R21 in Matrix-M adjuvant in UK malaria-naive adult men and non-pregnant women aged 18–45 years: an open-label, partially blinded, phase 1–2a controlled human malaria infection study

Navin Venkatraman, Daniel Silman, Duncan Bellamy, Lisa Stockdale, Georgina Bowyer, Nick J Edwards, Oliver Griffiths, Fernando Ramos Lopez, Jonathan Powlson, Catherine Mair, Pedro M Folegatti, Mehreen S Datoo, Richard Morter, Angela M Minassian, Ian Poulton, Katharine A Collins, Florian Brod, Philip Angell-Manning, Eleanor Berrie, Nathan Brendish, Greg Glenn, Louis Fries, Jake Baum, Andrew M Blagborough, Rachel Roberts, Alison M Lawrie, Brian Angus, David J M Lewis, Saul N Faust, Katie J Ewer, Adrian V S Hill

Summary

Background R21 is a novel malaria vaccine, composed of a fusion protein of the malaria circumsporozoite protein and hepatitis B surface antigen. Following favourable safety and immunogenicity in a phase 1 study, we aimed to assess the efficacy of R21 administered with Matrix-M (R21/MM) against clinical malaria in adults from the UK who were malaria naive in a controlled human malaria infection study.

Methods In this open-label, partially blinded, phase 1–2A controlled human malaria infection study undertaken in Oxford, Southampton, and London, UK, we tested five novel vaccination regimens of R21/MM. A standard three-dose regimen (groups 1 and 6) was compared with a reduced (fractional) third dose (groups 2 and 5) of R21/MM, concomitant administration with viral vectors ChAd63-MVA expressing ME-TRAP (group 3), and a two-dose R21/MM regimen (group 7). Controlled Human Malaria Infection (CHMI) was delivered by mosquito bite at Imperial College London, London, UK, 3–4 weeks after final vaccination (or 18 months after final vaccination for group 6) alongside unvaccinated controls (groups 4A and 4B). The primary outcome measures were to assess safety of the vaccines in healthy malaria-naive volunteers and the efficacy (occurrence of blood-stage malaria infection) of the different vaccine regimens compared with non-vaccinated controls after CHMI. The trial was registered with ClinicalTrials.gov (NCT02905019).

Findings 66 volunteers were enrolled with 59 undergoing subsequent CHMI. All vaccination schedules were well tolerated. The highest level of protection against CHMI was observed in participants receiving the standard three-dose regimen of R21/MM (group 1, nine of 11 volunteers protected) with protection maintained in three of five volunteers re-challenged by CHMI 7·5 months later. Protection against malaria was also seen in group 2, group 3, and group 5 compared with unvaccinated control participants. Total IgG antibody responses to the NANP repeat region of circumsporozoite protein peaked after the third dose of R21/MM in all volunteers and were well maintained to 90 days after challenge. Reducing the third dose did not affect protection or antibody concentrations.

Interpretation Our study shows that R21/MM elicits high-level efficacy against clinical malaria in a controlled human infection model of malaria in adults who are malaria naive. These data supported the evaluation of R21/MM in field efficacy trials in the target population of young children in malaria-endemic areas.

Funding EU Horizon 2020, the UK Medical Research Council, the European Commission, the UK National Institute of Health Research, the Imperial NIHR Clinical Research Facility, the Oxford NIHR Biomedical Research Centre, and the Wellcome Trust.

Introduction

Malaria remains a major public health problem, especially in sub-Saharan Africa. Although several species of plasmodium are responsible for causing the disease, *Plasmodium falciparum* is responsible for 95% of global cases.[1] In 2022, an estimated 249 million malaria cases occurred in 85 endemic countries, resulting in 608 000 deaths, with 75% of deaths occurring in children younger than 5 years.[1] The past 20 years have seen a substantial reduction in global mortality associated with malaria, although disruption of public health services because of the SARS-CoV-2 pandemic caused a 10% increase in malaria deaths in 2020.[1] This trend is threatened by the emergence and spread of parasite resistance to artemisinins and vector resistance to insecticides.[2] Therefore, developing durable and highly efficacious malaria vaccines remains a global health priority.[3] RTS,S/AS01$_E$ (Mosquirix; GSK Biologicals, Rixensart, Belgium) and now R21 administered with Matrix-M (R21/MM) are licensed malaria

Lancet Microbe 2025; 6: 100867

Published Online January 10, 2025 https://doi.org/10.1016/S2666-5247(24)00083-1

Jenner Institute, University of Oxford and the NIHR Oxford Biomedical Research Centre, Oxford, UK (N Venkatraman DPhil, D Silman MSc, D Bellamy DPhil, L Stockdale PhD, G Bowyer PhD, N J Edwards BSc, J Powlson BSc, C Mair MSc, P M Folegatti MSc, M S Datoo DPhil, R Morter MBChB, A M Minassian DPhil, I Poulton DipHE, K A Collins DPhil, F Brod DPhil, R Roberts MSc, A M Lawrie PhD, Prof K J Ewer PhD, Prof A V S Hill FRS); **Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Oxford, UK** (N Venkatraman, D Silman, O Griffiths BSc, F R Lopez MSc, P M Folegatti, M S Datoo, A M Minassian, I Poulton, R Roberts, A M Lawrie, B Angus MD); **Clinical Biomanufacturing Facility** (P Angell-Manning MSc, E Berrie PhD) **and Department of Biochemistry** (A M Minassian), **University of Oxford, Oxford, UK; NIHR Southampton Clinical Research Facility and Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust, Southampton, UK** (N Brendish PhD, S N Faust PhD); **Faculty of Medicine and Institute for Life Sciences, University of Southampton, Southampton, UK** (N Brendish, S N Faust); **Novavax, Gaithersburg, MD, USA** (G Glenn MD, L Fries MD); **School of Biomedical Sciences, UNSW Sydney, Sydney, NSW, Australia** (Prof J Baum PhD); **Department of Pathology, University of Cambridge, Cambridge, UK**

(A M Blagborough PhD); **NIHR/ Wellcome Trust Imperial Clinical Research Facility, Imperial Centre for Translational and Experimental Medicine, London, UK**
(Prof D J M Lewis MD)

Correspondence to:
Prof Katie Ewer, Jenner Institute, University of Oxford, and the NIHR Oxford Biomedical Research Centre, Oxford OX3 7DQ, UK
katie.j.ewer@gsk.com

Research in context

Evidence before this study

Malaria caused by *Plasmodium falciparum* remains a leading cause of childhood death in sub-Saharan Africa, accounting for 608 000 deaths in the region in 2022. Improved tools for malaria control, targeting both the vector and the parasite, are urgently needed to reduce morbidity and mortality from malaria. There are now two vaccines licensed against malaria; Mosquirix from GSK and R21/Matrix M from the University of Oxford, Oxford, UK. We searched PubMed from database inception to the date of the search, 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. Following demonstration of protective efficacy against disease among 6000 children aged 5–17 months in a phase 3 clinical trial, RTS,S/AS01B (Mosquirix) was further assessed in pilot implementation studies over 4 years in 900 000 children. Following positive results, WHO issued a policy recommendation for the use of RTS,S (four doses of 50 g) for the prevention of *P falciparum* malaria for children in regions of moderate-to-high malaria transmission in 2021. The durability of the antibody response to the standard three-dose regimen of RTS,S wanes rapidly during the first year and the response is only partially restored by booster doses, therefore malaria vaccines that can induce longer-lasting antibody and memory-B-cell responses would be valuable. Reducing the third dose in the primary series (giving a fractional third dose) has been shown to improve the efficacy of RTS,S in Controlled Human Malaria Infection trials and therefore assessment of differing dose regimens could improve efficacy of durability of circumsporozoite-based vaccines. Combinations of different vaccine types, such as viral vectors, could also improve vaccine efficacy by improving T-cell responses to malaria antigens.

Added value of this study

R21 comprises the same components of the malaria CSP antigen as RTS,S, but without the additional hepatitis B molecules that were required to form RTS,S particles. R21 is administered with the Matrix M adjuvant, which has been used with other (non-malaria) vaccine candidates in more than 1 million people and using 4 million doses to date. We report here a phase 2A clinical trial of R21 in participants who are malaria naive in the UK, assessing several vaccine doses and regimens for efficacy against malaria in a controlled human malaria infection model. We demonstrate high-level efficacy of a three-dose regimen that uses a much lower dose than RTS,S (10 µg), but a fractional third dose did not improve protection in this study. Combination of a viral vectored malaria vaccine with R21/Matrix M did not improve vaccine efficacy; however, two doses of R21/Matrix M gave substantial protection against infection although lower than observed with three doses.

Implications of all the available evidence

R21 with Matrix-M shows high efficacy with particular immunisation regimens in UK adults as well as being used currently to prevent malaria in young African children.

vaccines that target the pre-erythrocytic sporozoite stage of the parasite that is transmitted to humans by the bite of infected *Anopheles* mosquitoes. Both are prequalified and recommended for widespread use by WHO having demonstrated a favourable safety profile and important vaccine efficacy.[4]

R21 was developed at the Jenner Institute, University of Oxford, Oxford, UK, and comprises particles formed from a single fusion protein. The fusion protein contains the central repeat and C terminus of the circumsporozoite protein fused to hepatitis-B surface antigen (HBsAg), and the particles are formed without the four-fold excess of unfused HBsAg protein molecules found in RTS,S.[5] We have shown that R21/MM elicited a similar humoral immunogenicity to RTS,S/AS01 at much lower doses, and reactogenicity was also significantly reduced.[6] Additionally, we have reported an acceptable safety and efficacy profile up to age 2 years in infants in Burkina Faso in a phase 2B study[7] and more recently in a phase 3 study.[8] Promising results from the phase 1 trials of R21 provided the basis for us to assess efficacy in a malaria sporozoite challenge study in adults who were malaria naive. Given a signal of increased efficacy associated with a fractional (one fifth) third dose using RTS,S/AS01, a fractional third dose of R21 that maintained the 50 µg dose of Matrix-M was included in this study.[9] The exact mechanisms for the additional efficacy obtained by this dose reduction using RTS,S/AS01 are unclear, but data from our group showed that the increased efficacy could be related to an increase in the functional ability of the induced antibodies to inhibit sporozoite invasion of liver cells rather than the magnitude of the antibody response.[10]

Given the complex lifecycle of malaria, and the variety of antigens presented at different parasite life stages, a long-term aim in the field has been a vaccine regimen targeting several stages of the parasite lifecycle. In addition to RTS,S and R21 that target the pre-erythrocytic stage, vaccines are being developed to target liver-stage, blood-stage, and mosquito-stage parasites. Ideally these vaccines would be combined into a multistage malaria vaccine regimen. In this study, we therefore evaluated the safety, immunogenicity, and efficacy of administering R21/MM with viral-vectored vaccines Chimpanzee adenovirus serotype 63 (ChAd63) and modified *vaccinia Ankara* virus (MVA) multiepitope Thrombospondin-related adhesion protein (ME-TRAP), which target the liver stage. The vaccines were administered in a staggered fashion (figure 1). It is hypothesised that a combination of different vaccine approaches, acting on

several antigens, and involved in different stages of the complex pathogen lifecycle, will provide additional protective efficacy, should the situation arise in which some sporozoites evade the initial protective antibody response.[11] Coadministration of R21 and TRAP-based viral vectors was shown to enhance protection in mouse models of malaria.[5] The viral-vectored vaccines used within this cohort are known to elicit potent antigen-specific T-cell responses in adults in the UK and adults and infants in malaria-endemic areas, and have an excellent track record of safety and tolerability in these populations.[12,13] The ChAd63 and MVA ME-TRAP malaria vaccine strategy has previously demonstrated partial yet durable efficacy in a controlled human malaria infection study in the UK[14] and reduced the risk of malaria infection by 67% in a randomised controlled trial done in Kenyan male adults.[15]

This phase 1–2A study in healthy UK volunteers assesses the safety and efficacy against malaria sporozoite challenge of R21/MM vaccination using different doses, a reduced third dose, and incorporating combination dosing with ChAd63 and MVA ME-TRAP.

Methods

Study design and participants

We did an open-label, partially blinded, phase 1–2A controlled human malaria infection study in healthy adult men and non-pregnant women who were malaria naive between the ages of 18 years and 45 years. The trial was split into two distinct Controlled Human Malaria Infection (CHMI) studies, comprising challenge A on Jan 30 and Jan 31, 2017 and challenge B on Sept 18 and Sept 19, 2017, testing a total of six different vaccination schedules. Commencing in July, 2016, recruitment and vaccination were done at the Centre for Clinical Vaccinology and Tropical Medicine at the University of Oxford, the Wellcome Trust Clinical Research Facilities in Southampton, UK, and Imperial College London, London, UK. CHMI was delivered by mosquito bite at Imperial College London, London, UK, 3–4 weeks after final vaccination (or 18 months after final vaccination for group 6) alongside unvaccinated controls (groups 4A and 4B).

The inclusion and exclusion criteria are listed in the supplementary appendix (p 21). All participants gave written informed consent before participation, and the study was done according to the principles of the Declaration of Helsinki and in accordance with Good Clinical Practice (GCP).

The study was approved by the UK National Research Ethics Service, Committee South Central–Berkshire (16/SC/0261), the Medicines and Healthcare Products Regulatory Agency (21584/0360/001-0001), and the Oxford University Clinical Trials and Research Governance team, who independently and externally monitored compliance with GCP guidelines. Viral-vectored vaccine use was authorised by the Genetically Modified Organisms Safety Committee of the Oxford University Hospitals UK National Health Service (NHS) Trust (GM 462.16.88). The trial was registered with ClinicalTrials.gov (NCT02905019) and an independent local safety monitor provided safety oversight. Additional methods are included in the appendix (pp 26–29).

Outcomes

The primary outcome measures were to assess the efficacy (occurrence of *P falciparum* parasitaemia, assessed by blood slide) of adjuvanted R21 at different doses and the combination malaria vaccine candidate regimen of adjuvanted R21 plus ChAd63 and MVA-encoding ME-TRAP, against malaria sporozoite challenge, in healthy volunteers who were malaria naive, and to assess the safety of adjuvanted R21 at different doses and the combination malaria vaccine candidate regimen of adjuvanted R21 plus ChAd63 and MVA-encoding ME-TRAP, in healthy volunteers who were malaria naive.

The secondary outcome measures were to assess immunogenicity generated in individuals who are malaria naive by adjuvanted R21 at different doses and the combination malaria vaccine candidate regimen of adjuvanted R21 plus ChAd63 and MVA-encoding ME-TRAP, and to assess the efficacy (measured as time to *P falciparum* parasitaemia assessed by blood slide, PCR, and parasite-density dynamics assessed by PCR) of adjuvanted R21 at different doses and the combination malaria vaccine candidate regimen of adjuvanted R21 plus ChAd63 and MVA-encoding ME-TRAP, against malaria sporozoite challenge, in healthy volunteers who are malaria naiive.

Procedures

The principal immunological readout was IgG antibody responses to the repeat region of the circumsporozoite protein (anti-NANP) as measured by ELISA. Anti-TRAP ELISA, in addition to ex-vivo interferon gamma ELISpot responses to circumsporozoite protein (all groups) and ME and TRAP (group 3 and controls only) were also done. The full methods used for immunological assays can be found in the appendix (pp 31–34).

Statistical analysis

Formal sample-size calculations were not done for this study because sample sizes reflect practical limitations on volunteer recruitment, ethical considerations limiting the number of volunteers that should receive a vaccine regimen without previous evidence of efficacy, and the desire to obtain a preliminary description of efficacy of vaccination regimes.

See Online for appendix

Data were analysed using GraphPad Prism version 10.0 for Windows and Stata 14.0. For immunological readouts, the Kruskal–Wallis analysis and the Friedman test were used to compare peak responses with the baseline and significance testing between two groups used Mann-Whitney analysis. A Wilcoxon matched-pairs analysis was used to compare timepoints within groups. A χ^2 test for trend was used to compare the safety data between different groups. A Fisher's exact test was used to assess likelihood of

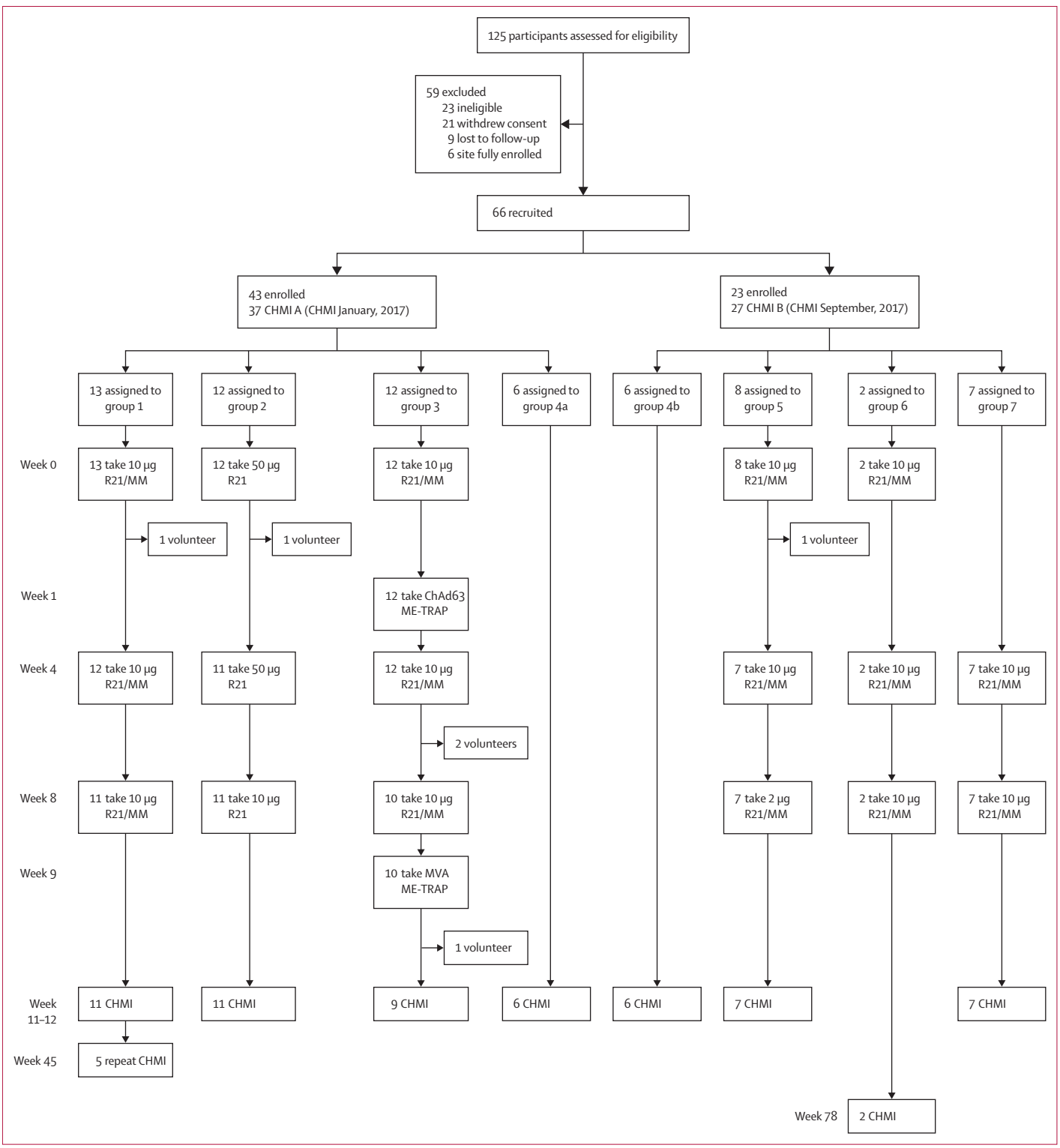

Figure 1: **Trial profile**

Healthy UK adults aged between 18 years and 45 years were enrolled and assigned to one of seven groups. Groups 1, 2, 3, and 4A underwent CHMI in January, 2017 and groups 4B, 5, 6, and 7 underwent CHMI in September, 2017. Participants in group 1 who did not develop malaria after challenge A were invited back for rechallenge in challenge B. CHMI=Controlled Human Malaria Infection. R21/MM=R21/Matrix M. MVA ME-TRAP=modified *vaccinia Ankara* virus multi-epitope thrombospondin-related adhesion protein.

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Dose regimen	10 µg, 10 µg, and 10 µg R21 + rechallenge	50 µg, 50 µg, and 10 µg R21	10 µg R21, ChAd63 ME-TRAP, 10 µg R21, 10 µg R21, MVA ME-TRAP	Infectivity controls	10 µg, 10 µg, and 2 µg R21	10 µg, 10 µg, 10 µg, and 2 µg R21 + delayed CHMI	10 µg and 10 µg R21
Number of participants	11	11	9	12	7	2	7
Age of participants							
Mean age in years (SD)	28·7 (9·07)	25·8 (5·36)	25·7 (4·95)	29·1 (7·96)	26 (6·45)	30·5 (0·71)	28·9 (8·41)
Age range	19–43	21–36	21–34	21–43	19–38	30–31	22–44
Sex							
Male	3 (27%)	4 (36%)	4 (44%)	5 (41%)	6 (86%)	1 (50%)	5 (71%)
Female	8 (72%)	7 (64%)	5 (56%)	7 (58%)	1 (14%)	1 (50%)	2 (29%)
Mean weight (kg)	82·9	79·3	66·5	70·2	75·2	76·2	74·6

Table 1: **Baseline demographic and clinical characteristics of participants**

participant protection above or below a set antibody titre measured in ELISA Units ([EU]/mL).

Vaccine efficacy was calculated as 1 minus the estimated relative risk (RR), in which RR was calculated by dividing the number of individuals diagnosed with malaria in the vaccinated group by the number of individuals diagnosed with malaria in the control group. Controls from both challenges were combined for analysis. Incidence rates were calculated as the number of participants with malaria divided by the number of those at risk. 95% CIs for vaccine efficacy were calculated with asymptotic estimates of the variances of the RR (precision-based estimates) without adjustment for multiple comparisons.

Differences in time to parasitaemia were calculated by log-rank (Mantel-Cox) analysis of Kaplan-Meier curves by comparing time to PCR positivity for group 1, group 2, and group 3 with unvaccinated controls from challenge A and group 5, group 6, and group 7 with unvaccinated controls from challenge B. $p<0·05$ was considered to indicate a statistically significant result.

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 this report.

Results

From Nov 7, 2016 to Sept 17, 2017, 66 volunteers (of the 125 who were screened for eligibility) were enrolled. Two vaccinated volunteers in group 1, one in group 2, and two in group 3 withdrew during the vaccination phase of the study because of changes in personal circumstances. One volunteer in group 1 was replaced. One further volunteer in group 3 withdrew after their fifth vaccination because of apprehensions concerning CHMI and one volunteer in group 5 was withdrawn after first vaccination at the discretion of the investigator. None of the withdrawals were related to the vaccination and there were no ongoing adverse events or abnormalities in safety blood tests.

37 volunteers (11 in group 1, 11 in group 2, nine in group 3, and six unvaccinated controls) underwent CHMI in challenge A on Jan 30 and Jan 31, 2017. 27 participants underwent CHMI in challenge B on Sept 18 and Sept 19, 2017: five protected volunteers from group 1, a further six unvaccinated controls, seven volunteers in group 5, two volunteers in group 6, and seven volunteers in group 7. All volunteers completed follow-up until 90 days after CHMI. We summarised participant enrolment and study design (figure 1). Baseline demographic and clinical characteristics are shown for all participants (table 1).

The majority of adverse events reported were self-limiting and mild in severity. The reactogenicity profiles that we observed were similar to previous studies for both the R21/MM[6] and the ChAd63/MVA ME-TRAP.[16] Vaccine site pain was the most common local adverse event, reported by between 60% and 100% of participants and was predominantly mild in severity. Local and systemic solicited adverse events are detailed in the appendix (pp 10–15). No serious adverse reactions or suspected unexpected serious adverse reactions occurred and no predefined study stopping or holding rules were activated. There was one serious adverse event in the course of the study (admission to hospital because of acute appendicitis), which was deemed to be unrelated to the vaccine. Unsolicited adverse events were predominantly mild in nature and those that were deemed definitely, probably, or possibly related to vaccination are tabulated (appendix p 5). Laboratory adverse events were predominantly grade 1 in severity and are tabulated in the appendix (p 8).

For R21/MM, the highest frequency of solicited adverse events was seen after the second dose with systemic reactogenicity, notably stronger than after the first or third dose. Reactogenicity to the ChAd-vectored vaccine was similar to that observed for R21/MM, whereas the MVA vaccine elicited a higher frequency of adverse events than ChAd, in line with previous studies.[12,17]

In challenge A, five of six participants in the unvaccinated infectivity control group (group 4A) developed malaria with a median time to diagnosis by blood film of 11·25 days (IQR 11–12, SD 0·45; figure 2A). Nine of 11 volunteers in group 1, seven of 11 in group 2, and six of nine in group 3 were protected from malaria 21 days after CHMI (table 2). A substantial delay to parasitaemia was observed all vaccine groups compared with challenge A controls (per-protocol

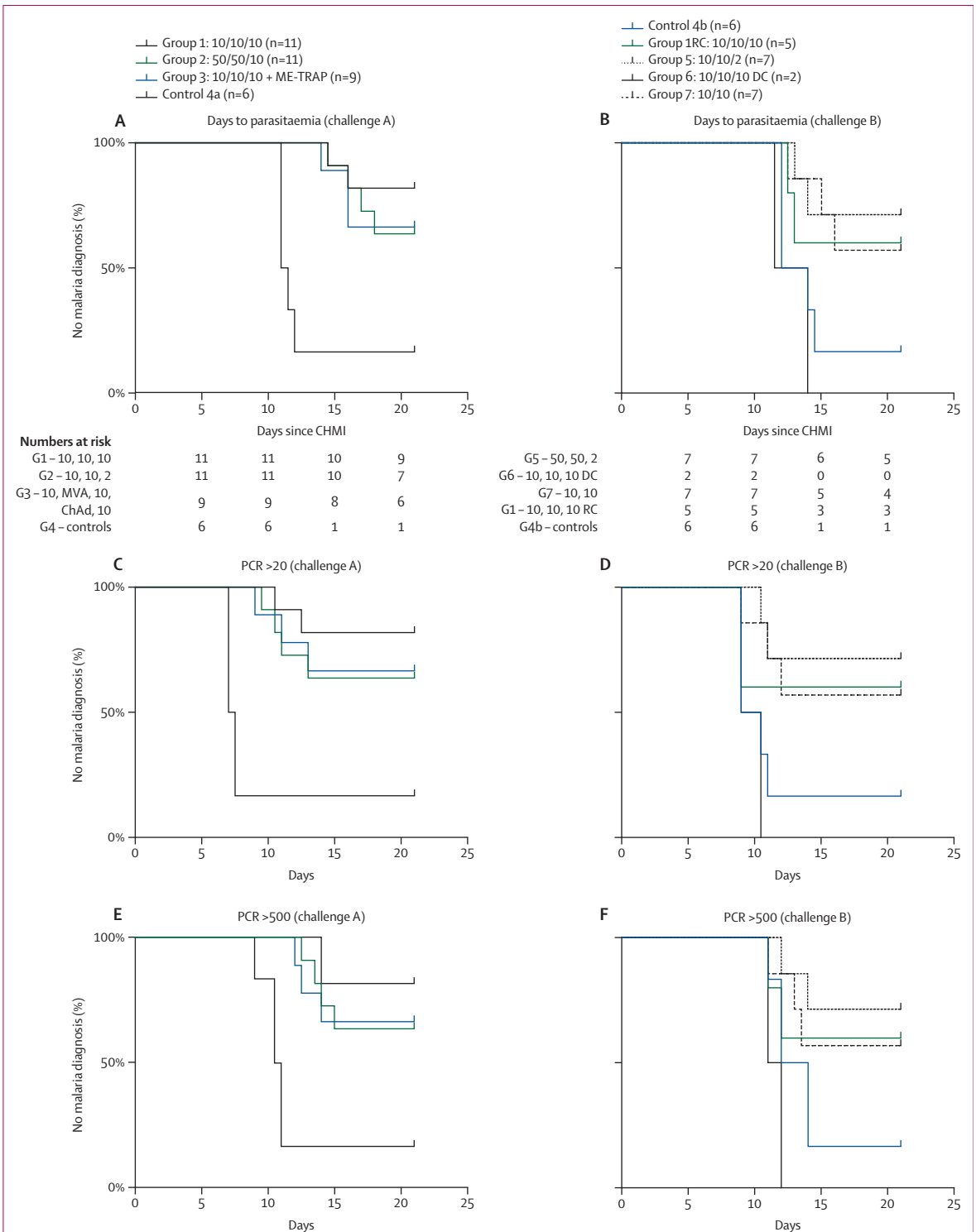

Figure 2: **Days to parasitaemia after vaccination with R21/MM and ChAd63-MVA ME-TRAP following mosquito-delivered *Plasmodium falciparum* 3D7 sporozoite challenge**

Kaplan-Meier analysis of time to diagnosis of malaria by blood-film detection for challenge A (A) and B (B). Kaplan-Meier analysis of time to first sample with more than 20 parasites per mL detected by qPCR for challenge A (C) and B (D). Kaplan-Meier analysis of time to first sample with more than 500 parasites per mL detected by qPCR for challenge A (E) and B (F). CHMI=controlled Human Malaria Infection. ChAd63-MVA ME-TRAP=Chimpanzee adenovirus seotype 63-modified *vaccinia Ankara* virus multi-epitope thrombospondin-related adhesion protein. qPCR=quantative PCR. R21/MM=R21/Matrix M.

	Regimen (dose R21 µg)	Total	Number infected	Number sterilely protected	p value*	Vaccine efficacy, % (95% CI)**	p value
Group 1	10 µg, 10 µg, and 10 µg	11	2	9 (82%)	0·001	80% (60 to 94)	0·003
Group 1	Participants who were rechallenged	5	2	3 (60%)	0·184	72% (–12 to 93)	0·117
Group 2	50 µg, 50 µg, and 10 µg	11	4	7 (64%)	0·004	63% (14 to 86)*	0·029
Group 3	10 µg R21, ChAd63 ME-TRAP, 10 µg R21, 10 µg R21, MVA ME-TRAP	9	3	6 (67%)	0·006	69% (17 to 90)	0·029
Group 5	10 µg, 10 µg, and 2 µg	7	2	5 (71%)	0·027	77% (21 to 94)	0·029
Group 6	10 µg, 10 µg, and 10 µg (delayed CHMI)	2	2	0	0·890	NA	NA
Group 7	10 µg and 10 µg	7	3	4 (57%)	0·079	65% (–8 to 89)	0·947
Infectivity controls	Five *Plasmodium falciparum*-infected bites	12	10	NA	NA	NA	NA

*Log-rank (Mantel–Cox) efficacy compared groups 1, 2, and 3 with six unvaccinated controls from challenge A and groups 5, 6, and 7 with six unvaccinated controls from challenge B, using days to parasitaemia by blood film for diagnosis.**Vaccine efficacy is equal to 1 – relative risk (one-tailed Fisher's exact test).

Table 2: **Vaccine efficacy by regimen**

log-rank [Mantel-Cox] analysis at all tested thresholds): diagnosis by blood film (figure 2A; p=0·0001); PCR >20 parasites per mL (figure 2C; p=0·0002); or PCR >500 parasites per mL (figure 2E, p<0·0001). Among participants diagnosed with malaria, a significant delay in patency was observed in all groups included in challenge A (groups 1, 2, and 3), with a mean time to diagnosis by blood film of 15·3 days (median 15·3, IQR 14·5–16·0, SD 1·1) for group 1, 16·4 days (16·5, 14·5–16·0, 1·5) for group 2, and 15·3 days (16·0, 14·0–16·0, 1·2) for group 3 (figure 2A).

In challenge B, five of six volunteers in the unvaccinated infectivity control group (group 4b) developed malaria with a median time to diagnosis of 13·0 days (IQR 12·0–14·5, SD 1·05; figure 2B). Among vaccinated participants, protection against malaria was exhibited in five of seven volunteers in group 5, none in group 6, and four of seven in group 7. When all vaccine groups from challenge B (groups 5, 6, and 7) were combined, we saw a significant delay to parasitaemia compared with challenge B controls (per-protocol log-rank [Mantel-Cox] analysis): for blood-film positivity only (figure 2B; p=0·034). However, PCR >20 and PCR >500 measures did not show significant delays to positivity (p=0·061, figure 2D; p=0·066, figure 2F). From challenge B, only group 5 demonstrated significant delay to parasitaemia compared with challenge B controls (table 2). The observed difference between PCR positivity in unvaccinated controls in challenge A compared with challenge B meant that we were unable to compare delay in time to positivity between vaccine groups included in different challenge groups. Nonetheless, log-rank tests show that, within the same challenge, delays in time to infection were unlikely to have occurred by chance.

The highest vaccine efficacy was found among participants in group 1 (80%, 95% CI 60–90; table 2). Because the sample sizes in each group were small, CIs for the estimates of vaccine efficacy were wide (table 2), resulting in insufficient statistical power to provide meaningful conclusions on group-to-group comparisons.

Five protected participants from group 1 agreed to undergo repeat CHMI (at challenge B) with 60% (three of five participants; p=0·079) remaining protected at 7·5 months by log-rank (Mantel-Cox test) analysis of primary outcome (table 2) compared with unvaccinated controls.

A significant increase in NANP-specific IgG concentrations was observed after the first, second, and third dose of R21/MM. NANP-specific IgG concentrations peaked 4 weeks after the third dose of R21 for groups 1, 2, 3, and 5, and 4 weeks after the second dose of R21 for group 7 (figure 3A). A fractional third dose (group 2 and 5) was not associated with any difference in peak NANP-specific IgG compared with standard dose regimens (figure 3B), and there was no significant difference between any of the R21 dose groups. Furthermore, the two-dose regimen (group 7) had similar NANP-specific IgG to the three-dose regimens 1 day before challenge (the day before challenge). The two participants in group 6 with a delayed challenge (at week 78 instead of week 12) had lower NANP-specific IgG at the day before challenge; however, statistical analysis was not undertaken because of the small sample size.

When analysed by challenge outcome, there was no significant difference in peak NANP-specific IgG concentrations between protected and unprotected participants (figure 3C). Individuals who had a peak NANP-specific IgG concentration higher than 1100 EU/mL were more likely to be protected after CHMI than those with peak NANP-specific IgG concentrations lower than 1100 EU/mL (p=0·01; Fisher's exact test; figure 3D). The NANP-specific IgG response waned during the 90 days after peak antibody response; however, this decrease was not significant compared with peak response for any group. Additionally, there were no differences in anti-NANP IgG between vaccinated groups 90 days after CHMI.

TRAP-specific IgG responses were measured only in participants in group 3 who received viral vectored ChAd63 and MVA-expressing ME-TRAP and unvaccinated controls in challenge A. Participants in group 3 had significantly higher TRAP-specific IgG and NANP-specific IgG antibody responses than controls at C – 1 (appendix p 19). A positive correlation between NANP-specific and TRAP-specific IgG

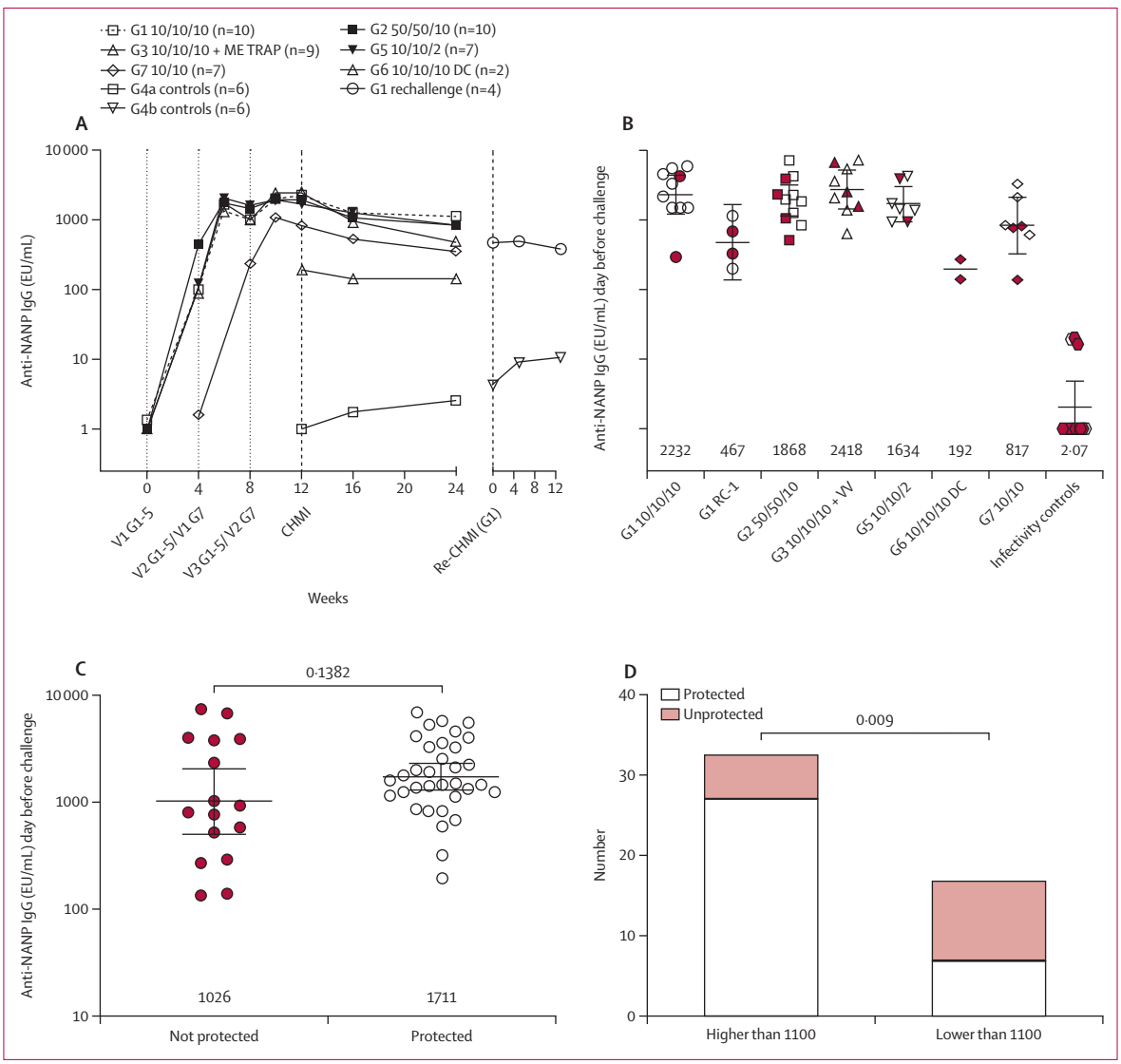

Figure 3: **Antibody responses to vaccination measured by ELISA**

(A) Anti-NANP IgG antibody responses over time. Geometric mean values are shown. Vertical dotted lines indicate timepoints of administration of vaccine. Vertical dashed lines indicate timepoints for challenge and rechallenge. (B) Anti-NANP6 IgG antibody responses 1 day before CHMI (the day before challenge). Geometric means (values shown above x-axis) with 95% CIs are shown for each group. Datapoints coloured in red indicate participants diagnosed with malaria during study follow-up. (C) Anti-NANP6 IgG antibody responses 1 day before CHMI (the day before challenge). Geometric means (values shown above the x-axis) with 95% CIs are shown; participants are stratified by malaria diagnosis. Datapoints coloured in red indicate participants diagnosed with malaria during study follow-up. (D) Participants stratified by anti-NANP IgG concentration either higher or lower than 1100 ELISA units/mL. CHMI=Controlled Human Malaria Infection.

was found among participants in group 3 (r=0·41; p=0·24; Spearman's rank test; appendix p 19). TRAP-specific IgG titres in group 3 were significantly lower than TRAP-specific IgG titres from a previous trial[18] assessing the same viral vectored regimen (p=0·018; appendix p 19).

Inhibition of sporozoite invasion (ISI; appendix p 34) was investigated using serum collected 1 day before CHMI (C – 1), which was the peak NANP-specific IgG timepoint. Because of assay capacity restrictions, six randomly selected participants from group 1 and 2 were assessed for ISI at baseline (day 0) and C – 1 to investigate vaccine induced changes in ISI. There was a significant increase in ISI between baseline (median 2·69%, IQR 0·001–7·69) and after three doses of R21/MM (median 24·4%, 20·2–29·6; p=0·031; Wilcoxon-matched pairs ranked test; appendix p 17).

A subset of samples from each group at the peak NANP-specific IgG timepoint were selected for ISI analysis (group 1, n=8; group 2, n=8; group 3, n=7; group 5, n=6; group 7, n=5; and group 1 rechallenge, n=4). There was no difference in the ability of serum from different vaccine regimens at the peak timepoint to inhibit sporozoite invasion (appendix p 17), despite a clear positive correlation between ISI and NANP-specific IgG concentrations (r=0·38; p=0·008; Spearman's rank test; appendix p 17). Additionally,

there was no difference between individuals who were protected or unprotected after challenge (appendix p 17).

Peripheral T-cell responses to circumsporozoite protein were weak and transient after every vaccination in groups 1, 2, 3, 5, 6, and 7 who received R21/MM (appendix p 18). Group 3 (who received ME-TRAP) showed a significantly increased number of spot-forming units (SFU) per 10^6 PBMC 1 month after one dose of vaccine (median 48 SFU for baseline or visit 1 and 499 SFU for week 4 or visit 2; p=0·0039; appendix p 18). A further increase was seen at Day 70, but this increase was not statistically significant (appendix p 17). On the day before CHMI, group 3 vaccinees who received three doses of R21/MM with one dose of ChAd63 ME TRAP and one dose of MVA ME-TRAP had significantly higher interferon-γ SFU to ME-TRAP peptides than non-vaccinated controls (median group 3, 731 SFU; median group 4, 65 SFU; p=0·0002; appendix p 18). By contrast, individuals receiving R21/MM vaccine (groups 1, 2, 3, 5, 6, and 7) did not show elevated T-cell reactivity to circumsporozoite protein peptides compared with non-vaccinated controls (appendix p 18).

Discussion

Here we report the high-level efficacy of a novel malaria vaccine candidate, R21 adjuvanted with Matrix-M. At a dose of 10 μg R21 and 50 μg MM, we report high efficacy against CHMI following three doses of vaccine, given 1 month apart. Our data confirm that this vaccine is safe and well tolerated in healthy UK adults with adverse events being predominantly mild in nature.

Our group previously reported, in the context of malaria-naive UK RTS,S recipients, that a reduced third dose gave better vaccine efficacy (89%) than the same dose given at all three visits (75%) with no concomitant increase in immunogenicity.[9] In this study, we do not see enhanced vaccine efficacy or immunogenicity associated with a reduced third dose (in either group 2, receiving 50 μg, 50 μg, and 10 μg R21 [63%], or group 5, receiving 10 μg, 10 μg, and 2μg [77%], over that seen with the three doses of 10 μg [80%]). A reduced third dose for group 2 (50 μg, 50 μg, and 10 μg) and group 5 (10 μg, 10 μg, and 2 μg) was not associated with any significant difference in peak NANP-specific antibody titre compared with a dose of 10 μg, 10 μg, and 10 μg (group 1). Although this regimen has shown promise in adults who are malaria naive using RTS,S, a large phase 2B study in Ghana and Kenya found lower efficacy of a reduced third dose regimen in children than with a standard regimen.[19]

Other groups have investigated the use of a fractional third dose in conjunction with a delay in giving the third dose. For RTS,S in adults who are malaria naive, this resulted in significantly higher immunogenicity and efficacy.[20] For a blood-stage malaria vaccine candidate, RH5.1, a delayed fractional third dose in adults who are malaria naive resulted in higher efficacy,[21] which was associated with higher levels of, and durability of, circulating vaccine-specific B cells and serum IgG1.[22] What immune mechanisms might be influencing the combination of a delay and fractional third dose remains unclear, and studies conducted thus far have been unable to disentangle the two aspects. A fractional third-dose regimen is considered logistically difficult to deploy because of the need for different doses within the primary series, which is not mitigated by a clear improvement in vaccine efficacy and hence is not likely to progress. Regimens with a delayed third standard dose of R21/MM are still in clinical evaluation.

All regimens induced strong humoral responses against the central NANP repeat region of the circumsporozoite protein vaccine antigen with no significant differences between the study groups at the peak timepoint measured 1 day before CHMI. Although the magnitude of the NANP-specific IgG response did not predict vaccine efficacy after CHMI on an individual basis, we identified a concentration of 1100 EU/mL that was associated with a higher likelihood of being protected after CHMI, which provides a useful benchmark for future clinical studies. The magnitude of NANP-specific IgG response observed here was similar to that achieved after RTS,S given to UK adults who are malaria naive;[9,16] however, this concentration is lower than seen in children immunised in Burkina Faso with R21/MM. A 5 μg dose of R21 with 50 μg MM elicited a mean peak NANP-specific IgG concentration of approximately 11 000 EU/mL[7] and an ELISA titre of 6618 EU/mL was associated with protection in the first year of follow-up.[23] These findings suggest that qualitative measures of the humoral response, in addition to quantitative antibody concentrations, are important.

Among all groups receiving RTS,S vaccine,[9] the ability of sera after vaccination to inhibit sporozoite invasion into a hepatocyte cell line was better among those who were protected after CHMI than those who were unprotected (p=0·017).[10] The fact that we do not see a similar finding in this study with R21/MM might be evidence of differences in protective mechanisms between RTS,S and R21, despite both vaccines targeting the same circumsporozoite protein antigen, or because of differences in formulation, adjuvant, or because of variability in mosquito-administered sporozoite dose or limited statistical power.

There was no difference in NANP-specific IgG antibodies between group 1 (10 μg, 10 μg, and 10 μg) and group 3 who received the same dose of R21 but with the addition of viral vectors, suggesting no interference effect. Hypothesised synergy between cell-mediated immunity of the liver-stage-targeting viral vectored vaccines, and humoral immunity induced by R21, which targets the sporozoite stage before hepatocyte invasion, remains biologically plausible, despite this study showing no additional protection from CHMI. There were minimal inteferon-γ ELISpot responses to circumsporozoite protein induced by R21/MM, which is similar to previous experience with RTS,S/AS01, for which low level CD4 and no CD8 T-cell responses were observed.[24]

This study registered the first cases (one in each challenge) of unvaccinated control volunteers not developing clinical

malaria in a CHMI study conducted at the University of Oxford, Oxford, UK, although similar instances have been reported in several other CHMI trials undertaken at other centres.[25,26] Mean time to patency in the control groups from challenge A (11·3 days) and challenge B (13 days) is otherwise within the expected range and both volunteers had a high mosquito infectivity score. Five infectious bites were 100% infective in most cases with a control group size of six; however, several studies have reported one infectivity control per control group that did not develop parasitaemia, probably because of chance.[27] These occurrences pose no substantial limitation to the principal aim of the study, which was to provide a measure of efficacy in phase 2 testing of this vaccine candidate, given that estimates of vaccine efficacy using relative risk take into account non-infected controls.

We have previously reported on the R21/MM 10 μg, 10 μg, and 10 μg schedule in African adults, which showed that the reactogenicity profile was significantly improved and humoral responses after the first and second vaccination were similar to those of UK volunteers.[6] There was no significant boosting of the humoral response after the third vaccination and the response at 28 days after the final vaccination was significantly lower in the African adults exposed to malaria than in UK adults who were malaria naive. A two-dose vaccine schedule has merited exploration because it might be sufficient to provide useful protection to people living in malaria-endemic regions who have been primed through natural exposure. The 65% efficacy seen in group 7 is among the highest reported for a two-dose malaria vaccine regimen.[28–30] The small group size here resulted in a wide CI. Despite a lower mean NANP-specific IgG concentration in group 7, this finding was not statistically significantly different to the groups receiving three doses, with similar ISI activity into a hepatocyte cell line. This two-dose regimen could merit further development.

Of the group 1 volunteers who were protected upon first CHMI, three of five remained protected at rechallenge. Evidence of durable protection at this timepoint is important given the rapid waning of efficacy for RTS,S/AS01 after the first year. Delaying the first booster dose, and combining R21/MM with a vaccine-inducing blood-stage immunity, could improve durability of efficacy and circumvent the suggested phenomenon of rebound malaria in the fifth year after vaccination seen with RTS,S in high-exposure populations.[31]

Limitations of this study include the limited sample size, absence of randomisation, and that only ten of 12 participants in the infectivity control group developed parasitaemia. CHMI studies are, by design, small proof-of-concept studies to accelerate down selection of promising vaccines by generating an early indication (or no indication) of efficacy. Sample size is limited by cost, supply of GMP-grade mosquitoes as a challenge agent, and clinical trial site capacity to undertake the intensive follow-up required. Recruitment to CHMI studies can be challenging because of stringent safety requirements, inflexible timelines because of the need to challenge all participants simultaneously, and time requirements for participants to attend several visits. Additional limitations to participation, such as random assignment of volunteers to groups, can further restrict the available pool of volunteers and delay enrolment.

We have demonstrated clinical efficacy of the vaccine candidate R21/MM in this phase 1–2A malaria sporozoite challenge study in adults who are malaria naive. Following subsequent clinical development, including a phase-3 licensure trial,[8] R21/MM is now prequalified and recommended for use in young children by WHO. R21/MM is being manufactured at the Serum Institute of India at a large scale and will be a cost-effective tool for the reduction of malaria morbidity and mortality in children in sub-Saharan Africa.

Contributors

NV, KJE, and AVSH contributed to conceptualisation; NV, GB, and KJE to the methodology; NV, DS, DB, LS, GB, NJE, and KJE to the formal analysis; NV, DS, OG, FRL, JP, CM, PMF, MSD, RM, AMM, IP, NB, JB, AMB, and BA to the investigation; KAC, FB, PA-M, EB, GG, and LF to the resources; NV to the writing of the original draft; DB, LS, AVSH, and KJE to the writing, review, and editing; NV, DB, LS, and KJE to the visualisation; AML, BA, DJML, SNF, KJE, and AVSH to the supervision; RR and AML to the project administration; and RR and AVSH to the funding acquisition. All authors had full access to all the data in the study, reviewed the final manuscript, and had final responsibility for the decision to submit for publication. KJE directly accessed and verified the underlying data reported in the manuscript.

Declaration of interests

KAC, NV, KJE, and AVSH are named as co-inventors or contributors on patent filings related to the R21 vaccine candidate. KJE was an employee of the University of Oxford, Oxford, UK at the time of the work and is now an employee of GSK. KJE holds restricted shares in the GSK group of companies. All other authors declare no competing interests.

Data sharing

De-identified participant data will be made available upon request directed to the chief investigator AVSH (adrian.hill@ndm.ox.ac.uk). 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.

Acknowledgments

This manuscript is dedicated to the late Navin Venkatraman. The team would like to thank all the study volunteers. This project has received funding from the European Union Horizon 2020 research and innovation programme under grant agreement 733273 and is part of the MultiMalVax programme supported by the EU (305282). Vaccine manufacture was funded by the UK Medical Research Council (MR/JOO8680/1) and the European Commission FP7 programme (305282). The work was also supported by the UK National Institute of Health Research through the Oxford Biomedical Research Centre (http://oxfordbrc.nihr.ac.uk/; A91301 Adult Vaccine), the Imperial National Institute for Health Research (NIHR) Clinical Research Facility, and the Wellcome Trust (https://www.wellcome.ac.uk/; 084113/Z/07/Z). The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health. We are grateful to Chris Janse and the late Shahid Khan at Leiden University Medical Centre for provision of transgenic parasite lines and to Alexandra Spencer, Ahmed Salman, and Marta Ulaszewska for immunopotency assays and preparation of infected mosquitoes. We thank Philip Angell-Manning for his contribution to R21 manufacturing. We are also grateful to Ruth Payne and Saranya Sridhar for contributing to clinical care of participants and to Catherine Mair and Kate Harrison for assistance with sample processing. We thank the Centre for Clinical Vaccinology and

Tropical Medicine clinic nursing staff Raquel Lopez Ramon, Megan Baker, and Celia Mitton for their maintenance of volunteer clinic appointments and taking safety and immunology bloods. We thank Nicola Williams of the Nuffield Department of Primary Health Care Sciences at the University of Oxford for statistical review of the manuscript. AMB is supported by the Medical Research Council (MR/N00227X/1 and MR/W025701/1), Global Health Innovative Technology Fund, Rosetrees Trust (G109130), and Royal Society (RGS/R1/201293; IEC/R3/19302). JB acknowledges support from the Wellcome Trust (100993/Z/13/Z). SNF is an NIHR Senior Investigator.

References

1 WHO. World malaria report 2023. 2023. https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2023 (accessed Nov 7, 2024).
2 Plowe CV. Malaria chemoprevention and drug resistance: a review of the literature and policy implications. *Malar J* 2022; **21:** 104.
3 WHO. Malaria vaccines: preferred product characteristics and clinical development considerations. 2022. https://www.who.int/publications/i/item/9789240057463 (accessed July 12, 2024).
4 WHO. Full evidence report on the RTS,S/AS01 malaria vaccine. *SAGE Yellow B* 2021; **1:** 1–90.
5 Collins KA, Snaith R, Cottingham MG, Gilbert SC, Hill AVS. Enhancing protective immunity to malaria with a highly immunogenic virus-like particle vaccine. *Sci Rep* 2017; **7:** 1–15.
6 Venkatraman N, Tiono AB, Bowyer G, et al. Phase I assessments of first-in-human administration of a novel malaria anti-sporozoite vaccine candidate, R21 in matrix-M adjuvant, in UK and Burkinabe volunteers. *medRxiv* 2019; published online Oct 18. https://doi.org/10.1101/19009282.
7 Datoo MS, Natama MH, Somé A, et al. Efficacy of a low-dose candidate malaria vaccine, R21 in adjuvant Matrix-M, with seasonal administration to children in Burkina Faso: a randomised controlled trial. *Lancet* 2021; **397:** 1809–18.
8 Datoo MS, Dicko A, Tinto H, et al. Safety and efficacy of malaria vaccine candidate R21/Matrix-M in African children: a multicentre, double blind, randomised, phase 3 trial. *Lancet* 2024; **403:** 533–44.
9 Rampling T, Ewer KJ, Bowyer G, et al. Safety and efficacy of novel malaria vaccine regimens of RTS,S/AS01B alone, or with concomitant ChAd63-MVA-vectored vaccines expressing ME-TRAP. *Vaccines* 2018; **3:** 1–9.
10 Bowyer G, Grobbelaar A, Rampling T, et al. CXCR3+ T follicular helper cells induced by co-administration of RTS, S/AS01B and viral-vectored vaccines are associated with reduced immunogenicity and efficacy against malaria. *Front Immunol* 2018; **9:** 01660.
11 Long C, Zavalani F. Malaria vaccines and human immune responses. *Curr Opin Microbiol* 2016; **32:** 96–102.
12 O'Hara GA, Duncan CJA, Ewer KJ, et al. Clinical assessment of a recombinant simian adenovirus ChAd63: a potent new vaccine vector. *J Infect Dis* 2012; **205:** 772–81.
13 Afolabi MO, Tiono AB, Adetifa UJ, et al. Safety and immunogenicity of ChAd63 and MVA ME-TRAP in West African children and infants. *Mol Ther* 2016; **24:** 1470–77.
14 Ewer KJ, O'Hara GA, Duncan CJA, et al. Protective CD8 + T-cell immunity to human malaria induced by chimpanzee adenovirus-MVA immunisation. *Nat Commun* 2013; **4:** 4–13.
15 Ogwang C, Kimani D, Edwards NJ, et al. Prime-boost vaccination with chimpanzee adenovirus and modified vaccinia Ankara encoding TRAP provides partial protection against Plasmodium falciparum infection in Kenyan adults. *Sci Transl Med* 2015; **7:** 1–20.
16 Rampling T, Ewer KJ, Bowyer G, et al. Safety and high level efficacy of the combination malaria vaccine regimen of RTS,S/AS01B with chimpanzee adenovirus 63 and modified vaccinia Ankara vectored vaccines expressing me-trap. *J Infect Dis* 2016; **214:** 772–81.
17 Bliss CM, Bowyer G, Anagnostou NA, et al. Assessment of novel vaccination regimens using viral vectored liver stage malaria vaccines encoding ME-TRAP. *Sci Rep* 2018; **8:** 1–17.
18 Hodgson SH, Ewer KJ, Bliss CM, et al. Evaluation of the efficacy of ChAd63-MVA vectored vaccines expressing circumsporozoite protein and ME-TRAP against controlled human malaria infection in malaria-naive individuals. *J Infect Dis* 2015; **211:** 1076–86.
19 Samuels AM, Ansong D, Kariuki SK, et al. Efficacy of RTS,S/AS01E malaria vaccine administered according to different full, fractional, and delayed third or early fourth dose regimens in children aged 5–17 months in Ghana and Kenya: an open-label, phase 2b, randomised controlled trial. *Lancet Infect Dis* 2022; **22:** 1329–42.
20 Regules JA, Cicatelli SB, Bennett JW, et al. Fractional third and fourth dose of RTS,S/AS01 malaria candidate vaccine: a phase 2a controlled human malaria parasite infection and immunogenicity study. *J Infect Dis* 2016; **214:** 762–71.
21 Minassian AM, Silk SE, Barrett JR, et al. Reduced blood-stage malaria growth and immune correlates in humans following RH5 vaccination. *Medicine* 2021; **2:** 701–19.
22 Nielsen CM, Barrett JR, Davis C, et al. Delayed boosting improves human antigen-specific Ig and B cell responses to the RH5.1/AS01B malaria vaccine. *JCI Insight* 2023; **8:** 163859.
23 Datoo MS, Natama HM, Somé A, et al. Efficacy and immunogenicity of R21/Matrix-M vaccine against clinical malaria after 2 years' follow-up in children in Burkina Faso: a phase 1/2b randomised controlled trial. *Lancet Infect Dis* 2022; **22:** 1728–36.
24 Lalvani A, Moris P, Voss G, et al. Potent induction of focused Th1-type cellular and humoral immune responses by RTS,S/SBAS2, a recombinant *Plasmodium falciparum* malaria vaccine. *J Infect Dis* 1999; **180:** 1656–64.
25 Seder RA, Chang LJ, Enama ME, et al. Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. *Science* 2013; **341:** 1359–65.
26 Ishizuka AS, Lyke KE, DeZure A, et al. Protection against malaria at 1 year and immune correlates following PfSPZ vaccination. *Nat Med* 2016; **22:** 614–23.
27 Shibeshi W, Bagchus W, Yalkinoglu Ö, Tappert A, Engidawork E, Oeuvray C. Reproducibility of malaria sporozoite challenge model in humans for evaluating efficacy of vaccines and drugs: a systematic review. *BMC Infect Dis* 2021; **21:** 1–15.
28 Kester KE, McKinney DA, Tornieporth N, et al. A phase I/IIa safety, immunogenicity, and efficacy bridging randomized study of a two-dose regimen of liquid and lyophilized formulations of the candidate malaria vaccine RTS,S/AS02A in malaria-naïve adults. *Vaccine* 2007; **25:** 5359–66.
29 Kester KE, McKinney DA, Tornieporth N, et al. Efficacy of recombinant circumsporozoite protein vaccine regimens against experimental *Plasmodium falciparum* malaria. *J Infect Dis* 2001; **183:** 640–47.
30 Moon JE, Ockenhouse C, Regules JA, et al. A phase IIa controlled human malaria infection and immunogenicity study of RTS,S/AS01E and RTS,S/AS01B delayed fractional dose regimens in malaria-naive adults. *J Infect Dis* 2020; **222:** 1681–91.
31 Olotu A, Fegan G, Wambua J, et al. Seven-year efficacy of RTS,S/AS01 malaria vaccine among young African children. *N Engl J Med* 2016; **374:** 2519–29.