S limits its cost-effectiveness relative to existing control measures, such as seasonal malaria chemoprevention and insecticide-treated nets.

### Added value of this study

R21 is a new malaria vaccine candidate, based on the same malaria antigen as RTS,S, but without the additional hepatitis B protein molecules required to form RTS,S particles. R21 is administered with the Matrix-M adjuvant which has been tested with other (non-malaria) vaccine candidates and has shown safety in millions of COVID-19 vaccinees. We report here the first two phase 1 clinical trials of R21, one conducted in malaria-naive individuals in the UK and the other in semi-immune adults from an area of very high malaria transmission in Burkina Faso. A favourable vaccine safety profile was identified in both UK and Burkinabe adults with minimal reactogenicity and a much reduced incidence of post-vaccination fever compared with RTS,S. Antibody responses to malaria induced by R21/Matrix-M were not different to those induced by RTS,S even when using five-fold and 25-fold less immunogen, which could reduce vaccine costs and increase the number of vaccine doses that could be provided.

### Implications of all the available evidence

R21/Matrix-M is an alternative malaria vaccine to RTS,S with similar or better efficacy recently demonstrated in ongoing safety and efficacy trials. Lower manufacturing costs and reduced reactogenicity after vaccination could make R21/Matrix-M a useful future tool for malaria control and eradication efforts.

deployment of rapid diagnostic tests to target treatment with artemisinin-based combination therapy, scale-up of seasonal malaria chemoprevention, and use of intermittent preventive treatment in pregnant people have all contributed to a reduction in cases since 2010, although progress has been highly variable. The emergence and spread of resistance to artemisinins and insecticides[2] threatens malaria control efforts and there remains an urgent need for highly efficacious malaria vaccines.[1]

The first WHO-recommended malaria vaccine, RTS,S/AS01$_B$ (RTS,S) elicits antibodies to the pre-erythrocytic circumsporozoite protein (CSP) and has completed testing in a large phase 3 clinical trial.[3–6] RTS,S has shown significant vaccine efficacy of 46% over 18 months in children aged 5–17 months after three doses. However, this efficacy falls short of the overall goal set by the Malaria Vaccine Technology Roadmap for the development of a suitable vaccine with at least 75% efficacy against clinical malaria by 2030.[7] The combination of RTS,S vaccination administered seasonally with seasonal malaria chemoprevention has proven more efficacious than seasonal malaria chemoprevention or vaccination alone, showing the potential for improvements in efficacy by combining approaches.[8]

R21, designed and developed at the Jenner Institute, University of Oxford in 2011,[9] is a redesigned version of RTS,S aiming for improvements in immunogenicity, efficacy, and cost of goods. R21, like RTS,S, uses the HBsAg scaffold to display the CSP and is formed from a CSP-HBsAg fusion protein that contains the central repeat and the C-terminus of CSP. Unlike RTS,S, R21 does not contain the four-fold excess of unfused HBsAg protein, which was required for RTS,S particle formation.[10] CSP comprises around 20% of the total protein content in RTS,S and a large proportion of the antibody response induced by RTS,S is towards the HBsAg. By contrast, R21 contains only CSP-HBsAg fusion protein, with no unfused HBsAg, increasing the density of CSP antigens on the particle surface. As a result, a 50 µg dose of R21 contains about 25 µg of CSP antigen, compared with 10 µg of CSP antigen in a standard adult 50 µg dose of RTS,S. Formation of a particle with only the CSP-HBsAg fusion protein was done by expressing R21 in an improved yeast expression system, *Pichia pastoris*, and more recently *Hansenula polymorpha*,[11] rather than in *Saccharomyces cerevisiae*. At the C-terminus of R21, a four amino acid sequence, glu-pro-glu-ala (C-tag), was added for efficient immunochromatographic purification of R21 for the clinical trials reported here. This sequence is found many times in the proteome of malaria parasites and humans but, to our knowledge, has not been used previously in any vaccine administered to humans.[12] In the currently deployed R21 there is no C-tag as this has been removed.

Adjuvants can enhance the immunogenicity and efficacy of protein or particle vaccines, and Matrix-M, as with other

adjuvant formulations of Quillaja saponins, shows acceptable safety in large numbers of recipients[13] and the ability to enhance both cellular and humoral immune responses to a range of antigens.[14,15] In addition to the saponin, QS21, the adjuvant system-01 (known as AS-01) adjuvant used with RTS,S also contains 50 μg of the immunostimulant, monophosphoryl lipid A. No such TLR4 ligand is present in the Matrix-M adjuvant, which could lead to an improved safety profile and lower costs of manufacture for Matrix-M. Therefore, we initially conducted a phase 1, first-in-human, open-label clinical trial to assess the safety and immunogenicity of R21 administered alone and with Matrix-M, in healthy UK individuals. Based on an encouraging safety profile and similar humoral immunogenicity to RTS,S/AS01$_B$, we tested the lower dose of 10 μg R21 and 50 μg Matrix-M in Burkinabe adult individuals and extended the UK study to assess the immunogenicity of an even lower dose of 2 μg R21 and 50 μg Matrix-M. Here we report safety and immunogenicity in two clinical trials and compare our results with data from a previous UK clinical trial of participants receiving three 50 μg doses of RTS,S/AS01$_B$.[16]

## Methods

### Study design, participants, and randomisation and masking

In these phase 1, first-in-human trials, the first trial was phase 1a. This trial was conducted in healthy adults aged 18–50 years in the UK and was at the Centre for Clinical Vaccinology and Tropical Medicine (CCVTM) at the University of Oxford (Oxford, UK) and the National Institute for Health and Care Research Imperial Clinical Research Facility (London, UK). The trial was an open-label, first-in-human, clinical trial of R21 (University of Oxford Clinical Biomanufacturing facility, Oxford, UK) at a range of doses in Matrix-M adjuvant. The CCVTM enrolled participants and was responsible for them throughout the study.

Inclusion criteria required participants to be aged 18–50 years; able and willing to comply with all study requirements; consent to the investigator discussing their medical history with their general practitioner; and to agree not to donate blood during the study.[17] Female participants were also required to use continuous effective contraception for the course of the study and to provide a negative pregnancy test on the day of screening and the day of vaccination. Written informed consent was required.

Eligible participants were assigned to one of four groups and received three doses of R21, 4 weeks apart. The dose groups were 50 μg R21 only, 50 μg R21/Matrix-M, 10 μg R21/Matrix-M, and 2 μg R21/Matrix-M (Novavax, Uppsala, Sweden). When given, Matrix-M was always dosed at 50 μg. The first three vaccinations in groups receiving 10 μg or more of R21 occurred in a staggered manner and interim safety reviews of the first three participants in each group were conducted after the first vaccination by the local safety monitor before progression to the higher dose group. Three participants were recruited for the 10 μg R21/Matrix-M group first and, following safety review of the third participant, the first three participants in groups 2 and 3 were enrolled in parallel. Once a safety review of the first three participants in either group 1, 2, or 3 was complete, the remainder of the groups were recruited for. Allocation to groups was not randomised. The 2 μg group was added towards the end of the trial based on the similar immunogenicity of the 10 μg and 50 μg vaccinees and enrolment was not staggered or restricted as the antigen had already been administered at higher doses by this stage of the study. Full details regarding the study conduct are provided in the protocol, which is available online.[17]

The study protocol and associated documents were reviewed and approved by the UK National Research Ethics Service, Committee South Central–Berkshire B (reference number 15/SC/0386), the Medicines and Healthcare Products Regulatory Agency (reference number 21584/0352/001-0001), and the Oxford University Clinical Trials and Research Governance team, who independently monitored compliance with Good Clinical Practice guidelines. This phase 1a trial is registered with ClinicalTrials.gov, NCT02572388, and an independent local safety monitor provided safety oversight.

Based upon data from the phase 1a study, clinical development of R21/Matrix-M progressed to a phase 1b study, conducted in healthy Burkinabe adults aged 18–45 years, with the same inclusion criteria as for the phase 1a trial[17] at the Centre National de Recherche et de Formation sur le Paludisme (CNRFP) research unit (Banfora, Burkina Faso). Participants were enrolled at the site by clinic staff. All participants provided written informed consent. The phase 1b study was a single-blind, block randomised, controlled trial assessing three 10 μg doses, 4 weeks apart, of R21/Matrix-M in Burkinabe adults compared with a saline placebo. Again Matrix-M was dosed at 50 μg. Investigators were unaware of group assignment throughout the study duration. The phase 1b study only commenced after a satisfactory Data Safety and Monitoring Board review of the interim safety report for the 10 μg and 50 μg dose of R21/Matrix-M given to participants in the phase 1a study. Participants were randomly assigned to receive R21/Matrix-M or normal saline placebo by a team member not involved further in the trial. Full details regarding the study conduct are provided in the protocol which is available online.[11]

The study protocol was approved by the Burkina Faso regulatory authorities, the Burkina Faso Ministry of Health Ethical Committee for Biomedical Research (reference number 2014-10-118), the institutional review board of the Centre National de Recherche et de Formation sur le Paludisme, and Oxford Tropical Research Ethics Committee (reference number 36-15). The trial was registered with ClinicalTrials.gov, NCT02925403. An independent data safety monitoring board provided oversight and reviewed preliminary safety data before vaccinations continued

during the vaccination period. The trial was monitored by an external organisation (Margan Clinical Research Organization). Both studies were conducted according to the principles of the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines.

Group 2 of a previously conducted phase 2a, open-labelled, partly randomised malaria challenge trial was used as a comparator group.[18] The trial consisted of 16 healthy, malaria-naive adults who received three doses of 50 μg RTS,S (GSK, Rixensart, Belgium) at 4-week intervals (appendix p 20). Approvals for the study were granted by the UK National Research Ethics Service, Committee South Central–Oxford A (reference number 13/SC/0208), the Western Institution Review Board (reference number 20130698), and the UK Medicines and Healthcare Products Regulatory Agency (reference number 21584/0317/001-0001). The trial was registered with ClinicalTrials.gov, NCT01883609. The Local Safety Committee provided safety oversight, and Good Clinical Practice guideline compliance was independently monitored externally by the Clinical Trials and Research Governance Team of the University of Oxford.

See Online for appendix

## Procedures

R21 was thawed to room temperature and administered intramuscularly into the deltoid muscle within 1 h of removal from the freezer, either alone, or mixed with 50 μg Matrix-M adjuvant at the bedside, immediately before administration.

Participants were observed for 60 min following vaccine administration.

In the phase 1a study, follow-up visits including immunology blood sampling were scheduled for days 1, 7, 14, 28, 35, 42, 56, 63, 70, 84, and 238, with an additional visit at day 3 after vaccination for the first three participants in groups receiving 10 μg or more R21. All participants recorded their temperature daily and any solicited local and systemic adverse events for 7 days after vaccination and unsolicited adverse events for 28 days after vaccination using an electronic diary. A review of solicited and unsolicited adverse events occurred at each follow-up visit. Safety bloods including full blood count, renal function and liver function tests were done on visits at days 0, 7, 28, 35, 56, 63, 84, and 238 by local laboratories.

In the phase 1b study, participants were visited at home daily for 6 days after each vaccination by a field worker for assessment and recording of any solicited and unsolicited adverse events in diary cards. They were also seen in clinic at day 7 and day 28 after each vaccination and attended a final follow-up visit 1 year after enrolment. Safety bloods including full blood count, creatinine, and alanine aminotransaminase were done in clinic at days 0, 7, 28, 35, 56, 63, 84, 140, and 365 by local laboratories. Immunology bloods were taken at days 0, 28, 56, 84, 140, and 365 by clinic staff. Severity grading of adverse events and the assignment of a causal relationship for adverse events were conducted according to predefined guidelines stated in the protocol, which were harmonised across both clinical trials for grading of solicited adverse events. For unsolicited adverse events, MedDRA terminology and the DAIDS adverse event grading table was used.[19] A local safety monitor provided oversight of the trials.

IgG antibody titres to the NANP repeat region of the CSP antigen were measured by ELISA in the same laboratory by the same operator for both R21 trials. IgG antibody avidity was assessed by sodium thiocyanate-displacement ELISA. To assess whether antibodies to the C-tag used for R21 purification were induced by vaccination, N-terminal biotinylated peptides were constructed for the C-tag (glu-pro-glu-ala), the C-tag plus the four adjacent amino acids in the R21 construct (trp-val-tyr-ile-glu-pro-glu-ala) and the C-tag plus the 11 adjacent amino acids in the R21 construct (leu-pro-ile-phe-phe-cys-leu-trp-val-tyr-ile-glu-pro-glu-ala). This was done in the phase 1a study only, as the immunogenicity results from the phase 1a trial indicated that it did not need to be done for the phase 1b. For hepatitis B, antibodies to the HBsAg were measured using the Abbot Architect 2000i chemiluminescent micro-particle immunoassay. An antibody concentration of 100·0 mIU/mL or greater was considered positive. Ex-vivo IFN-γ ELISpot responses to CSP were assessed on samples from days 0, 42, and 84 (appendix p 17).

## Outcomes

The primary objective of the phase 1a trial was to assess the safety and tolerability of R21 with and without adjuvant Matrix-M. The primary objective of the phase 1b trial was to assess the safety and tolerability of R21 with Matrix-M. Primary outcome measures included the occurrence of solicited local and systemic reactogenicity signs and symptoms for the day of vaccination and the 7 days following vaccination, occurrence of unsolicited adverse events for the day of vaccination and the 28 days following vaccination, change from baseline for safety laboratory measures, and occurrence of serious adverse events during the whole study duration. The secondary outcome for the phase 1a study was cellular and humoral immunogenicity of R21 with and without the adjuvant Matrix-M. For the phase 1b study, the secondary outcome was humoral immunogenicity. The IgG response to the NANP repeat region was the primary immunogenicity readout, as this measure has been associated with RTS,S efficacy previously.[20]

## Statistical analysis

Data were analysed using GraphPad Prism version 10 for Mac and Stata 14.0. Geometric mean titre (95% CI) was used to describe serological measurements.

The functional activity of NANP-specific antibodies was assessed in vitro by measuring ISI into a human hepatoma cell line via serum. Median (IQR) was used to describe inhibition of sporozoite invasion (ISI) and cellular immunology. Immunogenicity data were tested for normal distribution by the D'Agostino–Pearson

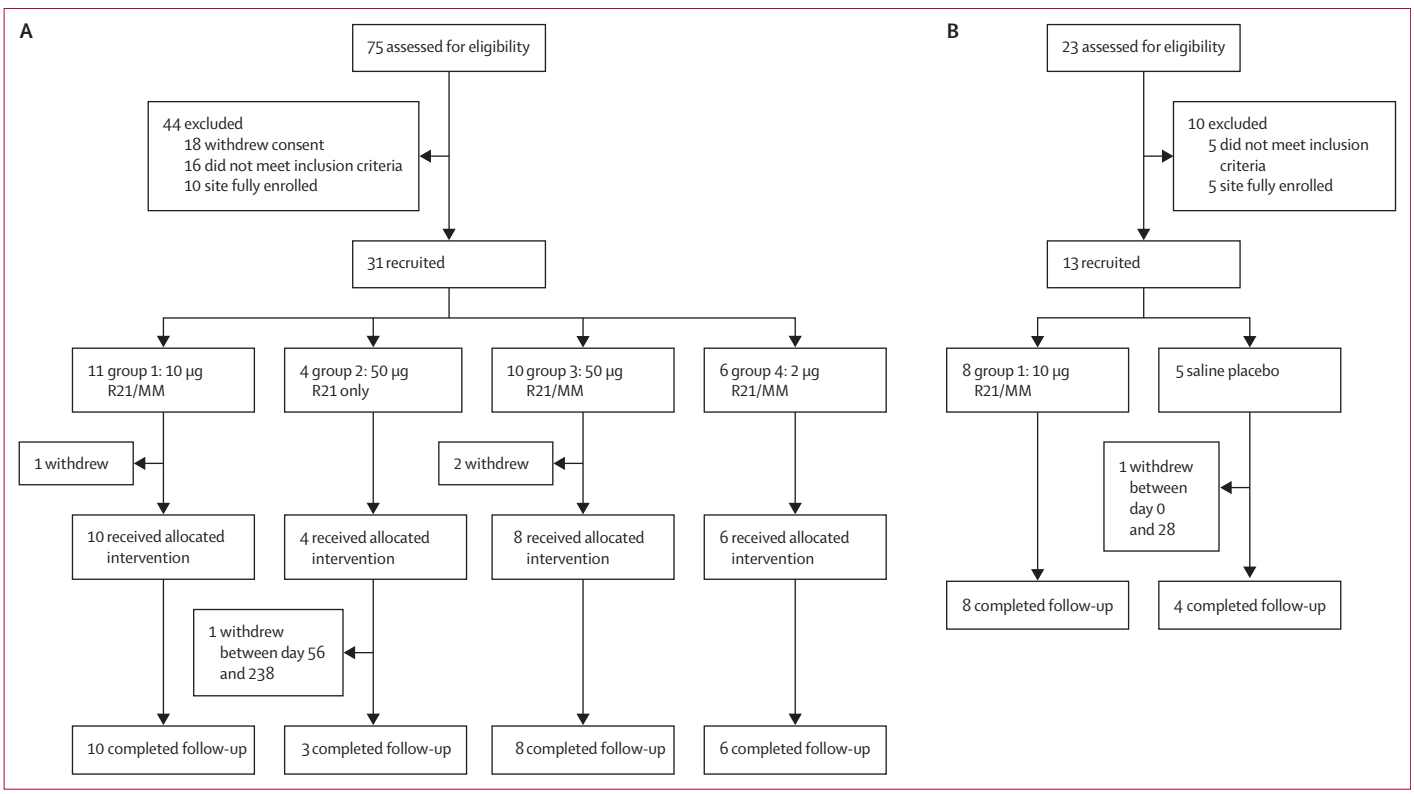


***Figure 1:*** **Trial profile**
(A) VAC053 phase 1a trial in UK Adults. (B) VAC060 phase 1b trial in Burkinabe Adults. MM=Matrix-M.

omnibus normality test. Mann–Whitney *U* analyses were used for significance testing of differences between two groups, and Kruskal–Wallis analyses with Dunn's multiple comparisons were used for more than two groups. A Wilcoxon matched-pairs analysis was used to compare between timepoints within groups. $\chi^2$ (Pearson) test for trend was used to compare the safety data between different groups. Spearman's rank was calculated for correlations. A value of $p<0{\cdot}05$ was considered statistically significant; all p values are two-tailed.

### Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

## Results

Recruitment for the phase 1a study took place between Oct 1, 2015, and Jan 3, 2017. 75 individuals were screened for eligibility and 31 were enrolled (figure 1). Four participants (one receiving 10 µg R21/Matrix-M, one receiving 50 µg R21 only, and two receiving 50 µg R21/Matrix-M) withdrew after their first vaccination and were not replaced (figure 1). At withdrawal there were no ongoing adverse events and safety bloods were normal for all who withdrew.

Recruitment for the phase 1b study took place between Aug 26, 2016, and Sept 28, 2017. 23 individuals were screened for eligibility, of whom 13 were enrolled. Eight participants completed follow-up after receiving three doses of 10 µg R21/Matrix-M in addition to five participants who received a saline placebo. One participant in the placebo group withdrew after the first dose and was not replaced.

Participant enrolment into group 2 of the UK phase 2a RTS,S study (NCT01883609) is available in the appendix (p 20).

No serious adverse reactions or suspected unexpected serious adverse reactions occurred in the UK phase 1a study. Two serious adverse events occurred in the UK phase 1a study; the first in the 10 µg R21/Matrix-M was caused by worsening of previously undisclosed or undiagnosed palindromic rheumatism and was deemed unlikely to be related to vaccination and the second in the 2 µg R21/Matrix-M group was hospital admission for an unplanned excision of a pre-existing Bartholin's cyst, also unrelated to vaccination (figure 2; appendix p 4). The majority of solicited adverse events reported were mild in severity and self-limiting. As expected, the addition of Matrix-M increased the reactogenicity of the 50 µg dose compared with administration of 50 µg R21 alone. There was a significant trend for more reactogenicity in the higher dose groups than in the very low dose 2 µg group ($p<0{\cdot}0001$, $\chi^2$ test for trend across doses in the phase 1a study) where minimal reactogenicity was observed. Injection site pain was the most common local adverse event and was predominantly mild in severity (table)

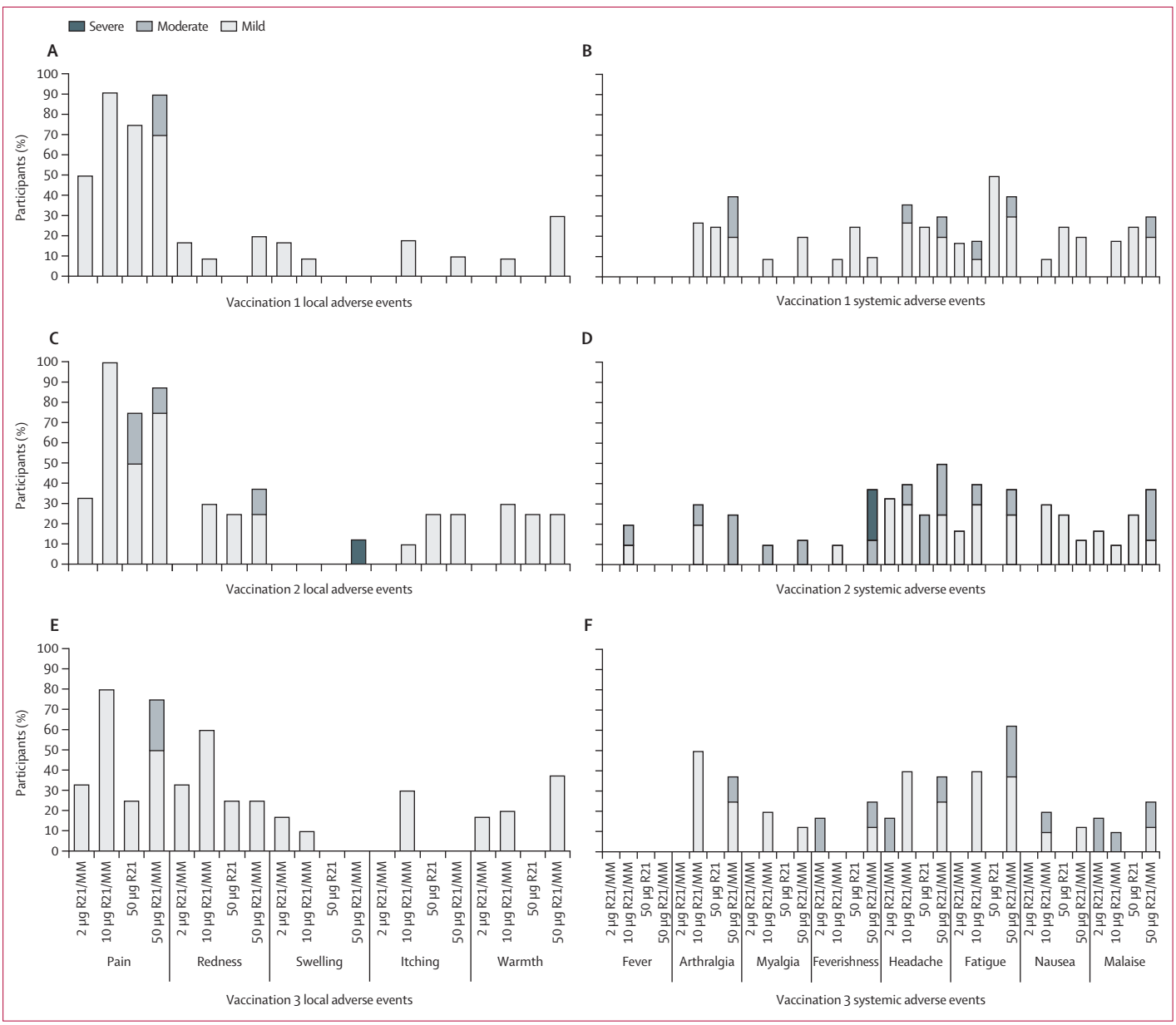


***Figure 2:*** **Local and systemic solicited adverse events reported by UK participants**

Reports were from electronic diary cards in the first 7 days after a given vaccination. Only the highest intensity of each adverse event per individual is listed. Data are combined for all adverse events for all participants receiving the same vaccine at the stated timepoint. (A) Local adverse events for the first vaccination. (B) Systemic adverse events for the first vaccination. (C) Local adverse events for the second vaccination. (D) Systemic adverse events for the second vaccination. (E) Local adverse events for the third vaccination. (F) Systemic adverse events for the third vaccination.

in the phase 1a study. One participant receiving 10 µg R21/Matrix-M in the phase 1a study reported a mild fever (37·7°C) and another participant receiving the same dose in the same study reported a moderate fever (38·1°C). Both fevers occurred after the second vaccination and resolved within 24 h. One participant receiving 50 µg R21 and no Matrix-M in the phase 1a study reported a fever of 39°C associated with multiple flu-like symptoms starting 8 days after their first vaccination, which resolved within 24 h. Severe solicited adverse events were reported by only three individuals who were receiving 50 µg R21/Matrix-M, and these resolved within 48 h (appendix p 4).

No severe local or systemic solicited adverse events were reported following 10 µg R21/Matrix-M in the phase 1b study (figure 3). Very few solicited systemic adverse events were reported and most local adverse events were mild in nature; overall reactogenicity was significantly reduced compared with UK participants receiving the same dose ($p<0·0001$, $\chi^2$ test; table; appendix p 7). Injection site pain was also the most reported local adverse event in the phase

1b study. There were no reports of fever associated with vaccination in the Burkinabe cohort. No solicited adverse events were recorded in the saline placebo group.

Of note, the reactogenicity profile observed in the 50 µg R21/Matrix-M group was significantly milder (p<0·0001, Pearson's $\chi^2$ test for trend) in the phase 1a trial compared with that observed following the same dose schedule in a phase 2a clinical trial.[18] This difference was mainly due to a significantly lower number of systemic solicited adverse events reported, and a higher incidence of moderate and severe adverse events reported by participants receiving RTS,S (table). There were no post-vaccination fevers in the 50 µg R21/Matrix-M group (0% compared with 26% for 50 µg RTS,S in the phase 1a trial, p=0·004 $\chi^2$ test).

Unsolicited adverse events collected for 28 days after each vaccination and those deemed possibly, probably, and definitely causally related to vaccination were predominantly mild in nature for the phase 1a trial (appendix p 10). Laboratory adverse events were predominantly grade 1 in the phase 1a trial (appendix p 11). In the phase 1b trial, all unsolicited adverse events were deemed unlikely to be or unrelated to vaccination (appendix pp 11–12).

In the phase 1a trial, NANP-specific IgG was induced with all doses of R21 administered, with similar kinetics to 50 µg of RTS,S (figure 4A). At the peak of the humoral immune response (day 84), there were no significant differences in NANP-specific IgG levels between any R21/Matrix-M dose groups in UK participants, nor between the 50 µg RTS,S/AS01 and any R21/Matrix-M dose (figure 4B). There was also no significant difference in peak (day 84) R21-induced NANP IgG between the phase 1a (geometric mean titre [GMT] 1613, 95% CI 674·3–3858, n=10) and phase 1b participants (2531, 1125–5693, n=8; p=0·52) at the 10 ug R21/Matrix-M dose.

The durability of NANP-specific IgG responses was similar between phase 1a (day 238) and 1b (day 365) individuals receiving R21 when the latest timepoints were compared (figure 4C). Specifically, at the 10 µg R21/Matrix-M dose, NANP IgG GMT was 375·4 (95% CI 168·6–835·8) for phase 1a participants at day 238 and 575·8 (246·5–1345) for phase 1b participants at day 365 (p=0·56). There were no comparable timepoints for the RTS,S phase 2a study since all participants underwent controlled human malaria infection at day 84.

Pre-vaccination IgG titres to NANP were higher in Burkinabe participants (phase 1b; GMT 63·4, 95% CI 14·2–284·0, n=8) compared with UK participants (phase 1a; 7·0, 2·4–2·05, n=11; p=0·0024), likely due to differences in previous malaria exposure (figure 4D). After day 0, there were no significant differences in NANP IgG between UK and Burkinabe adults receiving three doses of 10 µg R21/Matrix-M at any timepoint (figure 4D). Burkinabe individuals who received a saline injection did not show any increase in NANP IgG concentrations.

The avidity of NANP-specific IgG increased significantly between day 0 and day 84 in the phase 1b trial (p=0·008, Wilcoxon matched-pairs test), and this vaccine-induced avidity increase was higher at day 84 in the phase 1b compared with the phase 1a trial at the same timepoint, within the two 10 µg R21/Matrix-M groups (p=0·001, Mann–Whitney *U*; figure 4E). There was no correlation between the concentration of NANP IgG and NANP IgG avidity, when assessed at day 84 for either the phase 1a (*r*=0·20, p=0·59) or phase 1b (*r*=0·29, p=0·50) 10 µg R21/Matrix-M dose recipients (figure 4G).

Avidity of NANP-specific IgG antibodies at the peak of the immune response (day 84) was compared between the phase 1a R21/Matrix-M participants and RTS,S/AS01 phase 2a participants. There was no evidence of a difference in NANP IgG avidity at day 84 between the 50 µg RTS,S dose and the 50 µg R21/Matrix-M dose (p=0·75). Similarly, there was no difference between the 50 µg R21/Matrix-M and 10 µg R21/Matrix-M doses in the phase 1a study (p=0·67). The 50 µg RTS,S resulted in higher NANP IgG avidity compared with the 10 µg dose of R21/Matrix-M (Kruskal–Wallis test with Dunn's multiple comparisons, p=0·023; figure 4F).

The functional activity of NANP-specific antibodies was assessed via ISI from the serum of six phase 1a participants and seven phase 1b participants who received 10 µg R21/Matrix-M. Baseline serum ISI before vaccination was similar between phase 1a and 1b participants, with median ISI of 16·5% and 16·8%, respectively (Mann–Whitney *U*; appendix p 21). Vaccination with 10 µg R21/Matrix-M significantly increased ISI activity at day 84 compared with baseline in the phase 1a trial (16·5%, 14·3–17·6 at baseline; median 40·8%, IQR 29·3–45·5 at day 84; p=0·03, Wilcoxon matched pairs) and the phase 1b trial (16·8%, 12·9–21·2 at baseline; 27·3%, 26·2–35·5 at day 84; p=0·02, Wilcoxon matched pairs).

ISI activity among UK participants and Burkinabe participants at day 84 was similar (p=0·11). Despite low-level activity at baseline, ISI activity increased in all participants after three doses of 10 µg R21/Matrix-M (appendix p 21). A significant association was detected between NANP IgG antibody titre and ISI activity in both the phase 1a and phase 1b cohorts (*r*=0·71, p=0·008, Spearman's rank test, appendix p 21); however, these data are based upon small sample sizes (n=6 for phase 1a and n=7 for phase 1b).

| | Location | Adverse events | | | | Pearson's $\chi^2$ |
|---|---|---|---|---|---|---|
| | | Mild | Moderate | Severe | Total | |
| **10 µg groups** | | | | | | p<0·0001 |
| Phase 1a, 10 µg R21/Matrix-M (n=11) | UK | 94 | 9 | 0 | 103 | |
| Phase 1b, 10 µg R21/Matrix-M (n=8) | Burkina-Faso | 5 | 5 | 0 | 10 | |
| **50 µg groups** | | | | | | p<0·0001 |
| Phase 2a, 50 µg RTS,S/AS01$_B$ (n=17) | UK | 166 | 83 | 23 | 272 | |
| Phase 1a, 50 µg R21/Matrix-M (n=10) | UK | 65 | 26 | 3 | 94 | |
| **Other** | | | | | | |
| Phase 1a, 50 µg R21 alone (n=4) | UK | 19 | 2 | 0 | 21 | p=0·002* |
| Phase 1a, 2 µg R21/Matrix-M (n=6) | UK | 18 | 3 | 0 | 21 | p=0·0001* |
| Phase 1b, placebo (n=5) | Burkina-Faso | 0 | 1 | 2 | 0 | .. |

Data for the phase 2a study are from Rampling.[18] *Compared with the 50 µg R21/Matrix-M group.

***Table:*** **Summary of local and systemic solicited adverse event frequency in the UK and Burkina Faso**

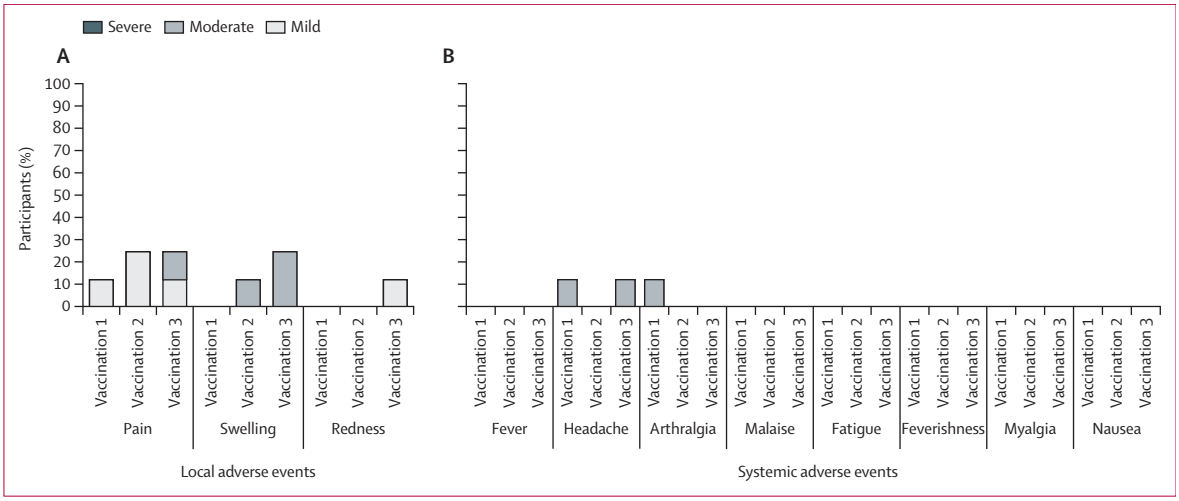


***Figure 3:*** **Local and systemic solicited adverse events reported by Burkinabe participants**
Reports were in the first 7 days after a given vaccination. Only the highest intensity of each adverse event per individual is listed. Data are combined for all adverse events for all participants receiving the same vaccine at the stated timepoint. No solicited adverse events were observed in the saline placebo group. (A) Local adverse events. (B) Systemic adverse events.

40–50% of participants in each phase 1a group were seropositive for HBsAg before R21 vaccination, presumably due to previous vaccination against hepatitis B (appendix p 21). Of those who were seronegative at vaccination, 0 of six participants seroconverted for HBsAg after three doses of 2 µg R21/Matrix-M; one (17%) of six seroconverted after three doses of 10 µg R21/Matrix-M; and three (50%) of six seroconverted after three doses of 50 µg R21/Matrix-M. This finding contrasts with vaccination with three doses of RTS,S, which induced seroconversion in seven (100%) of the seven seronegative vaccinees (appendix p 21). In the phase 1b cohort, seroconversion was detected in only one (17%) of the seven participants who were seronegative before vaccination.

During manufacture at the University of Oxford Clinical Biomanufacturing facility (Oxford, UK), a four amino acid label (glu-pro-glu-ala) was added to the C-terminal of the R21 construct (C-tag) to facilitate protein purification during biomanufacture. C-tag IgG responses were not induced by vaccination with R21 in any group, although one participant had a very weak response to glu-pro-glu-ala before vaccination, which was not detected after vaccination at day 84 (appendix p 21).

T-cell responses to CSP were enumerated by ex vivo IFN-γ ELISpot and were weak in individuals who received R21/Matrix-M (appendix p 21). There was no significant difference in magnitude of responses between R21 recipients in any group and RTS,S at any timepoint measured. Responses peaked at day 42, 2 weeks after the second vaccine dose, and there were no significant differences between all R21 vaccinated groups (appendix p 21).

## Discussion

We report here the safety and immunogenicity of the first-in-human administration of the novel malaria vaccine candidate, R21, administered with the saponin-based adjuvant, Matrix-M, in UK and Burkinabe adults. These trials demonstrate that the R21/Matrix-M vaccination approach was safe and well tolerated. Initial data suggest that low doses of R21/Matrix-M can induce high antibody levels, similar to those previously associated with protection in humans via RTS,S/AS01$_B$. The reactogenicity profile of 50 µg R21/Matrix-M was significantly improved compared with the standard adult 50 µg RTS,S dose in healthy adult UK participants[18] and, importantly, post-vaccination fever was not detected. Effective vaccines will be essential tools for malaria elimination and eradication and the absence of fever after vaccination is a substantial benefit in the context of mass administration campaigns. Minimal reactogenicity was detected in Burkinabe adults, which was significantly lower than in UK adults at the same dose of 10 µg R21/Matrix-M.

Anti-NANP antibody titres have been established as a correlate of vaccine-induced protection, with higher titres associated with protection against malarial disease.[16] The rate of waning of antibody responses following vaccination is associated with the duration of efficacy over time.[20] Despite this waning, substantial variation in protection exists among individuals with high concentrations of anti-NANP IgG, indicating that alternative quantitative as well as qualitative measures of humoral response might be important as correlates of protection.[21] Observed humoral responses to the conserved central NANP repeat region of CSP are comparable to previous RTS,S data from a clinical trial conducted in the UK where the same ELISA was used. In these small samples of UK and Burkinabe adults, we did not see an association between anti-NANP IgG and antibody avidity; however, a positive association between inhibition of sporozoite invasion and anti-NANP IgG concentrations was seen.

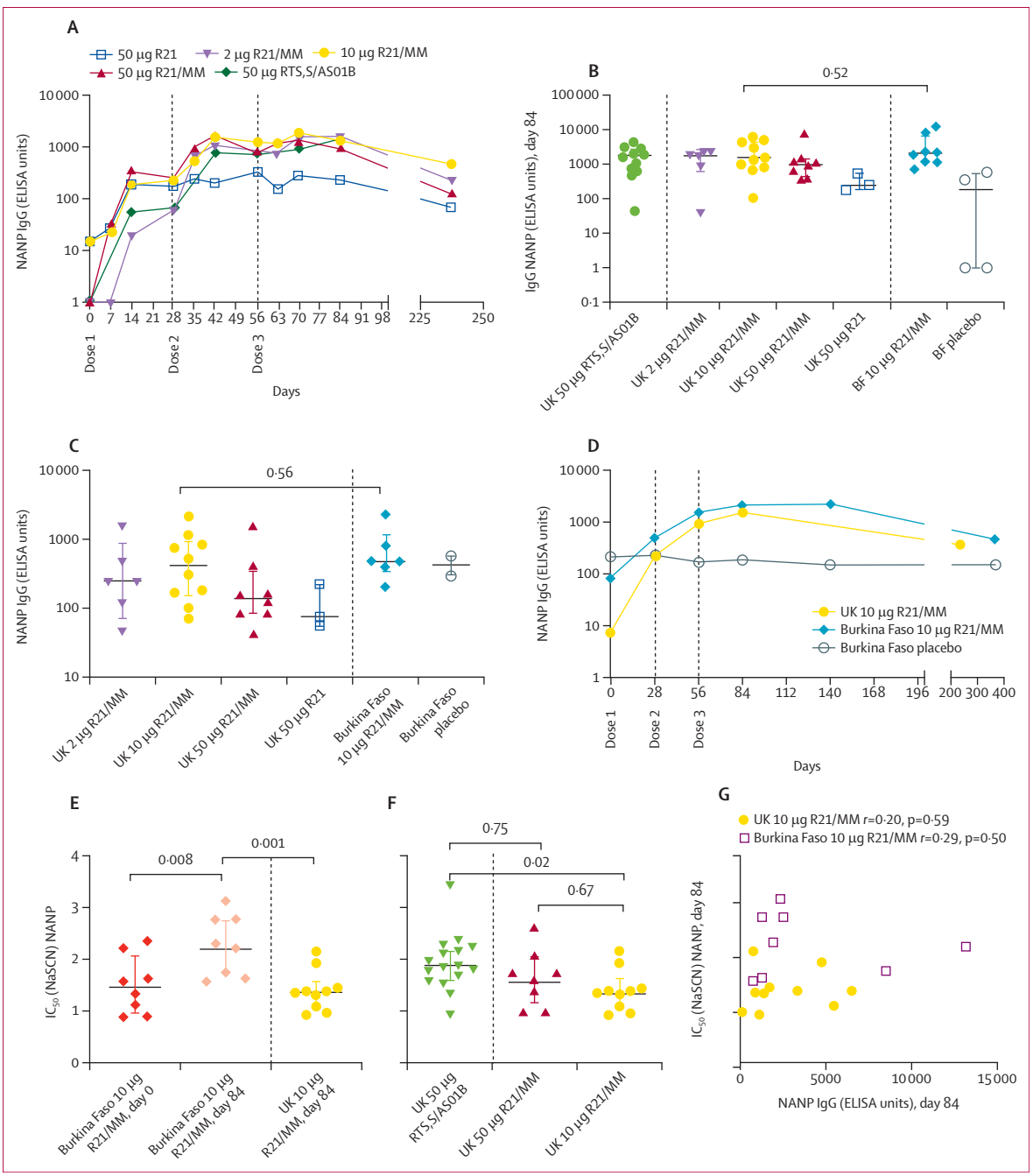


***Figure 4:*** **Antibody responses to R21/Matrix-M**

Bars represent 95% CIs. (A) Geometric mean NANP IgG in UK adults. (B) Peak NANP IgG titres at day 84 for all R21/Matrix-M groups in UK and Burkinabe participants and individuals vaccinated with three doses of 50 µg RTS,S/AS01$_B$ from a previous trial carried out in the UK.[18] Comparisons between RTS,S/AS01B and other groups was done by Kruskal–Wallis with Dunn's multiple comparisons. Mann–Whitney *U* was used to compare 10 µg R21/Matrix-M in UK and Burkinabe adults. (C) Individual NANP IgG responses at the final timepoint in the study (day 238 in the UK and day 365 in Burkina-Faso). Mann–Whitney *U* was used to compare 10 µg R21/Matrix-M in UK and Burkinabe adults. (D) NANP IgG responses for Burkinabe adults in placebo and 10 µg R21/Matrix-M and UK adults receiving 10 µg R21/Matrix-M dose. (E) Avidity of NANP-specific IgG at baseline (day 0) and at peak (day 84) timepoint in Burkinabe 10 µg R21/Matrix-M recipients (compared using Wilcoxon matched pairs) and day 84 for UK 10 µg R21/Matrix-M recipients (compared with Burkinabe adults at the same timepoint using Mann–Whitney *U*). (F) Avidity of NANP-specific IgG at the peak (day 84) timepoint in the 10 µg and 50 µg R21/Matrix-M groups and 50 µg RTS,S/AS01$_B$ from a previous trial.[18] Comparisons between RTS,S/AS01B and R21 dose groups was done by Kruskal–Wallis with Dunn's multiple comparisons. Mann–Whitney *U* was used to compare 10 µg and 50 µg R21/Matrix-M in UK adults. (G) Association between day 84 NANP IgG titres and day 84 NANP IgG avidity in UK and Burkinabe adults vaccinated with 10 µg R21/Matrix-M (Spearman rank analysis). BF=Burkina Faso. $IC_{50}$=half maximal inhibitory concentration. MM=Matrix-M. NaSCN=Sodium thiocyanate.

Although high antibody titres do not necessarily correlate with protection for anti-sporozoite vaccines at the individual level, high antibody titres are associated overall with increased protection. Further studies to investigate the probably multifaceted immunological mechanisms of protection using systems serology will likely be required, as was done for RTS,S.[22,23] In both the phase 1a and 1b studies we see similar anti-NANP IgG responses were elicited at 28 days after the third vaccination even at the very low doses of 2 μg and 10 μg of R21/Matrix-M, which could have substantial dose-sparing and cost-saving implications for vaccine production.

Durable NANP-specific antibody responses were observed at 6 months after the final vaccination for all doses of R21 tested in the phase 1a trial and at 9 months after the final vaccination in the phase 1b trial at the 10 μg R21/Matrix-M dose; however, studies with RTS,S and R21/Matrix-M have shown that booster doses might be required to maintain the efficacy levels observed soon after the primary doses. In pneumococcal and meningococcal disease, higher priming antigen doses favour production of antigen-specific plasma cells that only have a short lifespan, whereas lower doses can preferentially drive the induction of immune memory.[24,25] A few of these plasma cells differentiate into long-lived plasma cells in the absence of subsequent antigen exposure, and the proportion of long-lived plasma cells generated by a vaccine is predictive of the durability of the antibody response.[26]

New safe, high-efficacy, and low-cost malaria vaccines are needed to help reduce malaria morbidity and mortality in the many tens of millions of children born each year in regions that are moderately to highly endemic for malaria and for other indications such as malaria in pregnancy and malaria elimination. These initial data suggest an improved safety profile of R21 with Matrix-M adjuvant compared with the standard RTS,S regimen, along with moderate durability of immune response after a lower dose R21/Matrix-M, which is encouraging. Since this study was undertaken, clinical development of R21/Matrix-M has continued and results of a phase 2b efficacy trial in Burkina Faso identified a vaccine efficacy of 77% against clinical malaria over 1 year of follow-up in children aged 5–17 months.[27] Following a booster dose at 1 year, efficacy was maintained throughout the second year at 80%. A multisite phase 3 trial recently reported promising efficacy over 12–18 months. Based on these data, several African countries have now licensed R21/Matrix-M.[28,29] R21 was out-licensed by Oxford University to the Serum Institute of India for manufacturing and commercialisation, who can supply up to 200 million doses per year.[28] In conclusion, these phase 1 clinical trials showed that the malaria vaccine candidate, R21, administered with Matrix-M adjuvant has an acceptable safety profile with strong immunogenicity and was well tolerated in both UK and Burkinabe adults, supporting further clinical development.

#### Contributors

Conceptualisation and study design was performed by NV, ABT, SBS, KJE, and AVSH. The literature search was done by KJE. The formal analysis was performed by NV, GB, DGB, LKS, MSD, DS, KJE, and AVSH. The funding acquisition was undertaken by ABT, EBI, RR, KJE, and AVSH. Investigation was performed by NV, ABT, GB, DGB, LKS, JP, KAC, SC, MSD, DS, AO, IN, FB, PF, ED-C, SJ, DW, AD, CMB, RM, MB, IP, BA, and KJE. Project administrators were EBI, ECB, NKV, and RR. Trial resources were managed by GG, LFF, JMR, KL-B, SM, EB, NG, EM, and SCG. Trial supervision was performed by ABT, MSD, DS, IP, SM, EB, NG, EM, BA, AL, DJML, SBS, KJE, and AVSH. Immunological assay validation was performed by GB, DGB, LKS, ED-C and KJE. Manuscript visualisation was done by NV, DGB, LKS, and KJE. The original manuscript draft was written by NV and KJE. Writing review and editing were performed by ABT, GB, DGB, LKS, JP, KAC, SC, MSD, DS, AO, IN, FB, PF, ED-C, SJ, ECB, DW, AD, CMB, RM, GG, LFF, JMR, KL-B, MB, IP, SM, EB, NG, EM, NKV, BA, AL, RR, SCG, DJML, SBS, KJE, and AVSH. NV, KJE, DGB, LKS and AVSH had full access to all the data in the study and were responsible for the decision to submit for publication. NV and ABT accessed and verified the data.

#### Declaration of interests

KAC, NKV, SCG, KJE, and AVSH are named as co-inventors or contributors on patent filings related to the R21 vaccine candidate; all are or were University of Oxford students or employees and might thereby benefit from any royalty stream to Oxford University. LFF and JMR are or were employees of Novavax and JMR is named as co-inventor on patents related to adjuvant formulations for the R21 vaccine. LFF, KL-B, and JMR hold shares in Novavax. NKV is an employee of EVI and a recipient of European Commission grants supporting malaria vaccine development, in addition to being a Member of the Scientific Committee, European and Developing Countries Clinical Trials Partnership 3 (Global Health EDCTP3) Joint Undertaking. KJE was an employee of the University of Oxford at the time of the work and is now an employee of GSK and owns restricted shared in GSK. All other authors declare no competing interests.

#### Data sharing

De-identified participant data will be made available upon requests directed to the chief investigator, Adrian Hill. Proposals will be reviewed and approved by the sponsor, chief investigator, and collaborators on the basis of scientific merit. After approval of a proposal, data can be shared through a secure online platform after signing a data access agreement.

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