Clinical Study Report

A Phase I/IIa Sporozoite Challenge Study to Assess the Protective Efficacy of Two Prime-Boost Malaria Vaccine Candidates: ChAd63 and MVA encoding ME-TRAP and the same Viral Vectors encoding CS

VAC045

REC Reference: 12/SC/0037
EudraCT number: 2011-005477-24
CTA number: 21584/0293/001-0001

CONFIDENTIAL

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1. DECLARATION

I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.

Signed: Date: 06/11/13

Print name: Professor A.V.S. Hill

Chief Investigator

Signed: Date: 06/11/13

Print name: Dr T. Rampling

Lead Clinician & Report Author

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Malaria Trials coordinator

2. OVERVIEW

Study title:	A Phase I/IIa Sporozoite Challenge Study to Assess the Protective Efficacy of Two Prime-Boost Malaria Vaccine Candidates: ChAd63 and MVA encoding ME-TRAP and the same Viral Vectors encoding CS
Trial code:	VAC045
Study description:	Open label observational challenge study.
Test IMPs:	ChAd63 ME-TRAP, MVA ME-TRAP, ChAd63 CS, MVA CS
Indication studied:	Safety, immunogenicity and efficacy
Sponsor:	University of Oxford
Chief Investigator:	Professor Adrian V.S. Hill; Centre for Clinical Vaccinology & Tropical Medicine, University of Oxford, UK
Co-Investigators:	Dr Saul N. Faust; NIHR Wellcome Trust Clinical Research Facility University of Southampton, UK
Study centres:	Centre for Clinical Vaccinology and Tropical Medicine University of Oxford, Churchill Hospital Old Road Headington Oxford OX3 7LE NIHR Wellcome Trust Clinical Research Facility University of Southampton C Level, West Wing Mailpoint 218 University Hospital Southampton NHS Foundation Trust Tremona Road SO16 6YD Infection and Immunity Section Sir Alexander Fleming Building Imperial College of Science, Technology and Medicine Imperial College Road London SW7 2AZ
Clinical Phase:	I/IIa
Study dates planned:	April 2012 – November 2012

Study dates actual:	April 2012 – November 2012
Enrolment:	Completed
Publication:	In preparation
GCP Statement:	This study was performed in compliance with ICH Good Clinical Practice (GCP) including the archiving of essential documents.

3. PROTOCOL SYNOPSIS

Objectives	<u>Primary Objective:</u> To assess the protective efficacy in malaria naïve individuals of heterologous prime boost vaccination with ChAd63-MVA expressing the pre-erythrocytic antigen CS or ME-TRAP. <u>Secondary Objectives:</u> To assess the safety and immunogenicity generated in malaria naïve individuals following heterologous prime boost vaccination with ChAd63-MVA expressing the pre-erythrocytic antigen CS or ME-TRAP. <u>Tertiary Objective:</u> To assess the long term protective efficacy of heterologous prime-boost vaccination with ChAd63-MVA expressing the pre-erythrocytic antigen CS or ME-TRAP.
Trial design	Open label, partially randomised, multi-centre phase I/IIa controlled human malaria infection study. This may be conducted in two phases if volunteer availability and/or other logistic considerations make this preferable.
Sample Size	<u>Group 1</u> 15 volunteers; 1 dose of ChAd63 CS 5 x 10^{10} vp intramuscularly and 1 dose MVA CS 2 x 10^{8} pfu intramuscularly 8 weeks later followed by controlled human malaria infection 14-28 days later. <u>Group 2</u> 15 volunteers; 1 dose of ChAd63 ME-TRAP 5 x 10^{10} vp intramuscularly and 1 dose MVA ME-TRAP 2 x 10^{8} pfu intramuscularly 8 weeks later followed by controlled human malaria infection 14-28 days later. <u>Group 3</u> 6-18 unvaccinated control volunteers who undergo controlled human malaria infection. Total: 36 - 48 volunteers
Main criteria for inclusion	Volunteers must satisfy all the following criteria to be eligible for the study: • Healthy adults aged 18 to 45 years. • Able and willing (in the Investigator's opinion) to comply with all study requirements. • Willing to allow the investigators to discuss the volunteer's medical history with their General Practitioner. • Women only: Must practice continuous effective contraception for the duration of the study. • Agreement to refrain from blood donation during the course of the

	study and for at least 3 years after the end of their involvement in the study. • Written informed consent to participate in the trial. • Reachable (24/7) by mobile phone during the period between CHMI and completion of antimalarial treatment. • Willingness to take a curative anti-malaria regimen following CHMI. • For volunteers not living in Oxford: agreement to stay in a hotel room close to the trial centre during a part of the study (from at least day 6.5 post mosquito bite until anti-malarial treatment is completed). • Answer all questions on the informed consent quiz correctly.
Duration of treatment	All volunteers in groups 1 & 2 received one vaccination at enrolment, one vaccination 8 weeks later and sporozoite challenge 14-28 days later (experimental malaria infection). Six volunteers underwent sporozoite challenge only.
Criteria for Evaluation of Objectives	<u>Primary Objective:</u> The number of vaccinees who develop malaria infection and the time between exposure and parasitaemia as detected by thick-film blood smear, compared with controls. <u>Secondary Objective:</u> • Analysis of actively and passively collected data on adverse events from diary cards, clinical review of volunteers and laboratory measurements. • Immunological assays of cellular and humoral immunity. <u>Tertiary Objective:</u> To assess long term protective efficacy of ChAd63 ME-TRAP, ChAd63 CS, MVA CS and MVA ME-TRAP in heterologous prime boost regimens by re-challenging any volunteers protected at initial malaria challenge.
Statistical methods	Kaplan Meier analysis of efficacy data. Descriptive analysis of safety and immunology data.
Blinding	Non-Blinded
Controls	Controlled
Randomisation	Non Randomised

4. ETHICS AND REGULATORY APPROVAL

INDEPENDENT ETHICS COMMITTEE APPROVAL

Ethical approvals for the study was granted by the UK National Research Ethics Service, Committee South Central – Oxford A (Ref: 12/SC/0037) on 28/02/12. In addition, ethical approval was also granted by the Western Institution Review Board (Ref: 20120266) as a condition of funding.

ETHICAL CONDUCT OF THE STUDY

The study was performed in accordance with the declaration of Helsinki and in agreement with the International Conference on Harmonisation (ICH) guidelines on Good Clinical Practice (GCP).

VOLUNTEER INFORMATION & CONSENT

The volunteer information sheet detailed the procedures involved in the study (aims, methodology, potential risks and anticipated benefits) and the Investigator explained these verbally to each volunteer prior to obtaining consent. The volunteer then signed and dated the informed consent form to indicate that they fully understood the information, and were willing to participate in the study. Volunteers were given copies of the signed consent form to keep for their records. The original consent forms are kept in a confidential file in the Investigators' records. All volunteers provided written informed consent to participate in the study prior to being screened.

REGULATORY APPROVAL

The study was approved by the UK Medicines and Healthcare products Regulatory Agency (Ref: 21584/0293/001-0001) on 22/02/12. Vaccine use was authorized by the Genetically Modified Organisms Safety Committee (GMSC) of the Oxford University Hospitals NHS Trust (Reference number GM462.11.65). The trial was registered with ClinicalTrials.gov (Ref: NCT01623557). The Local Safety Committee provided safety oversight and GCP compliance was independently monitored by an external organization (Appledown Clinical Research Ltd, Great Missenden, UK).

5. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Title	Name and affiliation
Chief Investigator & Principal Investigator – Oxford and London	Professor A.V.S. Hill – University of Oxford
Principal Investigator - Southampton	Dr S.N. Faust – NIHR Wellcome Trust Clinical Research Facility, University of Southampton
Trial Clinicians	Dr S Sheehy – University of Oxford Dr T Rampling – University of Oxford Dr N Anagnostou – University of Oxford Dr E de Barra – Royal College of Surgeons in Ireland Dr T Havelock – NIHR Wellcome Trust Clinical Research Facility, University of Southampton
Project Managers	Dr A Lawrie – University of Oxford Ms R Roberts – University of Oxford
Monitor	Appledown Clinical Research Ltd
Laboratory Investigators	Dr K Ewer Ms C Bliss Mr N Edwards Ms S de Cassan Ms P Mange Ms K Collins

6. DESCRIPTION OF INVESTIGATIONAL PRODUCTS

CS Insert

Circumsporozoite protein (CSP) is expressed by sporozoites and liver schizonts and plays a key role in the attachment phase of sporozoite invasion into hepatocytes. The poor immunogenicity of the standard full length CSP insert used in previous vectors in clinical trials (CSO), suggest that there may be an important difference in the intrinsic immunogenicity of CSO compared to the ME-TRAP insert. Using information from multiple sources, a novel CS antigen, was designed for use in this study, which omits the extreme C-terminus of the protein that encodes the GPI-anchor sequence and may down-modulate CS immunogenicity (see ChAd63 CS & MVA CS IMP-Ds for more details).

ME-TRAP Insert

ME-TRAP contains a fusion protein of multiple epitopes (ME) and the *P. falciparum* pre-erythrocytic thrombospondin-related adhesion protein (TRAP). The 'ME' is a string of 20 epitopes, mainly CD8 T cell epitopes from *P. falciparum* pre-erythrocytic antigens, fused to TRAP. The individual CTL epitopes which constitute the 'multiple epitope' part of ME-TRAP represent a variety (six) of potentially protective target antigens and are included to ensure an immune response to the vaccine in the majority of the population vaccinated. The ME string is fused to the entire sequence of the T9/96 strain of *P. falciparum* TRAP and the ME-TRAP hybrid is a 2398 base-pair insert which encodes for a single polypeptide of 789 amino acids. TRAP was selected as it is well characterized abundant pre-erythrocytic stage antigen and has a protective homologue in rodents.

ChAd63 CS & ChAd63 ME-TRAP were all manufactured under Good Manufacturing Practice conditions by the Clinical Biomanufacturing Facility (CBF) in Oxford where final certification and associated labelling also took place. Further details relating to batch release and manufacturing of these investigational products can be found in the relevant IMP-Ds.

MVA CS & MVA ME-TRAP were all manufactured under Good Manufacturing Practice conditions by Impfstoffwerk Dessau-Tornau (IDT), Germany. Final certification of these products and associated labelling took place at the Clinical Biomanufacturing Facility (CBF) in Oxford. Further details relating to batch release and manufacturing of these investigational products can be found in the relevant IMP-Ds.

The vials of all vaccines were stored between –70°C and –90°C, in a locked freezer, at either the Clinical Biomanufacturing Facility, the Centre for Clinical Vaccinology & Tropical Medicine or the Pharmacy at Southampton General Hospital. All movements of the study vaccines between sites or from locked freezers to clinic rooms were fully documented.

7. STUDY POPULATION

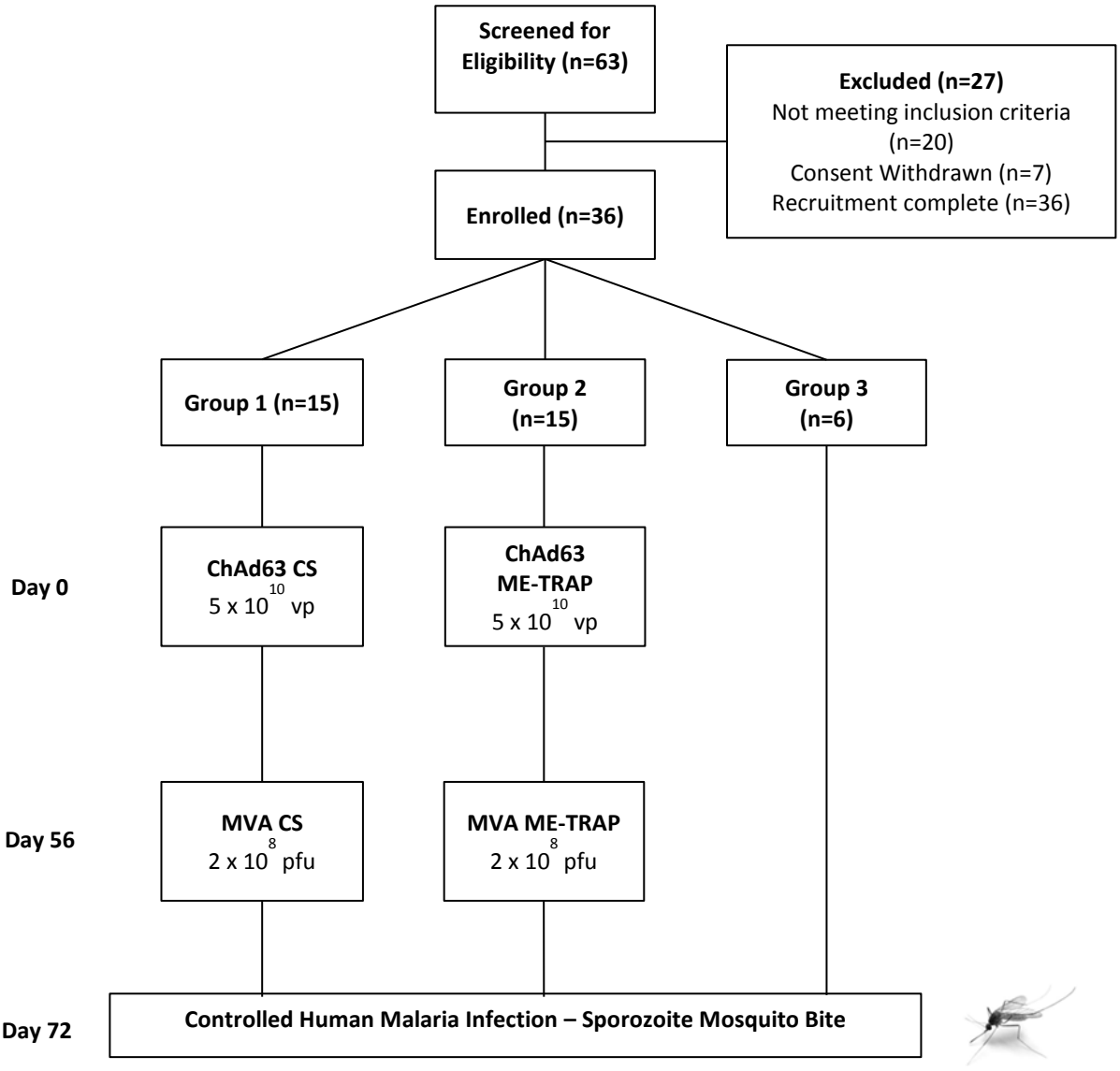


Figure 1. Flow chart of study design and volunteer recruitment. *20 volunteers were excluded following screening for the following reasons; psychiatric history (n=3), no GP screening letter returned (n=3), multiple medical problems (n=2), alcohol excess (n=2), syncope (n=1), connective tissue disease (n=1), iron deficiency (n=1), raised ALT (n=1), poor venous access (n=1), gastrointestinal problems under investigation (n=1), family history of heart disease (n=1), lost to follow up (n=1), unavailable during challenge (n=1), history of recreational drug use (n=1). Furthermore, 7 volunteers withdrew consent after screening, but prior to enrolment. All immunizations were administered intramuscularly with sequential vaccines administered into the deltoid of alternating arms. No volunteers withdrew from the study and all volunteers completed study visits as scheduled.*

8. PROTOCOL DEVIATIONS

9 protocol deviations occurred in total during this trial.

Protocol deviations at Oxford site

7 protocol deviations were recorded at the Oxford site. They were as follows:

- C+90 visit did not take place for 2 volunteers
- C+90 visit occurred outside the +/-14 day window for 2 volunteers
- 1 volunteer received the 48hr dose of malarone at 48hrs + 1hr 15mins (therefore outside the +/- 1 hr window)
- 1 volunteer received the 36hr dose of riamet at 32hrs (therefore outside the +/- 1 hr window)
- Incorrect bleed volume for PCR at visit 6.5 or visit 7.5 for 11 volunteers

Protocol deviations at Southampton site

2 protocol deviations were recorded at the Southampton site. They were as follows:

- Day 14 visit occurred outside of the specified window for 1 volunteer
- Investigators were unable to obtain the full 77ml of blood at the review on the day before CHMI (C-1). This was because of a difficult venepuncture.

9. RESULTS

9.1 DEMOGRAPHICS OF STUDY POPULATION

Volunteer group	Mean age at Screening (range)	Gender (% Male)
1 (n=15)	27.5 (19-39)	9m 6f (60)
2 (n=15)	25.7 (18-33)	11m 4f (73)
3 (n=6)	25.9 (20-37)	1m 5f (17)

Table 2: Demographics of volunteers.

9.2 ADVERSE EVENTS

(a) Serious Adverse Events (SAEs)

One SAE occurred during the study;

MVT-10451420 – Female – Group 1 - Recruited at Oxford Site
This volunteer underwent CHMI on 16th July 2012 by mosquito bite. She was diagnosed with malaria on 28th July 2012 following positive blood film and symptoms. She was commenced on oral malarone as per protocol. Following her first dose she vomited and was given a further 2 tablets. 24hrs later she was given her second dose of malarone in clinic, and vomited 3hrs later, but the clinical team were not informed at the time. 48hrs after diagnosis she was given her next dose of malarone in clinic and vomited again. She was therefore admitted on 30th July 2012 to the NHS infectious diseases ward for inpatient observation, anti-emetics and IV rehydration. She was started on oral Riamet, which was well tolerated and she was discharged on 31st July 2012 and made a complete recovery.

(b) Adverse Events Related to ChAd63 vectored vaccines

No unexpected or serious AEs related to ChAd63 vaccination occurred. The local (Figure. 2) and systemic (Figure 3) reactogenicity profile of each vaccine was similar to Phase Ia data (VAC038 and MAL034). The majority of ChAd63 related AEs were mild.

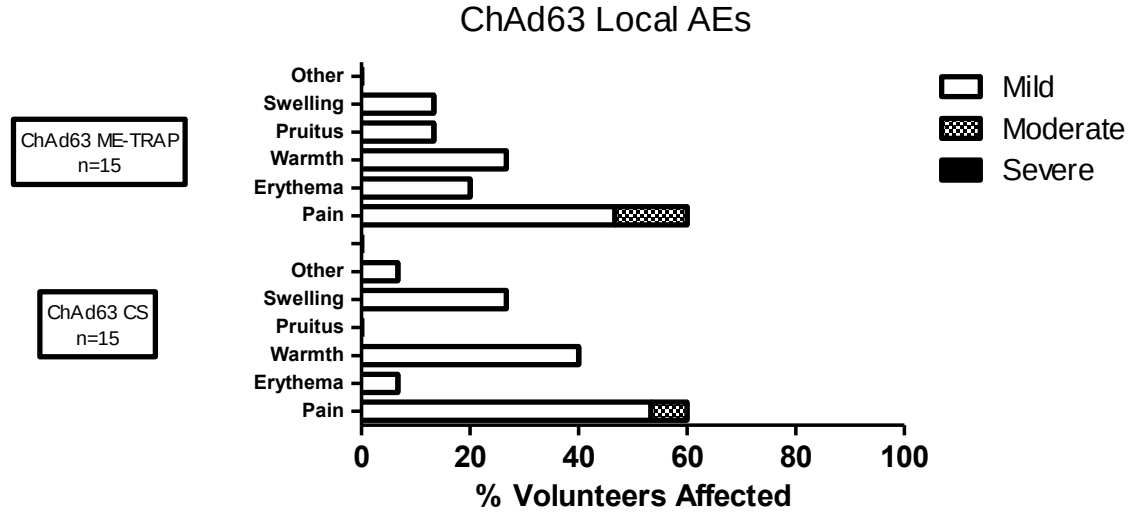


Figure 2: Local AEs after administration of ChAd63 CS (Group 1) and ChAd63 ME-TRAP (Group 2). The "Other" local AE in Group 1 was mild vaccine site paraesthesia. The highest intensity adverse event per subject is listed.

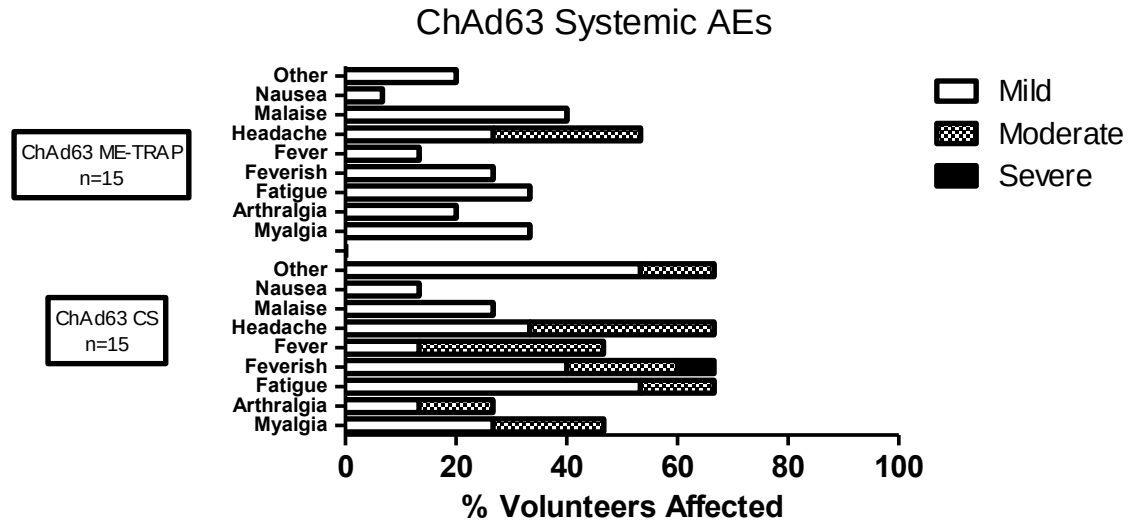


Figure 3: Systemic AEs after administration of ChAd63 CS (Group 1) and ChAd63 ME-TRAP (Group 2). "Other" systemic AEs in Group 1 were mild insomnia, diarrhoea, abdominal cramps, neck pain, upper back pain, leucopenia (3.3 x 10^9/l), elevated ALT (70 IU/l), and moderate abdominal pain and lymphopenia (0.91 x 10^9/l). "Other" systemic AEs in Group 2 were mild rash, pharyngitis and coryzal symptoms.

(c) Adverse Events Related to MVA vectored vaccines

The majority of local AEs post MVA vectored vaccines in this study were mild in severity and all resolved (Figure 4). The AE profile was similar for each vaccine and to the AE profile seen in other studies using these vaccines.

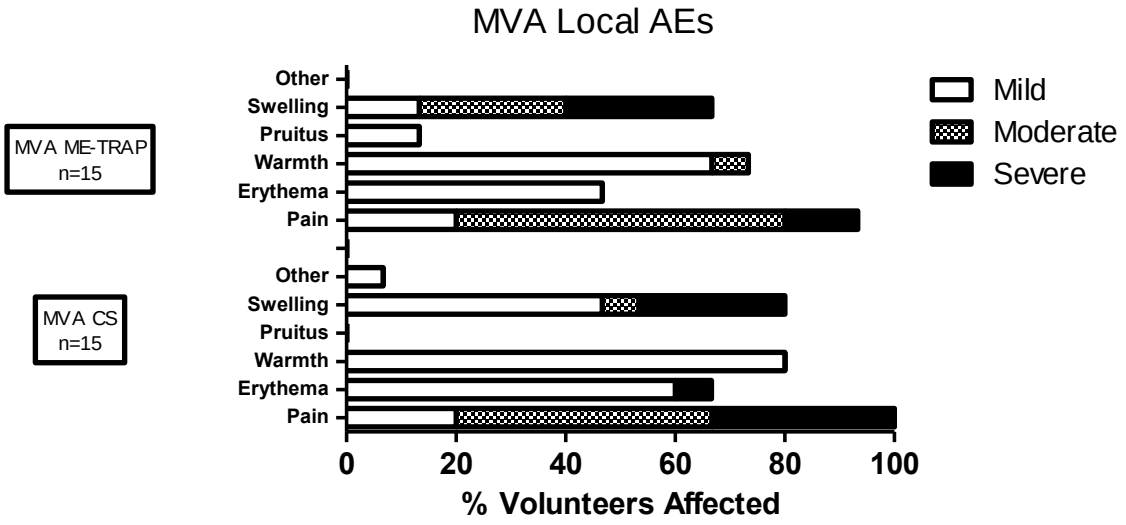


Figure 4: Local AEs after administration of MVA CS (Group 1) and MVA ME-TRAP (Group 2). The "Other" local AEs in Group 1 was mild vaccine site bruising.

The majority of systemic AES following MVA vaccination were mild in severity, and all resolved spontaneously (Figure 5).

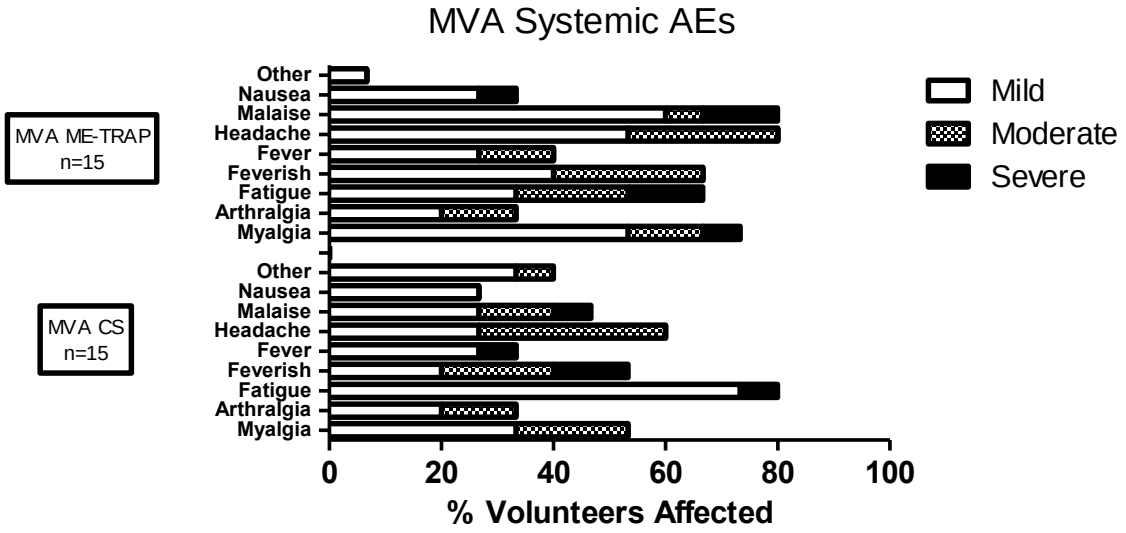
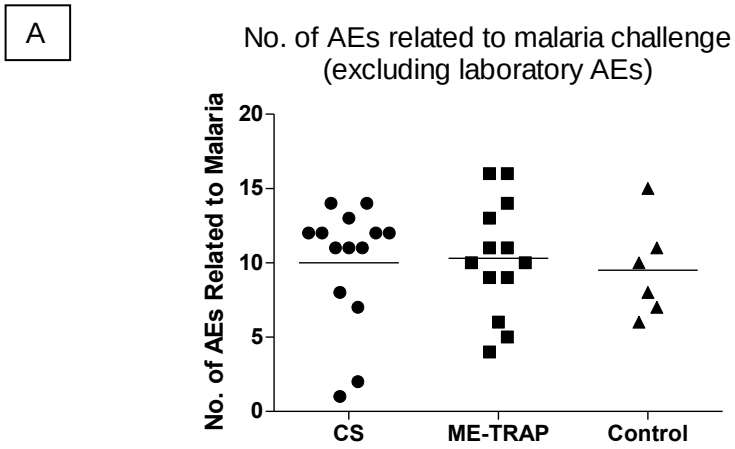


Figure 5: Systemic AEs after administration of MVA CS (Group 1) and MVA ME-TRAP (Group 2). "Other" systemic AEs in Group 1 were mild light headedness, disorientation, bruising at injection site, leucopenia (3.18 x 10^9/l) and episode of vasovagal syncope. The "Other" systemic AE in Group 2 was a mild rash.

(d) ChAd63-MVA safety of all regimens following sporozoite challenge

No unexpected clinical or laboratory AEs were observed in vaccinees post challenge. There was no difference between vaccinees and controls in the time that individuals were symptomatic prior to diagnosis (*p=0.43*) or the number of symptoms present at time of diagnosis (*p=0.65*) (Figure 6). Two of the 33 volunteers (6%) diagnosed with malaria post CHMI had no symptoms of malaria infection at diagnosis.

The total duration of symptoms in volunteers with symptomatic malaria infection ranged from 1-19 days (median 6 days) with no significant difference in duration of symptoms following CHMI between vaccinees and controls (*p=0.33*). Twenty-eight volunteers (84.8%) experienced at least one AE post challenge that was severe in intensity. One volunteer in Group 1 was admitted for in-patient management of vomiting secondary to anti-malarial therapy two days post malaria diagnosis, and was discharged the next day with no long term sequelae. Safety bloods taken at dC+9, dC+35, dC+90 and within 24 hours of diagnosis demonstrated transient abnormalities at frequencies and severities expected following *P. falciparum* infection



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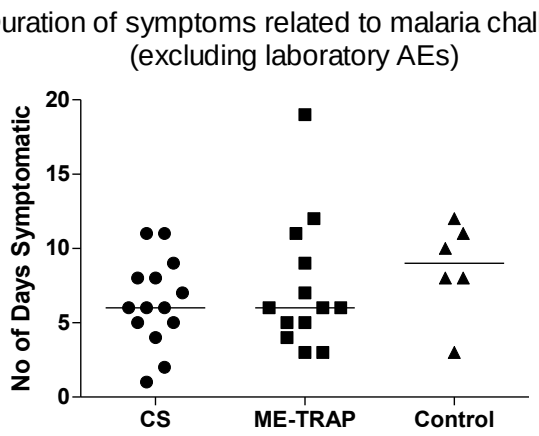

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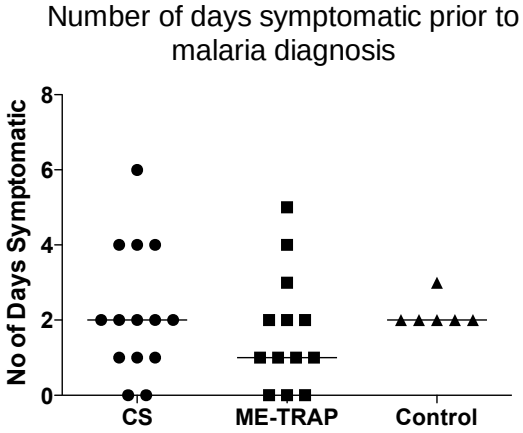

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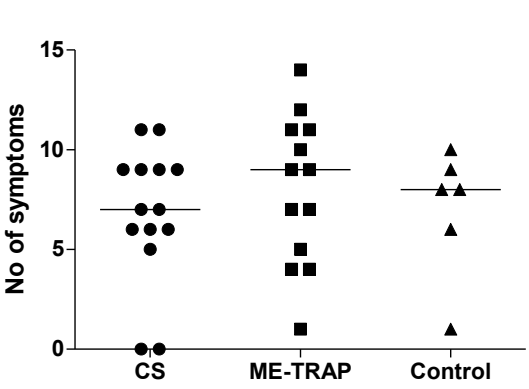


Figure 6: **(A)** Comparison of the number of AEs deemed possibly, probably or definitely related to malaria infection in individuals diagnosed with malaria in Group 1 (CS vaccinees, *n=14*) and Group 2 (ME-TRAP vaccinees, *n=13*) and unvaccinated controls (*n=6*). CS vaccinees: mean=10 AEs, median=11.5 AEs; ME-TRAP vaccinees: mean= 10.31 AEs, median=10 AEs: Controls: mean=9.5 AEs, median=9 AEs. Comparison across all 3 groups by Kruskal-Wallis test: *p=0.720*. Median value is represented by a straight line through each plot. **(B)** Comparison of the duration

of symptoms deemed possibly, probably or definitely related to malaria infection in individuals diagnosed with malaria in Group 1 (CS vaccinees, *n=14*) and Group 2 (ME-TRAP vaccinees, *n=13*) and unvaccinated controls (*n=6*). CS vaccinees: mean=6.36 days, median=6 days; ME-TRAP vaccinees: mean=7.39 days, median=6 days: Controls: mean=8.67 days, median=9 days. Comparison across all 3 groups by Kruskal-Wallis test: *p=0.333.* Median value is represented by a straight line through each plot. **(C)** Comparison of the number of symptomatic days before malaria diagnosis between vaccinees in Group 1 (CS) and Group 2 (ME-TRAP) who underwent CHMI and were diagnosed with malaria (*n=27*) and unvaccinated controls (*n=6*). CS vaccinees: mean=2.21 days, median=2.00 days; ME-TRAP vaccinees: mean=1.69 days, median=1 day: Controls: mean=2.17 days, median=2.00 days. Comparison across all 3 groups by Kruskal-Wallis test: *p=0.428* Median value is represented by a straight line through each plot. **(D)** Comparison of the number of symptoms at malaria diagnosis between vaccinees in Group 1 (CS) and Group 2 (ME-TRAP) who underwent CHMI and were diagnosed with malaria (*n=27*) and unvaccinated controls (*n=6*). CS vaccinees: mean=6.79 symptoms, median=7 symptoms; ME-TRAP vaccinees: mean=8 symptoms, median=9 symptoms: Controls: mean=7 symptoms, median=8 symptoms. Comparison across all 3 groups by Kruskal-Wallis test: *p=0.654* Median value is represented by a straight line through each plot.

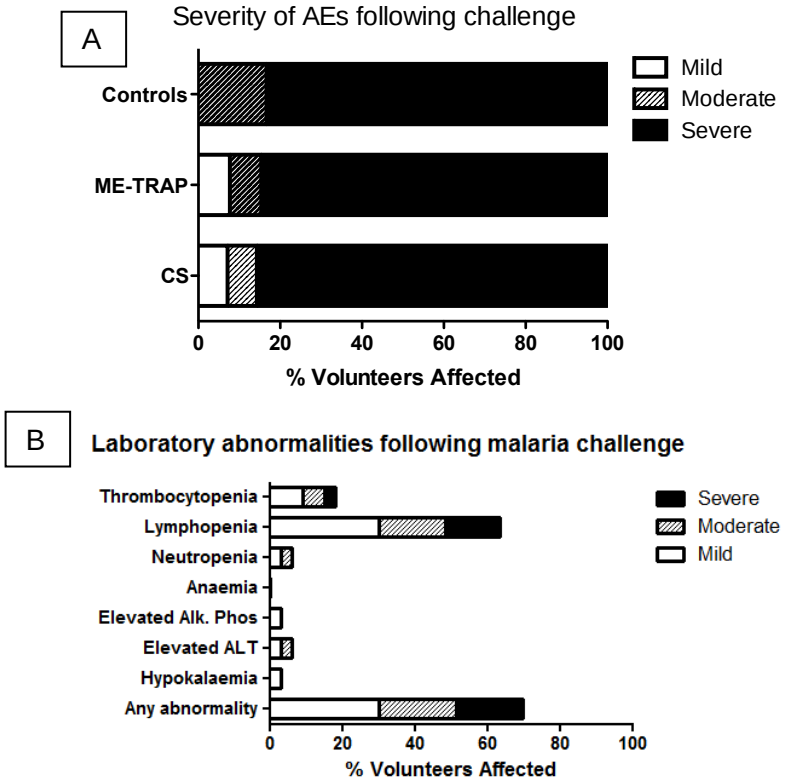


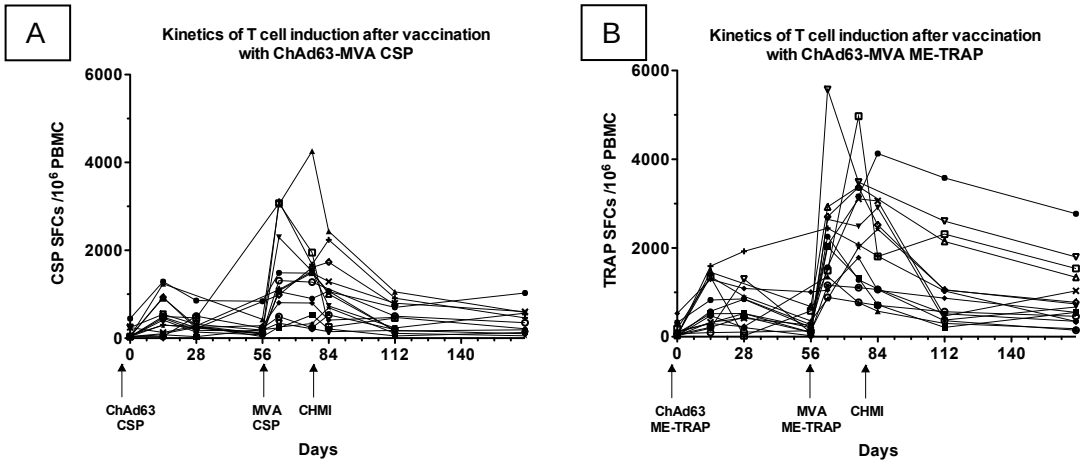
Figure 7: ***(A)*** *Comparison of maximum severity of any symptom of malaria infection between vaccinees diagnosed with malaria in Group 1 (CS vaccinees, n=14), Group 2 (ME-TRAP vaccinees, n=13) and unvaccinated controls (n=6).* ***(B)*** *Laboratory AEs after CHMI deemed possibly, probably or definitely related to Plasmodium falciparum infection. For "any laboratory abnormality" only the highest intensity AE per subject is counted.*

9.4 OTHER CLINICAL FINDINGS

There were no adverse events related to study interventions on-going at the end of the study.

9.5 IMMUNOLOGY

The *ex vivo* IFNγ ELISPOT was the primary readout for cellular immunogenicity in this trial.



Responses to priming and boosting vaccination as well as after CHMI are shown in Figure 8.

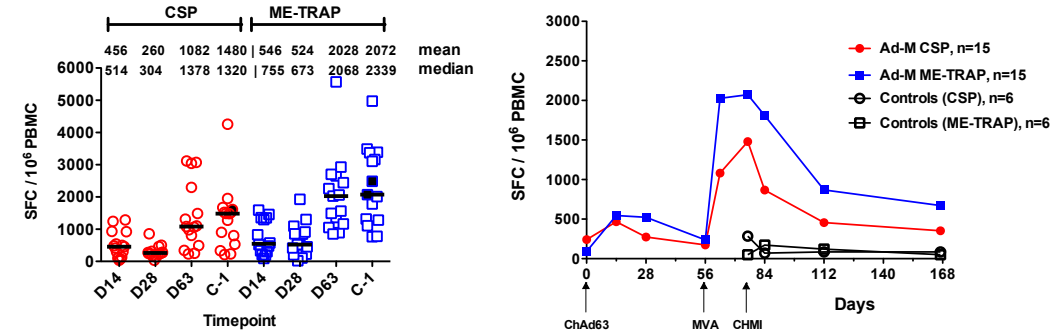


Figure 8: Ex-Vivo IFNγ ELISPOT responses to CS (A) and ME-TRAP (B) where each line represents individual volunteer. (C) Comparison of responses at key time points pre-CHMI, with mean & median values listed (D) Mean response plotted over the whole trial period.

Cellular immune responses peaked four weeks after administration of the ChAd63 vaccines (day 28) and three weeks after administration of the MVA vaccines (day 77 also C-1) in both groups. After immunisation, 13/14 (93%) vaccinees had a positive response to the priming vaccination at day 28, in both groups. Increases in ELISPOT response after priming were statistically significant for the ME-TRAP vaccinees when compared to pre-vaccination responses (day 0 *vs.* 28, $p<0.01$, Kruskal-Wallis test with Dunn's multiple comparison post-test). Responses to ChAd63 vector were stronger in the ME-TRAP group than the CS group (median at day 28 among ME-TRAP recipients 673 SFC per million PBMC compared with 304 SFC /10^6 PBMC for CS) (Figure 9).

After boosting with MVA, 100% of participants in the trial showed a positive cellular immune response by IFNγ ELISPOT. Increases were statistically significant for both groups compared to responses prior to MVA vaccination (day 56 *vs.* 77, p<0.01 for CS, p<0.001 for ME-TRAP, Kruskal-Wallis test with Dunn's multiple comparison post-test). Responses to the MVA vaccination were stronger in the ME-TRAP group than the CS group (median at day 77 among ME-TRAP recipients 2339 SFC per million PBMC compared with 1320 SFC /10^6 PBMC for CS). The reduced immunogenicity of the CS regime compared with ME-TRAP is most likely due to the difference in antigen size (334 amino acids for CS *vs.* 557 for TRAP).

Antigen-specific cytokine secretion was assessed at key time points with intracellular cytokine staining (ICS) using flow cytometry (graphs show responses from individual vaccinees, with the line representing group geometric mean).

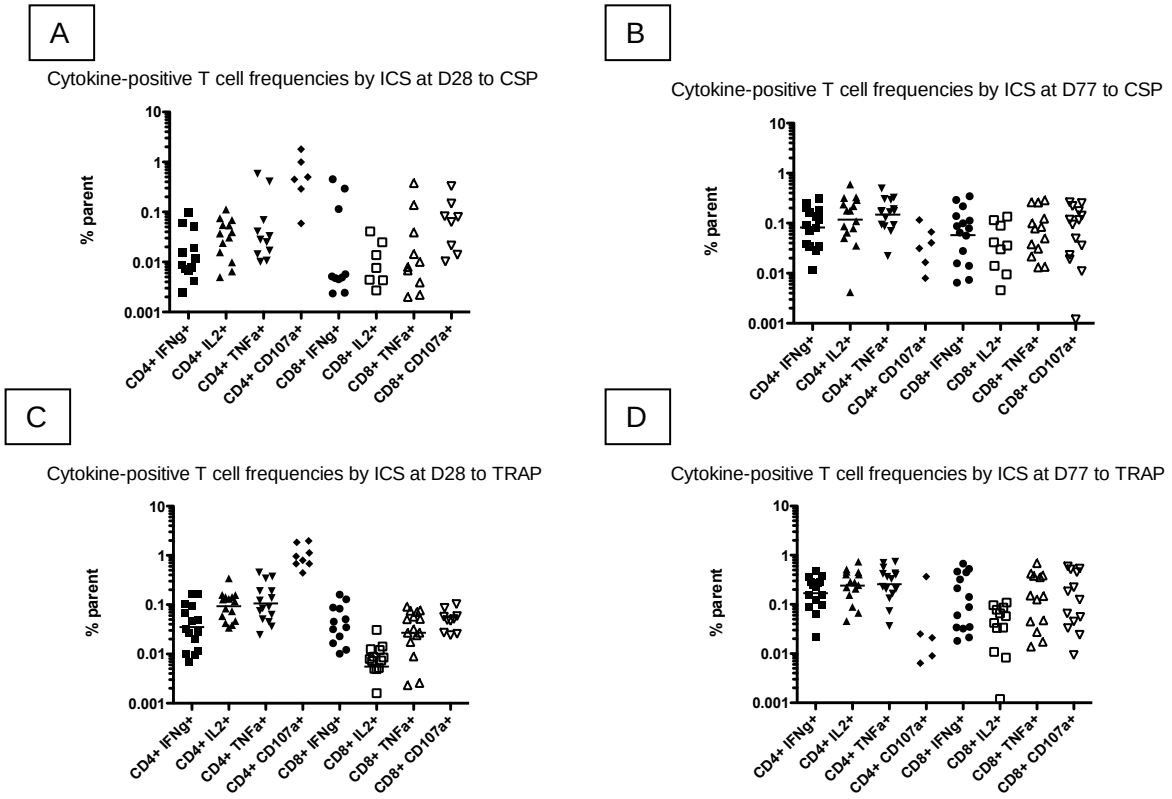


Figure 9: Antigen-specific cytokine secretion assessed at key time points with intracellular cytokine staining (ICS) using flow cytometry. (A) Responses to CS at D28. (B) Responses to CS at D77. (C) Responses to ME-TRAP at D28. (D) Responses to ME-TRAP at D77. Graphs show responses from individual vaccinees, with the line representing group geometric mean.

At D28, cytokine responses to CS were mostly detected from $CD4^+$ T cells, while after boosting an increase in cytokine secretion from $CD8^+$ T cells was noted at D77. Similar patterns were observed in responses to TRAP as measured by ICS, with an increase in the proportion of $CD8^+$ cytokine-secreting T cells after MVA vaccination.

Antibody responses to the NANP component of the CS antigen were quantified using an IgG ELISA against a peptide heptamer $NANP_6$. Responses were assessed in both groups as the ME component of ME-TRAP also contains this B cell-inducing epitope. Responses in the ME-TRAP group were very low compared to the CS group (figure 10a). Individual responses for each vaccinee are shown in figure 10b.

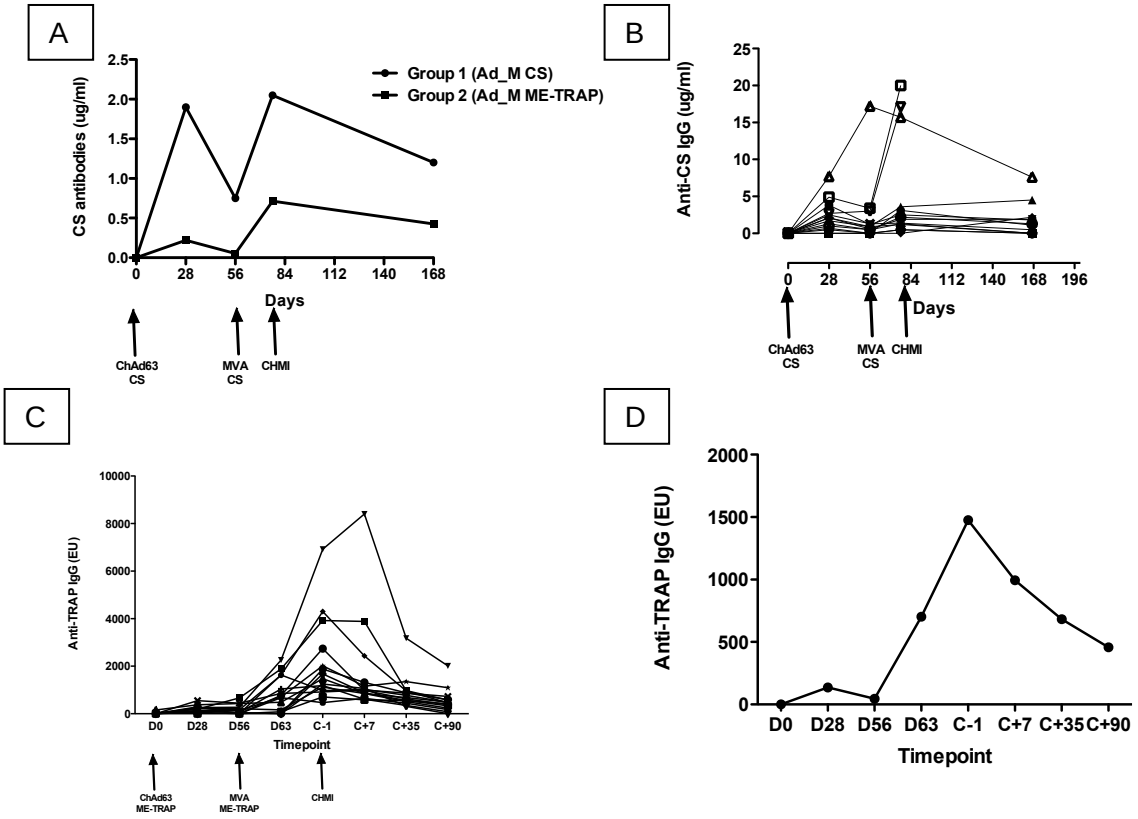


Figure 10: (A) Group median antibody responses against CS; (B) Individual anti-CS IgG responses, where each line represents an individual subject; (C) Individual anti-TRAP IgG responses for group 1 only; (D) Median anti-TRAP IgG responses for group 1 only

Antibodies to TRAP were measured using a standardised IgG ELISA against a recombinant TRAP protein. Responses were assessed only in ME-TRAP vaccinees; individual responses are shown in Figure 10c and the median for the group in figure 10d.
Vaccination with ChAd63 and MVA induced strong increases in IgG titres against both antigens.

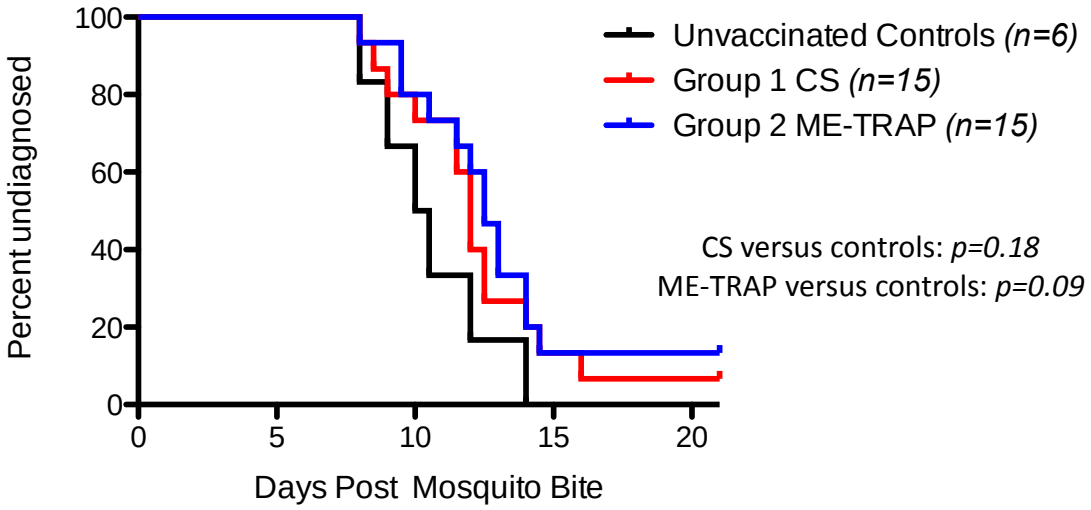
Substantial cellular and humoral immune responses were induced with this vaccination regime. Cytokine production from both $CD4^+$ and $CD8^+$ T cells was detected. Further analysis is underway to determine the role of these immune responses in protection against malaria challenge in this trial.

9.6 EFFICACY

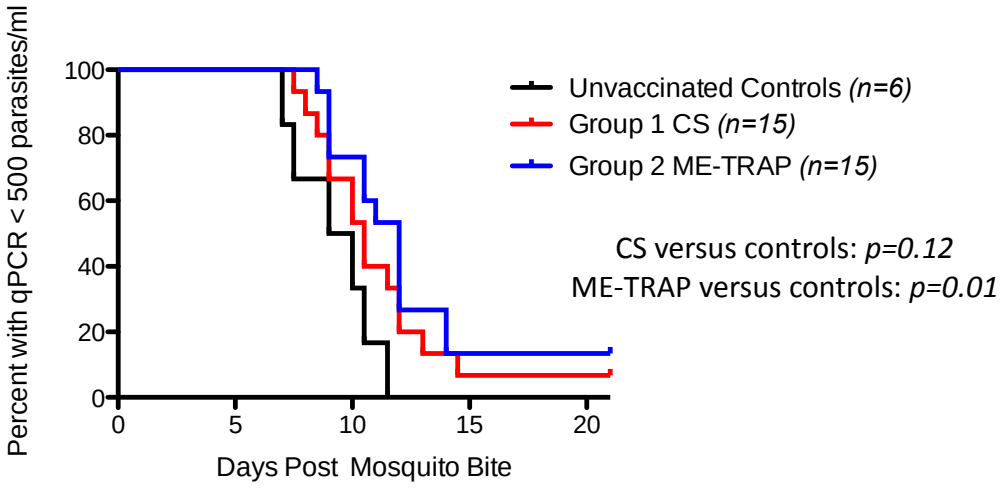
All volunteers in Groups 1 & 2 along with six unvaccinated control volunteers underwent sporozoite challenge 15-21 days after the MVA boost immunization. Following mosquito bite

CHMI, no unexpected AEs occurred. The infectivity controls (Group 3) and 27/30 vaccinees were diagnosed with malaria. One volunteer in Group 1 (ChAd63-MVA CS) and 2 volunteers in Group 2 (ChAd63-MVA ME-TRAP) were sterilely protected (Figure 11). The control volunteers (Group 3) were diagnosed after a median time of 10.3 days (range 8.0 – 14.0). There was no significant delay in time to diagnosis between unvaccinated controls and vaccinees assessed according to protocol specified end-point (treatment for malaria infection) (Figure 11a). However, on comparison of time to first sample post CHMI with either > 500 parasites/ml (Figure 11b) or > 20 parasites/ml (Figure 11c) a significant difference was seen between unvaccinated controls and vaccinees receiving ChAd63-MVA ME-TRAP (*p=0.01* and *p=0.02* respectively), but not between unvaccinated controls and vaccinees receiving ChAd63-MVA CS.

A



B



C

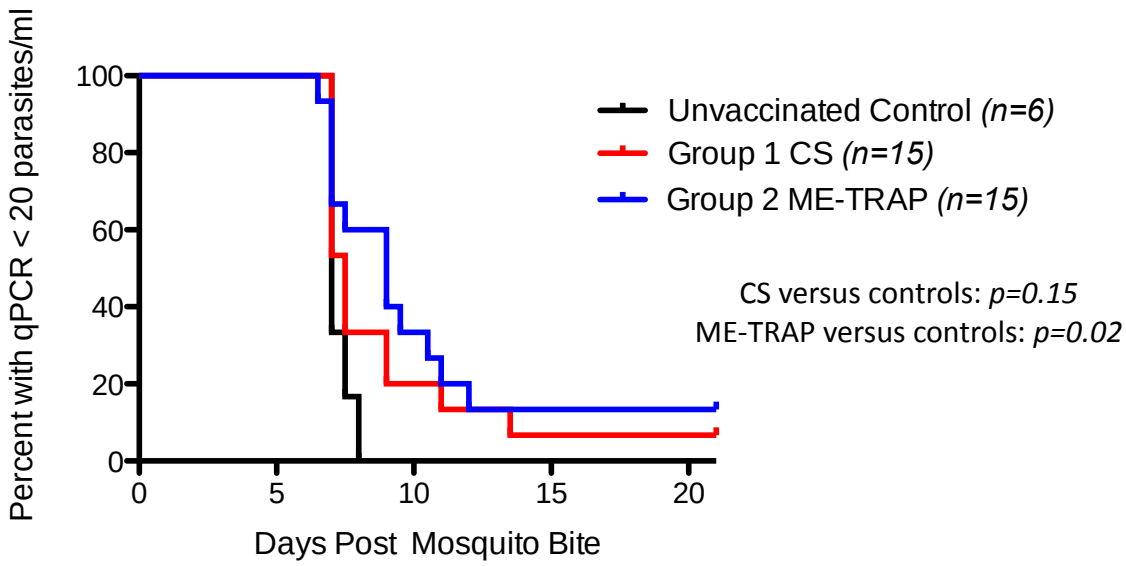
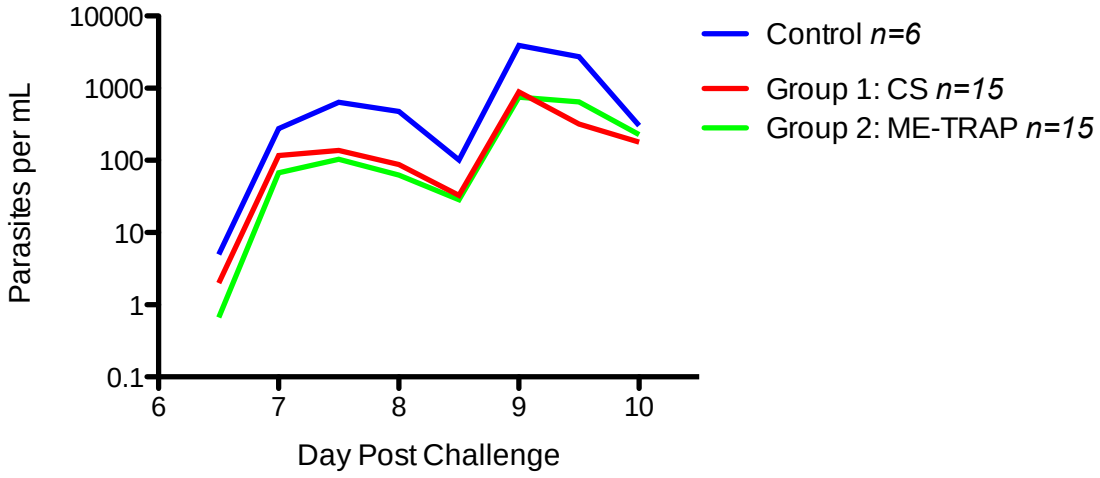


Figure 11: ***Efficacy of ChAd63-MVA CS & ChAd63-MVA ME-TRAP immunization following P. falciparum 3D7 strain sporozoite challenge.*** *Kaplan-Meier survival analysis of time to patent parasitemia in days. Log-Rank test P values for vaccinees versus unvaccinated control volunteers are shown.* ***(A)*** *Kaplan-Meier survival analysis of time to treatment in days. Median time = 12.0 days for Group 1 (CS), 12.5 days for Group 2 (ME-TRAP) and 10.25 days for unvaccinated controls.* ***(B)*** *Kaplan-Meier survival analysis of time to qPCR sample > 500 parasites/ml. treatment in days. Median time = 10.5 days for Group 1 (CS), 12.0 days for Group 2 (ME-TRAP) and 9.5 days for unvaccinated controls. (C) Kaplan-Meier survival analysis of time to qPCR sample > 20 parasites/ml. treatment in days. Median time = 7.5 days for Group 1 (CS), 9.0 days for Group 2 (ME-TRAP) and 7.0 days for unvaccinated controls.*



Group 1 (CS) versus Unvaccinated Controls; 79% Reduction; *p=0.09*
Group 2 (ME-TRAP) versus Unvaccinated Controls; 84% Reduction *p=0.02*

Figure 12. ***Comparison of Mean Parasite Density at 7.5 days post CHMI between Vaccinees and Unvaccinated Control Volunteers.*** *CS = circumsporozoite protein; ME-TRAP = multiple epitopes-thrombospondin related adhesion protein. Comparison of qPCR values at dC+7.5 between unvaccinated control volunteers and vaccinees (Mann Whitney test).*

10. CONCLUSIONS & DISCUSSION

Heterologous prime-boost with ChAd63-MVA expressing the malaria antigens CS and ME-TRAP administered intramuscularly is safe and immunogenic in healthy malaria naive adults, and both regimens demonstrated partial efficacy in this sporozoite challenge study.

All vaccinations in this study were well tolerated. A variety of local and systemic adverse events were observed following vaccination, however, the majority of these were mild in nature, and all adverse events considered related to vaccination resolved spontaneously. The MVA vectored vaccines in this study were seen to be more reactogenic than the ChAd63 vectored vaccines. This is consistent with observations in prior studies.

Strong cellular immune responses to both antigenic inserts were seen after prime boost immunisation with ChAd63-MVA. Greater CD8+ T-Cell responses were seen against ME-TRAP in group 2 than were seen against CS in group 1 both after priming with ChAd63 and boosting with MVA. Both regimens also induced IgG responses against CS (group 1) and ME-TRAP (group 2). This is likely due to the difference in antigen size (334 amino acids for CS, 557 for TRAP)

Partial efficacy was demonstrated in both vaccine groups with 1/15 volunteers (6.7 %) in group 1 exhibiting sterile protection to day 21 and 2/15 volunteers (13.3 %) in group 2 showing sterile protection to day 21. Furthermore, a significant delay in time to PCR parasitaemia at both endpoints of 500 parasites/ml and 20 parasites/ml ($p=0.01$ & $p= 0.02$ respectively) was observed for the ChAd63-MVA ME-TRAP vaccinees as compared to controls. This study does not provide any evidence that these vaccines reduce the duration of malaria symptoms after infection, or the number of symptoms at diagnosis.

The study has also provided further evidence that strong T cell responses following ChAd63-MVA vaccination against pre-erythrocytic and liver stage antigens of *P. falciparum* are not associated with adverse outcome when the subject is subsequently exposed to natural antigen.

The findings in this study are encouraging for future studies in which vectors encoding these antigens may be combined with other vaccines targeting different antigens, and different stages of the *P. falciparum* life cycle. The ChAd63-MVA viral vectored vaccine regimen also continues to provide a safe and clinically relevant strategy for further development of vaccines against other infectious diseases where strong multi-faceted immune responses are likely to be required for protection.