

**Safety and immunogenicity of heterologous prime-boost immunization with viral-
vectored malaria vaccines adjuvanted with Matrix-M™**

Navin Venkatraman^{a,b*}, Nicholas Anagnostou^{a,b}, Carly Bliss^a, Georgina Bowyer^a, Danny Wright^a, Karin Lövgren-Bengtsson^c, Rachel Roberts^{a,b}, Ian Poulton^{a,b}, Alison Lawrie^{a,b}, Katie Ewer^a, Adrian V. S. Hill^a.

^aJenner Institute, University of Oxford and the NIHR Oxford Biomedical Research Centre, Oxford OX3 7DQ, UK.

^bCentre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Old Road, Headington, Oxford OX3 7LJ, United Kingdom.

^cNovavax AB, Kungsgatan 109, SE-753 18 Uppsala, Sweden.

**Corresponding author at:*

Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital

Old Road, Headington, Oxford OX3 7LJ, United Kingdom.

Telephone: +44 (0)1865 611410

Email: navin.venkatraman@ndm.ox.ac.uk

Abstract: 239 words (limit 300)

Text (body): 3068 words (limit 3000)

HIGHLIGHTS

- Viral vectored malaria vaccines adjuvanted with Matrix-M were well tolerated
- Addition of Matrix-M did not result in a reduction in cellular or humoral immunogenicity
- These vaccines adjuvanted with Matrix-M can be used safely in combination regimes with vaccines requiring an adjuvant

ABSTRACT

The use of viral vectors in heterologous prime-boost regimens to induce potent T cell responses in addition to humoral immunity is a promising vaccination strategy in the fight against malaria. We conducted an open-label, first-in-human, controlled Phase I study evaluating the safety and immunogenicity of Matrix-M adjuvanted vaccination with a chimpanzee adenovirus serotype 63 (ChAd63) prime followed by a modified vaccinia Ankara (MVA) boost eight weeks later, both encoding the malaria ME-TRAP antigenic sequence (a multiple epitope string fused to thrombospondin-related adhesion protein). Twenty-two healthy adults were vaccinated intramuscularly with either ChAd63-MVA ME-TRAP alone (n=6) or adjuvanted with 25µg (n=8) or 50 µg (n=8) Matrix-M. Vaccinations appeared to be safe and generally well tolerated, with the majority of local and systemic adverse events being mild in nature. The addition of Matrix-M to the vaccine did not increase local reactogenicity; however, systemic adverse events were reported more frequently by volunteers who received adjuvanted vaccine in comparison to the control group. T cell ELISPOT responses peaked at 7-days post boost vaccination with MVA ME-TRAP in all three groups. TRAP-specific IgG responses were highest at 28-days post boost with MVA ME-TRAP in all three groups. There were no differences in cellular and humoral immunogenicity at any of the time points between the control group and the adjuvanted groups. We demonstrate that Matrix-M can be safely used in combination with ChAd63-MVA ME-TRAP heterologous prime-boost immunization without any reduction in cellular or humoral immunogenicity.

Clinical Trials Registration NCT01669512

Keywords: malaria, vaccine, viral-vectored, Matrix-M

INTRODUCTION

Plasmodium falciparum malaria remains one of the leading infectious causes of mortality worldwide, predominantly affecting children and pregnant women in sub-Saharan Africa [1]. *Plasmodium falciparum* (Pf) is a complex and immunoevasive pathogen, therefore the development of a highly efficacious vaccine has remained difficult for decades. Lately however, there have been considerable advances; the leading vaccine candidate RTS,S/AS01, which predominantly elicits antibodies to the pre-erythrocytic circumsporozoite protein has completed testing in a large Phase III clinical trial [2-5] and the World Health Organisation (WHO) plan to commence pilot deployments in sub-Saharan Africa in 2018. Though RTS,S/AS01 has demonstrated significant short-term vaccine efficacy, there remains an urgent need to improve efficacy to achieve the goals set down by the Malaria Vaccine Technology Roadmap- development of a suitable vaccine with at least 75% durable efficacy against clinical malaria by 2030 [6].

Malaria immunity is complex, and in addition to humoral responses, T cell responses targeting pre-erythrocytic antigens can provide high level protection against malaria in animal models, [7-9] in the field [10], following irradiated sporozoite inoculation [11], and following vaccination in humans [12, 13]. In this regard, a leading alternative, and potentially complementary strategy is heterologous prime-boost immunization with sequential administration of viral-vectored vaccines chimpanzee adenovirus serotype 63 (ChAd63) and modified vaccinia Ankara (MVA), both encoding ME-TRAP (a multiple epitope string fused to the thrombospondin-related adhesion protein). In addition to eliciting antibody responses, this approach elicits potent T cell responses in adults in the UK [14], as

well as adults and infants in malaria endemic areas, and has an excellent track record of safety and tolerability in these populations [15-18].

The ChAd63-MVA ME-TRAP malaria vaccine strategy has demonstrated durable partial efficacy in a controlled human malaria infection (CHMI) study in the UK [12], and partial efficacy was again evident in two CHMI studies with *Pf*-infected sporozoites [19]; (Rampling, Payne et al. in preparation). Moreover, a randomised controlled single-blind trial undertaken in Kenyan male adults showed that vaccination reduced the risk of malaria infection by 67% [13]. More recently, this vaccination regime was also tested in the target population – African infants; these studies demonstrated acceptable reactogenicity and good immunogenicity [15, 16].

It is widely accepted that an efficacious malaria vaccine is likely to be a combination of different approaches acting on multiple antigens involved in different stages of its complex life cycle [20]. A recent clinical trial combined the leading malaria vaccine candidate, RTS,S/AS01 with ChAd63-MVA ME-TRAP in the same regimen for the first time, and tested efficacy against sporozoite challenge. This study demonstrated that combining these vaccines in the same regimen was not only safe and tolerable, but also highly immunogenic and efficacious [21].

A vaccine adjuvant is likely to be necessary for such combinations, and Matrix-M is an attractive candidate vaccine adjuvant for this purpose as it, and other particle formulations of *Quillaja* saponins, show acceptable safety [22], and the ability to enhance both cellular and humoral immune responses to a range of vaccines [23-27]. Therefore, we conducted

this study to evaluate the safety and immunogenicity of the ChAd63-MVA ME-TRAP vaccination regime adjuvanted with Matrix-M compared to ChAd63-MVA ME-TRAP given alone.

METHODS

Study participants

The study was conducted at the Centre for Clinical Vaccinology and Tropical Medicine at the University of Oxford. Participants were healthy, immunocompetent adults between the ages of 18 and 50 years all of whom provided written informed consent. Participant demographics and baseline characteristics are presented in Table 1.

Ethics and regulatory approval

The study protocol and associated documents were reviewed and approved by the UK National Research Ethics Service, Committee South Central–Oxford A (Ref: 12/SC/0036), the Medicines and Healthcare Products Regulatory Agency (Ref: 21584/0299/001-0001), and the Oxford University Clinical Trials and Research Governance team, who independently and externally monitored compliance with Good Clinical Practice guidelines. Vaccine use was authorised by the Genetically Modified Organisms Safety Committee (GMSC) of the Oxford University Hospitals NHS Trust (Reference number GM462.11.66). The study was conducted according to the principles of the Declaration of Helsinki (2008) and the International Conference on Harmonization Good Clinical Practice guidelines. An independent local safety

monitor provided safety oversight. The trial was registered with ClinicalTrials.gov (Ref: NCT01669512).

Study design

This was a Phase I controlled observational clinical trial to assess the safety and immunogenicity of ChAd63 ME-TRAP and MVA ME-TRAP adjuvanted with Matrix-M administered in a heterologous prime-boost regimen. Eligible participants were assigned to one of three dose groups: a control group who received ChAd63 ME-TRAP followed by MVA ME-TRAP 8 weeks later and two treatment groups who received the same regime adjuvanted with Matrix-M at doses of 25µg or 50µg, respectively. The first three vaccinations in the treatment groups occurred in a staggered manner and interim safety reviews were conducted prior to progression to the higher dose Matrix-M group. Full details regarding the study conduct are provided in the protocol (Supplementary appendix S1).

ChAd63 ME-TRAP and MVA ME-TRAP vaccines and Matrix-M adjuvant

Generation, manufacture and storage of the ChAd63 ME-TRAP (Batch no: 01S11-01) and MVA ME-TRAP (Batch no: 0080312) vaccines has been previously described [14, 28]. The antigen ME-TRAP contains a fusion protein of a multi-epitope string (ME), followed by the pre-erythrocytic thrombospondin-related adhesion protein (TRAP) from *Pf* strain T9/96. Matrix-M (Batch no: 12-104) is a patented adjuvant technology developed by Novavax: the production, manufacture and storage of this product have been previously described [29]. The vaccines were mixed with Matrix-M at the bedside immediately before administration.

All vaccines were administered intramuscularly into the deltoid using a 23G 1¼" needle.

Assessment of safety

Volunteers were observed for 45 minutes following vaccine administration and follow-up visits were scheduled as detailed in the protocol (S1). All participants recorded their temperature and any adverse events for 7 days post-vaccination. A review of solicited and unsolicited adverse events occurred at each follow-up visit. Safety bloods included a full blood count, renal function and liver function tests. Severity grading of adverse events and the assignment of a causal relationship were conducted according to predefined guidelines.

Cellular and humoral immunogenicity

Ex-vivo IFN- γ ELISPOT assays and antibody responses measured by anti-TRAP IgG ELISA were performed on samples from days 0, 14, 28, 56, 63, 84, 112 and 168. Cytokine production in response to stimulation with a pool of TRAP peptides was analysed at day 28 and day 63 for each group, using flow cytometry. The full methods used for these immunological assays have been previously described [12].

Statistical analysis

Data were analyzed using GraphPad Prism version 6.03 for Windows (GraphPad Software Inc., California, USA). Geometric means or medians with interquartile ranges for each group are described. Kruskal-Wallis analysis and the Friedman test were used to compare peak

immune responses with the baseline. Significance testing of differences between two groups used Mann-Whitney analysis. A Wilcoxon matched-pairs analysis was used to compare between time points within groups. A value of $p < 0.05$ was considered significant.

RESULTS

Study population

From 1st October, 2012 to 23rd June, 2014, a total of 23 volunteers (11 male, 12 female) of the 48 who were screened for eligibility were enrolled. Participant flow is summarised in Figure 1. One volunteer in the low dose group withdrew from the study 7 days after receiving their first vaccination for reasons unrelated to vaccination and was subsequently replaced. This volunteer was asymptomatic apart from mild fatigue when reviewed at 7 days post-vaccination. Safety bloods done at this time point were also normal. All the remaining 22 volunteers completed the study and were followed-up until Day 168.

Adverse events

The safety profile of heterologous prime-boost immunization with ChAd63-MVA ME-TRAP has been described [12, 14, 18]. No serious adverse events (SAEs) or suspected unexpected serious adverse reactions (SUSARs) occurred. The majority of adverse events (AEs) reported were self-limiting and mild in severity.

Local and systemic AEs deemed definitely, probably or possibly related to ChAd63 ME-TRAP vaccination either administered alone or mixed with Matrix-M are shown in Figures 2A and 2B, respectively. The majority of local adverse events were mild in nature. Vaccine site pain was the most common local adverse event and was predominantly mild in severity, with no clear pattern or attribution to the adjuvant. Five volunteers (one in the 25µg Matrix-M group and one in the 50µg Matrix-M group) reported a fever. Two of these volunteers (one in the low dose group and one in the high dose group) had an episode of severe fever, characterized as documented fever > 39°C. All episodes of fever occurred within the first day post-vaccination and did not persist for more than 24 hours. The majority of systemic AEs were mild in nature. Severe flu-like symptoms were reported by one volunteer each in the control group and high dose group. Two volunteers in the low dose group reported severe systemic symptoms.

Local and systemic AEs deemed definitely, probably or possibly related to MVA ME-TRAP vaccination either administered alone or mixed with Matrix-M are shown in Figures 3A and 3B, respectively. The majority of local AEs were mild in nature. Local reactogenicity was more pronounced compared to vaccination with ChAd63 ME-TRAP (3.6 AEs/volunteer vs 2.3 AEs/volunteer) again with no pattern attributable to the adjuvant. Vaccine site pain was reported by all volunteers receiving vaccination with MVA ME-TRAP. Three volunteers in the control group and two volunteers in the low dose group reported severe pain. Eight volunteers (three in the control group, two in the low dose group and three in the high dose group) reported a fever. Two of these volunteers (one in the control group and one in the high dose group) had an episode of severe fever. All episodes of fever occurred within the first day post-vaccination and did not persist for more than 24 hours. Severe systemic

symptoms were reported by two volunteers each in the control group, 25µg Matrix-M group and 50µg Matrix-M group, with no pattern attributable to the adjuvant.

One volunteer in the control group developed a mild transient leukopaenia at day 7 post-ChAd63 ME-TRAP vaccination, which resolved spontaneously. One volunteer each in the low dose group and the high dose group had a mild leukopaenia associated with neutropaenia present prior to their first vaccination and persisted intermittently through the course of the trial. There were no other biochemical or haematological abnormalities associated with vaccination.

Immunogenicity

Cell-mediated immunity

Vectored ME-TRAP immunisation was designed as a T cell inducing vaccination strategy; therefore T cell responses are of primary interest. Potent ME-TRAP-specific ELISpot responses were induced by ChAd63-ME-TRAP vaccination and peaked at day 14 [no Matrix-M and 25µg Matrix-M - geomean (95% CI) spot forming cells per million PBMC (SFC/10⁶ PBMC): 605.9 (156.5 – 2345) and 228.1 (144.8-359.2), respectively] or day 28 [50µg Matrix-M - geomean (95% CI) SFC/10⁶ PBMC: 469.2 (144.1 – 1527)]. T cell responses to ME-TRAP significantly increased after MVA boost in all groups, peaking 1 week after boost [day 63 geomean (95% CI) SFC/10⁶ PBMC: no Matrix-M 3802 (2294-6300), 25µg Matrix-M 3102 (2283 – 4215), 50µg Matrix-M 1874 (813.7 – 4316)]. T cell responses were well maintained between day 84 and day 168, where they remained at levels significantly above baseline and

comparable to levels at peak post-prime (Figure 4A, Wilcoxon matched pairs analyses for all groups combined $P < 0.0001$ and $P = 0.7216$ for baseline and day 14 respectively). There were no significant differences in post-prime or post-boost TRAP-specific T cell responses between the groups (Figure 4B, Kruskal-Wallis analyses $P = 0.0571$ at day 28 and $P = 0.4489$ at day 63). However, the dose of Matrix-M may have some effect on the durability of responses, with TRAP-specific T cell responses comparatively lower at day 168 in the group receiving 25 μ g of Matrix-M compared to the group that did not receive Matrix-M [Figure 4C, Geomeans (95% CI) SFC/ 10^6 PBMC: 183.4 (98.3-342.1) and 550 (193.3-1565) for 25 μ g Matrix-M and no Matrix-M groups, respectively; Mann-Whitney $P = 0.0480$]. However, the 50 μ g Matrix-M group had responses comparable to the group that did not receive Matrix-M and there were no significant differences across the three groups (Kruskal-Wallis with Dunn's post-test comparison; no significant differences between groups).

Flow cytometry for T-cell phenotypes and their cytokine profile

The proportion of total CD4⁺ T cells producing cytokine (IFN- γ , IL-2, TNF- α), or expressing CD107a (a marker of potential lytic activity) was not significantly different between groups at day 28 or day 63. However, only the group that received 50 μ g of Matrix-M had a significant increase in cytokine production by CD4⁺ T cells from day 28 to day 63 (Figure 5A; Wilcoxon $P = 0.0156$). Similarly, for CD8⁺ T cells, the percentage producing cytokines did not significantly differ across groups at day 28 or day 63 but the only group in which cytokine production in response to TRAP stimulation significantly increased from post-prime to post-boost was the group that received the 50 μ g dose of Matrix-M (Figure 5B; Wilcoxon $P = 0.0313$). The percentage of CD4⁺ T cells producing IFN- γ and/or IL-2 increased from post-

prime to post-boost in both the unadjuvanted and 50µg dose Matrix-M groups but remained low in the 25µg dose group (Figure 5C). The percentage of CD8+ T cells producing IFN-γ increased from post-prime to post-boost in both the adjuvanted Matrix-M groups but not in the unadjuvanted group (Figure 5D). The percentage of CD8+ T cells producing TNF-α or expressing CD107a (Figure 5D and 5E) increased from post-prime to post-boost only in the 50µg dose Matrix-M group (CD107a at D28 vs D63: Wilcoxon P=0.0156) and not when the vaccine was unadjuvanted or when a 25µg dose of Matrix-M was given.

TRAP-specific IgG

Anti-TRAP antibody responses were induced by ChAd63 ME-TRAP vaccination in all volunteers and MVA ME-TRAP boosted titres by over 10-fold from a geomean titre of 80.6 ELISA Units (EU) (95% CI 51.6 – 125.9) across all volunteers at peak post-prime (day 28) to 1030 EU (767.8 – 1383) at peak post-boost (day 84). Titres were maintained at levels significantly above baseline at 4 months after boost (Figure 6A; Wilcoxon test P<0.0001). There were no significant differences in post-prime or post-boost anti-TRAP IgG titres between the three groups (Figure 6B). Additionally, the avidity of anti-TRAP IgG at the peak time point (day 84) did not differ significantly between groups (Figure 6C).

DISCUSSION

We report here on the safety and immunogenicity of the first-in-human administration of the heterologous prime-boost regimen with ChAd63-MVA ME-TRAP administered with the saponin-based adjuvant, Matrix-M. Our data show that this vaccine approach was generally

well tolerated with AEs being predominantly mild in nature. MVA ME-TRAP was more reactogenic than ChAd63 ME-TRAP, which is in keeping with previous studies that have shown that MVA is a more reactogenic vector at the doses used [12, 18, 21, 30-32]. The addition of Matrix-M to the ChAd63 ME-TRAP vaccine did not result in increased local reactogenicity. However, moderate to severe systemic adverse events were reported more frequently by volunteers in the Matrix-M adjuvanted groups in comparison to the control group (0.8 AEs/volunteer vs 1.6 AEs/volunteer).

Similarly, the addition of Matrix-M to the MVA ME-TRAP vaccine did not increase the local reactogenicity. Volunteers in the Matrix-M adjuvanted groups experienced increased systemic adverse events such as nausea, myalgia and arthralgia in comparison to the control group (0.5 AEs/volunteer vs 1.25 AEs/volunteer); however, there was no overall increase in moderate or severe AEs in the groups receiving Matrix-M in comparison to the control group.

Matrix-M has been tested as an adjuvant to vaccines for other diseases, including influenza. It has been shown that addition of Matrix-M does result in increased local and systemic reactogenicity compared to controls who receive unadjuvanted vaccine [24]. Though we did not observe a similar effect for the local reactogenicity, we did observe increased reporting of severe systemic adverse events in volunteers receiving adjuvanted ChAd63 ME-TRAP in comparison to the control group. However, the majority of adverse events were mild or moderate in severity and resolved within 72 hours.

The need to induce cell-mediated immunity in addition to humoral immunity is widely recognized to be an important facet of vaccines being developed against a number of diseases, including malaria. A number of previous studies in UK and African adults and children have shown the ability of heterologous prime-boost immunisation regimes to elicit high levels of T cell responses [12, 14, 18]. In keeping with this, our study also showed that priming with ChAd63 ME-TRAP followed by a boost vaccination with MVA ME-TRAP given 8 weeks later resulted in a significant increase in the T cell response (> 2500 SFC/ 10^6 PBMC). As reported previously, median T cell ELISPOT responses peaked at 7-days post boost vaccination with MVA ME-TRAP in all three groups [12, 14, 18]. Responses were durable and well maintained at 6 months and the addition of an adjuvant did not alter T cell responses. The proportion of total CD4⁺ and CD8⁺ T cells producing cytokines (IFN- γ , IL-2, TNF- α), or expressing CD107a was not significantly different between groups at day 28 or day 63. The higher dose of the adjuvant was required to induce a significant increase in cytokine production by CD4⁺ and CD8⁺T cells from day 28 to day 63. Interestingly, the addition of the adjuvant resulted in an increased percentage of CD8⁺ T cells producing IFN- γ from post-prime to post-boost. In the first malaria sporozoite challenge study testing the same vaccines being used in this study, it was shown that the frequency of monofunctional IFN- γ producing CD8⁺ T cells, but not antibodies, correlated with efficacy. RTS,S/AS01 the leading malaria vaccine candidate induces potent antibody responses to circumsporozoite protein, but low level CD4⁺ and no CD8⁺ T-cell responses [33]. In this study, geometric mean anti-TRAP IgG antibody responses were highest at 28-days post boost vaccination with MVA ME-TRAP in all three groups and there were no differences in humoral immunogenicity at any of the time points between the control group and the adjuvanted groups.

In conclusion, this Phase I clinical trial showed that heterologous prime-boost immunization with viral-vectored vaccines adjuvanted with Matrix-M was safe and well tolerated. There was no evidence of reduction in vectored vaccine-induced immunogenicity when used in combination with an adjuvant. We did not assess immune responses to the viral vectors in this study; however, we do not expect them to be enhanced by Matrix-M as we have previously shown that anti-vector responses positively correlate with responses to the transgene [12]. It is highly likely that an efficacious protein or virus-like particle-based malaria vaccine will require an adjuvant and our data suggest that Matrix-M could be used mixed with viral vectors as part of this combination regime without leading to any deleterious effect on the immunogenicity of the vectored vaccines.

TABLE AND FIGURE LEGENDS

Table 1. Demographics and baseline characteristics of volunteers enrolled in the trial.

***BMI (body mass index) was not recorded in 4 volunteers.**

Figure 1. VAC048 flow chart of study design and volunteer recruitment. *Abbreviations: ChAd63, chimpanzee adenovirus serotype 63; ME-TRAP, multiple epitope string fused to the thrombospondin-related adhesion protein; MVA, modified vaccinia Ankara; ; pfu, plaque forming units; vp, viral particles. A total of 48 volunteers were screened and 23 were enrolled in total. One volunteer in the low dose group withdrew consent after their first vaccination and was subsequently replaced. A total of 22 volunteers completed the study. *The first two volunteers in the low dose group received MVA ME-TRAP at a dose of 1.44×10^8 pfu instead of 2×10^8 pfu.*

Figure 2. Local (A) and systemic (B) adverse events (AE) deemed definitely, probably or possibly related to ChAd63 ME-TRAP (5×10^{10} vp) vaccination either administered alone (-) or mixed with Matrix-M given at a dose of 25 μ g (25+) or 50 μ g (50+). *Only the highest intensity of each AE per subject is listed. Data are combined for all AEs for all volunteers receiving the same vaccine at the stated time point. 'Other' systemic AEs included mild coryzal symptoms, mild to moderate lower back ache, moderate to severe rigors, moderate to severe dizziness and moderate insomnia.*

Figure 3. Local (A) and systemic (B) adverse events (AE) deemed definitely, probably or possibly related to MVA ME-TRAP (2×10^8 pfu) vaccination either administered alone (-) or mixed with Matrix-M given at a dose of 25 μ g (25+) or 50 μ g (50+). *The first two volunteers in the 25 μ g dose group received MVA ME-TRAP at a dose of 1.44×10^8 pfu instead of 2×10^8 pfu. Only the highest intensity of each AE per subject is listed. Data are combined for all AEs for all volunteers receiving the same vaccine at the stated time point. 'Other' local AEs included mild bruising at the vaccine site and a severe burning sensation in the arm. 'Other' systemic AEs included mild to moderate abdominal pain, moderate dizziness, mild to moderate lower back pain, moderate chills, moderate to severe rigors, mild increased heart rate and mild to moderate feeling of shakiness.*

Figure 4. Ex-vivo IFN- γ ELISPOT responses to vaccination. *(A) TRAP IFN- γ ELISPOT time courses. Lines show geomeans. G1: no Matrix-M, n=6, G2: 25 μ g Matrix-M, n=8, G3: 50 μ g Matrix-M, n=8. Friedman test; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. (B) TRAP T cell responses measured by IFN- γ ELISPOT at peak post-prime and post-boost (D14 or D28 and D63). Kruskal-Wallis analysis. (C) Durability of TRAP-specific T cell responses, measured by IFN- γ ELISPOT at D168.*

Figure 5. Total cytokine responses in each group at peak post-prime, day 28 (D28) and peak post-boost, day 63 (D63). *Percentage of (A) CD4 $^+$ or (B) CD8 $^+$ T cells producing IFN- γ , IL-2, TNF- α or expressing CD107a. Medians with interquartile ranges are shown. Individual points are PBMC from individual vaccinees. Dotted lines indicate the lower limits of detection. **Distribution of cytokine responses at peak post-prime and post-boost in each group.** Geomean percentage of (C) CD4 $^+$ or (D) CD8 $^+$ T cells producing each cytokine within each group. (E) Percentage of CD8 $^+$ T cells expressing CD107a in each group at D28 and D63.*

*Wilcoxon matched-pairs analyses between time points within groups; Kruskal-Wallis analyses with Dunn's post-test comparison to compare between groups; ns = non-significant, * $P < 0.05$.*

Figure 6. Antibody responses to vaccination. (A) Anti-TRAP IgG time courses. Lines show geomeans + 95% CI. G1: no Matrix-M, $n=6$, G2: 25 μ g Matrix-M, $n=8$, G3: 50 μ g Matrix-M, $n=8$. Friedman test. (B) Peak anti-TRAP IgG responses post-prime and post-boost (D28, D84). (C) Avidity of anti-TRAP IgG at peak time point (D84).

Conflicts of Interest

A.V.S.H. is a named inventor on patent applications on malaria vectored vaccines and immunization regimens. K.L.B. is an employee of Novavax and employees may hold stock and/or stock options in the company.

ACKNOWLEDGEMENTS: This study was funded by the National Institute of Health Research through the Oxford Biomedical Centre vaccines theme. We thank J. Illingworth and A. Douglas for production of recombinant TRAP protein. We thank all the members of the clinical trials team at the Centre for Clinical Vaccinology and Tropical Medicine, including Raquel Lopez Ramon, Paula Marriott, Adrienne Cook, Oliver Griffiths, Mary Smith, Megan Baker, Natalie Lella and Charlotte Tyson.

REFERENCES

1. WHO, *World Malaria Report*. 2013.
2. *Efficacy and safety of the RTS,S/AS01 malaria vaccine during 18 months after vaccination: a phase 3 randomized, controlled trial in children and young infants at 11 African sites*. PLoS Med, 2014. **11**(7): p. e1001685.
3. *Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial*. Lancet, 2015. **386**(9988): p. 31-45.
4. Rts, S.C.T.P., et al., *A phase 3 trial of RTS,S/AS01 malaria vaccine in African infants*. N Engl J Med, 2012. **367**(24): p. 2284-95.
5. Agnandji, S.T., et al., *First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children*. N Engl J Med, 2011. **365**(20): p. 1863-75.
6. Malaria Vaccine Funders Group. *Malaria Vaccine Technology Roadmap*. 2013 20/3/13]; Available from: http://www.who.int/immunization/topics/malaria/vaccine_roadmap/TRM_update_nov13.pdf?ua=1.
7. Doolan, D.L. and S.L. Hoffman, *The complexity of protective immunity against liver-stage malaria*. J Immunol, 2000. **165**(3): p. 1453-62.
8. Khusmith, S., M. Sedegah, and S.L. Hoffman, *Complete protection against Plasmodium yoelii by adoptive transfer of a CD8+ cytotoxic T-cell clone recognizing sporozoite surface protein 2*. Infect Immun, 1994. **62**(7): p. 2979-83.
9. Romero, P., et al., *Cloned cytotoxic T cells recognize an epitope in the circumsporozoite protein and protect against malaria*. Nature, 1989. **341**(6240): p. 323-6.
10. Todryk, S.M., et al., *Correlation of memory T cell responses against TRAP with protection from clinical malaria, and CD4 CD25 high T cells with susceptibility in Kenyans*. PLoS One, 2008. **3**(4): p. e2027.
11. Malik, A., et al., *Human cytotoxic T lymphocytes against the Plasmodium falciparum circumsporozoite protein*. Proc Natl Acad Sci U S A, 1991. **88**(8): p. 3300-4.
12. Ewer, K.J., et al., *Protective CD8+ T-cell immunity to human malaria induced by chimpanzee adenovirus-MVA immunisation*. Nat Commun, 2013. **4**: p. 2836.
13. Ogwang, C., 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**(286): p. 286re5.
14. O'Hara, G.A., et al., *Clinical assessment of a recombinant simian adenovirus ChAd63: a potent new vaccine vector*. J Infect Dis, 2012. **205**(5): p. 772-81.
15. Afolabi, M.O., et al., *Safety and Immunogenicity of ChAd63 and MVA ME-TRAP in West African Children and Infants*. Mol Ther, 2016. **24**(8): p. 1470-7.
16. Bliss, C.M., et al., *Viral Vector Malaria Vaccines Induce High-Level T Cell and Antibody Responses in West African Children and Infants*. Mol Ther, 2017. **25**(2): p. 547-559.
17. Kimani, D., et al., *Translating the immunogenicity of prime-boost immunization with ChAd63 and MVA ME-TRAP from malaria naive to malaria-endemic populations*. Mol Ther, 2014. **22**(11): p. 1992-2003.
18. Ogwang, C., et al., *Safety and immunogenicity of heterologous prime-boost immunisation with Plasmodium falciparum malaria candidate vaccines, ChAd63 ME-TRAP and MVA ME-TRAP, in healthy Gambian and Kenyan adults*. PLoS One, 2013. **8**(3): p. e57726.

19. Hodgson, S.H., et al., *Evaluation of the efficacy of ChAd63-MVA vectored vaccines expressing circumsporozoite protein and ME-TRAP against controlled human malaria infection in malaria-naïve individuals*. J Infect Dis, 2015. **211**(7): p. 1076-86.
20. Long, C.A. and F. Zavala, *Malaria vaccines and human immune responses*. Curr Opin Microbiol, 2016. **32**: p. 96-102.
21. Rampling, T., 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**(5): p. 772-81.
22. Bigaeva, E., et al., *Meta-Analysis on Randomized Controlled Trials of Vaccines with QS-21 or ISCOMATRIX Adjuvant: Safety and Tolerability*. PLoS One, 2016. **11**(5): p. e0154757.
23. Bengtsson, K.L., et al., *Matrix-M adjuvant: enhancing immune responses by 'setting the stage' for the antigen*. Expert Rev Vaccines, 2013. **12**(8): p. 821-3.
24. Cox, R.J., et al., *Evaluation of a virosomal H5N1 vaccine formulated with Matrix M adjuvant in a phase I clinical trial*. Vaccine, 2011. **29**(45): p. 8049-59.
25. Cox, R.J., et al., *Matrix M H5N1 Vaccine Induces Cross-H5 Clade Humoral Immune Responses in a Randomized Clinical Trial and Provides Protection from Highly Pathogenic Influenza Challenge in Ferrets*. PLoS One, 2015. **10**(7): p. e0131652.
26. Bengtsson, K.L., et al., *Matrix-M adjuvant enhances antibody, cellular and protective immune responses of a Zaire Ebola/Makona virus glycoprotein (GP) nanoparticle vaccine in mice*. Vaccine, 2016. **34**(16): p. 1927-35.
27. Cox, F., et al., *Matrix-M adjuvation broadens protection induced by seasonal trivalent virosomal influenza vaccine*. Virol J, 2015. **12**: p. 210.
28. Bejon, P., et al., *A phase 2b randomised trial of the candidate malaria vaccines FP9 ME-TRAP and MVA ME-TRAP among children in Kenya*. PLoS Clin Trials, 2006. **1**(6): p. e29.
29. Hahn, T., *Rapid manufacture and release of a GMP batch of Zaire ebolavirus glycoprotein vaccine made using recombinant baculovirus-Sf9 insect cell culture technology*. BioProcess J, 2015. **14**(1): p. 6.
30. Sheehy, S.H., et al., *Phase Ia clinical evaluation of the safety and immunogenicity of the Plasmodium falciparum blood-stage antigen AMA1 in ChAd63 and MVA vaccine vectors*. PLoS One, 2012. **7**(2): p. e31208.
31. de Barra, E., et al., *A phase Ia study to assess the safety and immunogenicity of new malaria vaccine candidates ChAd63 CS administered alone and with MVA CS*. PLoS One, 2014. **9**(12): p. e115161.
32. Sheehy, S.H., et al., *Phase Ia clinical evaluation of the Plasmodium falciparum blood-stage antigen MSP1 in ChAd63 and MVA vaccine vectors*. Mol Ther, 2011. **19**(12): p. 2269-76.
33. Lalvani, A., 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**(5): p. 1656-64.