End Of Study Report

Title of study
A phase I/IIa sporozoite challenge trial to assess protection against malaria in healthy adults vaccinated with AdCh63 ME-TRAP alone, and as a heterologous boost with MVA ME-TRAP

Protocol ID
MAL034

Chief Investigator
Professor Adrian V.S. Hill

Sponsor
University of Oxford

Investigational Medicinal products
The candidate malaria vaccines, ChAd63 ME-TRAP and MVA ME-TRAP

Study design
A phase I/IIa open label challenge study in healthy adults aged 18-50 years

Study initiation date (first vaccination of first volunteer)
26/03/2009

Study completion date (last visit of the last volunteer)
28/02/2011

Date of this report
27/02/2012

Publication
Ewer et. al. (submitted).

This study was performed in compliance with Good Clinical Practice (GCP), including the archiving of essential documents.

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Protocol ID

MAL034

Study authors

Geraldine O'Hara, Christopher Duncan, Adrian Hill

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

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Dr Alison Lawrie (Clinical Project Manager)

Date: 27 Feb 2012

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Professor Adrian Hill (Chief Investigator)

Date: 27/2/2012

1. Abbreviations

ChAd63 (AdCh63)	Recombinant Chimpanzee Adenovirus 63
ChAd63 ME-TRAP	Recombinant Chimpanzee Adenovirus 63 encoding 'multiple epitopes and thrombospondin related adhesion protein'
AE	Adverse event
CCVTM	Centre for Clinical Vaccinology and Tropical Medicine
CBF	Clinical Bio manufacturing Facility
ELISPOT	Enzyme-linked immunospot
GCP	Good Clinical Practice
GMO	Genetically modified organism
GTAC	Gene Therapy Advisory Committee
IDT	Impfstoffwerk Dessau-Tornau
MHRA	Medicines and Healthcare products Regulatory Agency
MVA	Modified Vaccinia Virus Ankara
MVA ME-TRAP	Recombinant Modified Vaccinia Virus Ankara encoding 'multiple epitopes and thrombospondin related adhesion protein'
SAE	Serious adverse event
SUSAR	Suspected unexpected serious adverse reactions

2. Summary

Phase I clinical testing of immunisation with ChAd63 ME-TRAP alone, or followed by MVA ME-TRAP, began with the UK adult clinical trial, VAC033, in October 2007. Following preliminary safety and immunogenicity assessment and dose finding in VAC033, MAL034 was conducted in the UK as a Phase I/IIa adult clinical trial to evaluate the efficacy of these candidate vaccines against malaria infection in malaria-naïve adults, as well as to extend the safety and immunogenicity assessment of this approach. Following ethical and regulatory approvals, recruitment commenced in March 2009. The study was based at the CCVTM, Oxford, with volunteers attending the insectary facilities of Imperial College of Science, Technology and Medicine, London, to undergo *Plasmodium falciparum* malaria challenge by infected mosquito bite. The study was conducted in compliance with GCP. There were no protocol deviations that impacted significantly on the safety of the volunteers or on the scientific integrity of the trial. The objectives of the study were met.

Initial groups of volunteers received vaccination with ChAd63 ME-TRAP alone, or ChAd63 ME-TRAP / MVA ME-TRAP prime-boost immunisation, prior to undergoing malaria challenge alongside control volunteers. Protective efficacy was demonstrated in the prime-boost group. Following amendments to the study, further groups of volunteers were enrolled to determine whether the protective efficacy of the ChAd63 ME-TRAP / MVA ME-TRAP prime-boost regimen could be repeatedly demonstrated. Volunteers participating in the second challenge experiment did so after having received ChAd63 ME-TRAP / MVA ME-TRAP prime-boost vaccination, or novel multi-immunisation schedules with the ChAd63 ME-TRAP + MVA ME-TRAP Mixture Formulation. In this second malaria challenge experiment, protective efficacy was demonstrated amongst a second group of ChAd63 ME-TRAP / MVA ME-TRAP prime boost volunteers. Protective efficacy was not demonstrated amongst volunteers receiving ChAd63 ME-TRAP alone or the ChAd63 ME-TRAP + MVA ME-TRAP Mixture Formulation. In total, three prime-boost volunteers had sterile protection against malaria. These three volunteers underwent delayed rechallenge at eight months after initial vaccination and challenge: one was again sterilely protected, and two had a delay to parasitaemia, indicating durable efficacy of prime-boost vaccination.

Vaccinations were well tolerated. There were no serious adverse events related to vaccination. Adverse events related to vaccination were generally mild or moderate in grade, and all resolved. Local adverse events consisted mainly of injection site pain, swelling and erythema. In addition, injection site pruritus and scaling were commonly seen with MVA ME-TRAP, which was administered intradermally, though not with ChAd63 ME-TRAP or ChAd63 ME-TRAP + MVA ME-TRAP Mixture formulation, which were administered intramuscularly. Systemic adverse events related to vaccination generally consisted of flu-like symptoms such as pyrexia, arthralgia, myalgia, malaise, fatigue and headache.

Vaccinations generated potent TRAP-specific T cell responses, as measured by ELISPOT. MVA ME-TRAP boosting vaccination after ChAd63 ME-TRAP prime increased the magnitude and breadth (as measured by ELISPOT), and polyfunctionality (as measured by flow cytometry) of the T cell response. Cellular and antibody responses to prime-boost immunization were studied for association with delay to onset of blood stage infection following malaria challenge. The frequency of CD8-positive T cells secreting Interferon-gamma but not Interleukin-2 or Tumour Necrosis Factor-alpha correlated with efficacy.

The findings of this study support and inform the further development of vaccination strategies employing the candidate malaria vaccines, ChAd63 ME-TRAP and MVA ME-TRAP.

3. Protocol Synopsis

Objectives

Primary Objective: To assess if volunteers who received ChAd63 ME-TRAP alone, or as a heterologous boost with MVA ME-TRAP, or administered in a mixture formulation in homologous prime boost are protected wholly or partially against malaria infection in a sporozoite challenge model. This will be determined by noting the number of subjects who develop malaria infection and the time in hours between exposure and parasitaemia as detected by thick-film blood smear, and compared with controls.

Secondary Objective: To assess the safety of the immunisation regimes and to measure IFN-γ ELISPOT and antibody responses to the ME-TRAP antigen before and after malaria infection. If there is evidence of partial or complete protection by the vaccinations then we will explore immunological correlates of protective immunity.

Tertiary Objective: To assess long term efficacy of ChAd63 ME-TRAP and MVA ME-TRAP in a re-challenge of volunteers protected at initial malaria challenge

Study Groups

Group 1: 9 volunteers receiving ChAd63 ME-TRAP 5 x 10^{10} vp intramuscularly and 1 dose MVA ME-TRAP 2 x 10^{8} pfu intradermally 8 weeks later followed by sporozoite challenge 2 weeks later.

Group 2: 10 volunteers receiving single dose ChAd63 ME-TRAP at 5 x 10^{10} vp intramuscularly followed by sporozoite challenge 2 weeks later.

Group 3: 6 Non-vaccinated control volunteers for challenge of Groups 1 and 2

Group 4: 6 volunteers receiving priming vaccination with ChAd63 ME-TRAP 5 x 10^{10} vp intramuscularly, boosted with MVA ME-TRAP 2 x 10^{8} pfu intradermally at an interval of 8 weeks (range 6-12 weeks), followed by sporozoite challenge 11 weeks later (range 6-20 weeks).

Group 5: 6 volunteers receiving priming vaccination with ChAd63 ME-TRAP 5 x 10^{10} vp intramuscularly, boosted with MVA ME-TRAP 2 x 10^{8} pfu intradermally at an interval of 8 weeks (range 6-12 weeks), followed by sporozoite challenge 3 weeks later (range 2-5.5 weeks).

Group 6: 3 volunteers from with sterile protection from initial vaccination for rechallenge at approximately 6 months (range 2.5 – 10 months)

Group 7: 6 Non-vaccinated controls for challenge of Groups 4-6, 8-10.

Group 8: 4 volunteers receiving mixture formulation of ChAd63 ME-TRAP 5 x 10^{10} vp and MVA ME-TRAP 2 x 10^{8} pfu intramuscularly at enrolment, followed by homologous boosting with the same mixture formulation at 8 weeks (range 6-12 weeks) and again at a further 8 (range 6-12 weeks) weeks, followed by sporozoite challenge 3 weeks later (range 2-5.5 weeks).

Group 9: 4 volunteers receiving mixture formulation of ChAd63 ME-TRAP 5 x 10^{10} vp and MVA ME-TRAP 2 x 10^{8} pfu intramuscularly at enrolment, followed by homologous boosting with the same mixture formulation at 8 weeks (range 6-12 weeks), followed by sporozoite challenge 3 weeks later (range 2-5.5 weeks).

Group 10: 4 volunteers receiving mixture formulation of ChAd63 ME-TRAP 5 x 10^{10} vp and MVA ME-TRAP 2 x 10^{8} pfu intramuscularly at enrolment, followed by homologous boosting with the same mixture formulation at 4 weeks (range 2-6 weeks) and again at a further 4 (range 2-6 weeks) weeks, followed by sporozoite challenge 3 weeks later (range 2-5.5 weeks).

Investigational Medicinal Products Dose and Route

ChAd63 ME-TRAP: 5 x 10^{10} vp via intramuscular injection in the deltoid region of the arm

MVA ME-TRAP: 2 x 10^{8} pfu via intradermal or intramuscular injection in the deltoid region of the arm

4. Ethical and Regulatory Approvals

Initial approvals

EudraCT Number 2008-006804-46 was issued 16/10/2008.

The trial was registered with ClinicalTrials.gov, reference number NCT00890760.

Confirmation that the study can proceed as a Class I activity under the Genetically Modified (Contained Use) Regulations 2000 was given by the John Radcliffe Hospitals NHS Trust Genetic Modification Safety Committee on 20/11/2008. Reference number GM462.08.39.

Transfer of ethical application from Gene Therapy Advisory Committee to Oxfordshire Research Ethics Committee A was approved by the Chairman of GTAC on 05/12/2008. This occurred in light of the amendments to the Clinical Trials Regulations that came in to force in 2008 since the initial submission of the clinical trial for ethical approval.

Regulatory approval by the MHRA granted on 22/01/09.

Ethical approval by Oxfordshire REC A on 17/02/2009 subject to the provision of further information/clarifications. Favourable ethical opinion confirmed on 26/02/2009. The chairman of the ethics committee was Dr Brian Shine. The ethics committee study code is OXREC A 09/HO604/9.

Amendments

Substantial Amendment 1 and Non Substantial Amendment 1

Included in the initial ethical regulatory approval by OXREC A was a change to the study to use the intramuscular route of administration for the vaccine ChAd63 ME-TRAP. This change was submitted to the MHRA as Substantial Amendment 1, and approved by the MHRA on 06/03/09.

A non-substantial amendment was made on 17/3/09 to modify the GP Letter accordingly.

Substantial Amendment 2

Included in the initial regulatory approval by the MHRA, was a change to the study to change the first line antimalarial treatment from Chloroquine to Artemether-Lumefantrine. This change was submitted to OXREC A as Substantial Amendment 2, and approved by OXREC A on 11/03/09.

Substantial Amendment 3

In this amendment, approval was sought from the MHRA to list the Clinical Biomanufacturing Facility, rather than IDT, under point D8 “Site where the qualified person certifies batch release” on the CTA (Annex 1). This was to reflect where the certification of batch release for the IP MVA ME-TRAP would take place. Approval was granted by the MHRA on 03/04/09.

Substantial Amendment 4

Prior to this amendment, this trial (MAL034) had shown sterile protection against malaria sporozoite challenge in two prime-boost-vaccinated volunteers in Group 1. In Substantial Amendment 4, approvals were sought from OXREC A and MHRA to do the following:

- rechallenge of the two Group 1 volunteers who were protected at initial malaria challenge following prime-boost vaccination. These volunteers would now constitute Group 6 of the trial. The aim was to explore the durability of protection against malaria challenge following prime-boost vaccination.
- recruitment of six additional volunteers to act as controls for the rechallenge. These volunteers would constitute Group 7.
- recruitment of a further 12 volunteers (6 volunteers in Group 4, and 6 volunteers in Group 5) to receive the same vaccination strategy as those in Group 1. The aim was to increase the sample size and statistical power to try to confirm protective efficacy prior to entering larger Phase IIb studies.
- Group 6, 7, 4 and 5 volunteers would undergo malaria challenge concomitantly. For Group 4 volunteers, there was proposed an increase in the interval between final vaccination with MVA ME-TRAP and sporozoite challenge to 11 weeks to explore the effect this interval may have on the generation of T cell memory and its influence on levels of protection.
- Removal of seropositivity for antibodies to ChAd63 as an exclusion criterion for volunteers being screened for eligibility to participate in the trial as vaccinees.
- Addition of a safety and immunogenicity followup at Day 150 post challenge for all groups, to explore the generation of a T cell memory phenotype
- Use of a second lot, lot 0060109, of MVA ME-TRAP. Lot 0060109 was manufactured to GMP by IDT in Germany using the same process as previous lots of MVA ME-TRAP.

Ethical and regulatory approvals were granted on 29/07/09 and 23/07/09, respectively.

Substantial Amendment 5

Approvals were sought from OXREC A and MHRA to recruit 12 subjects to receive ChAd63 ME-TRAP in a mixture formulation with MVA ME-TRAP, administered intramuscularly. Ethical approval was granted on 14/08/2009. The amendment was rejected by the MHRA and resubmitted as a new amendment with additional supporting documents. Approval was granted by the MHRA on 29/09/09.

Non Substantial Amendment 2

A nonsubstantial amendment was made on 25/11/2009 to offer volunteers the choice of which arm to receive the mixture vaccination in. This was made so that the dominant arm was not used if that was the wish of volunteer, in order to reduce the impact of local reactogenicity on volunteers. This amendment was notified to MHRA and OXREC A on 07/12/09.

Non Substantial Amendment 3

Resubmission of "(ALL) Poster version 3.1 21-01-2009" to OXREC A on 07/12/09 to clarify the error in the submission letter to OXREC A dated 18/12/2009, which referred to this poster as "20090218 (ALL) Poster version v3.1".

Substantial Amendment 6

This amendment consisted of the update of the MVA ME-TRAP Investigator's Brochure to version 6.0. The updated version contained further safety data. The amendment was approved by OXREC A and MHRA on 22/12/09 and 15/12/09, respectively.

Substantial Amendment 7

This amendment consisted of the update of the ChAd63 ME-TRAP Investigator's Brochure to version 8.0. The updated version contained further safety data. The amendment was approved by OXREC A and MHRA on 22/12/09 and 15/12/09, respectively.

Substantial Amendment 8

Ethical and regulatory approvals were sought to change the bleed schedule post malaria challenge for the six unvaccinated control volunteers. This was to allow the exploration of T cell responses and cytokine profiles following blood stage malaria infection in naïve, unvaccinated individuals. An increase in the volume of blood per bleed, as well as additional bleeds were proposed. Approvals were granted by OXREC A and MHRA on 22/12/2009.

Non Substantial Amendment 4

Correction of a typographical error (omission of blood volume) on the VIS (Controls). The new document thus created is, "VIS (Controls)", v 5.1, 29/01/2010.

Non Substantial Amendment 5

The Investigator Brochure for the mixture formulation of ChAd63 ME-TRAP and MVA ME-TRAP was provided to the MHRA as part of the resubmission to MHRA of Substantial Amendment 5. The IB contained mainly further preclinical data which did not significantly affect the risk-benefit considerations for humans. As such, and further to discussion between Dr A Lawrie and OXREC A, this IB was provided to OXREC A as a nonsubstantial amendment (Non Substantial Amendment 5) on 18/09/2009 for the information of the committee.

Non Substantial Amendment 6

The IB for the mixture formulation of ChAd63 ME-TRAP and MVA ME-TRAP was updated (v2.0, 26/11/2010), and this was notified to OXREC A on 02/12/2010.

5. Investigators and Administrative Structure

Volunteers attended the Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), Churchill Hospital site, Old Road, Headington, Oxford, OX3 7LJ, UK, for study visits. The following staff, based at the CCVTM, worked on the trial, with the following roles:

- Clinical Research Fellows: Geraldine O'Hara, Stephen Aston, Matthew Hamill, Christopher Duncan, Anna Goodman, Susanne Sheehy, Joel Meyer, Patrick Lillie, Nick Anagnostou, Richard Antrobus.

- Research Nurses: Ian Poulton (Study Coordinator), Cynthia Bateman, Mary Smith.

- Project Manager: Alison Lawrie

- Programme Coordinator: Katherine Gantlett

- Volunteer Coordinator: Laura Dinsmore.

- Blood film microscopy (of volunteer blood samples post-malaria challenge): P Kalume, W Asava, S Correa, and K Konteh.

The Chief Investigator is Professor Adrian Hill.

The malaria challenge experiments were conducted at Infection and Immunity Section, Sir Alexander Fleming building, Imperial College of Science, Technology and Medicine, Imperial College Road, London SWY 2AZ. The experiments involved the participation and collaboration of the Division of Entomology, Walter Reed Army Institute of Research, USA. The following persons participated in the challenge experiments at the Imperial College site, with the following roles:

- Dissection: Simon Draper, Arturo Reyes, Andrew Blagborough, Robert Sinden
- Mosquito preparation: Jitta Murphy, Ken Baker
- Mosquito Support: J L Williams

Immunology investigations were done at the laboratories of the Jenner Institute at the Old Road Campus Research Building, University of Oxford, Old Road Campus, Oxford, adjacent to the Churchill Hospital site. The following staff undertook these analyses: Katie Ewer (Senior Immunologist), Carly Bliss, Fenella Halstead, Sean Elias, Katherine Collins.

Clinical laboratory tests on blood samples taken from volunteers were conducted at the clinical laboratories of the John Radcliffe Hospital, Oxford.

GCP compliance was externally monitored by Mrs C McKenna, of Appledown Clinical Research Ltd.

Dr Brian Angus, Honorary Consultant Physician and Director, Centre for Tropical Medicine at the University of Oxford, was the chair of the Local Safety Committee for MAL034. The Local Safety Committee had the role of reviewing SAEs deemed possibly, probably or definitely related to vaccination, and had the power to terminate the study if deemed necessary following a vaccine-related SAE.

6. Study Population, Study Groups

Screening of adult volunteers for eligibility to participate in the trial commenced on 09/03/2009 and ended on 21/01/2010. 76 volunteers from the Oxford area underwent screening. 58 eligible healthy adult volunteers were identified. 56 eligible volunteers were enrolled in to the study, into one of the groups listed in Tables 1 and 2 below.

Group	Number of volunteers enrolled	First vaccination (ChAd63 ME-TRAP)		Second Vaccination (MVA ME-TRAP)		Number of volunteers completing vaccinations	Number of volunteers undergoing malaria challenge	Number of volunteers completing follow up
		Dose	Route	Dose	Route			
1	9	5×10^{10} vp	IM	2×10^{8} pfu	ID	9	8	9
2	10	5×10^{10} vp	IM	-	-	10	10	10
3	6	No vaccination (control volunteers)					6	6
4	6	5×10^{10} vp	IM	2×10^{8} pfu	ID	6	6	6
5	6	5×10^{10} vp	IM	2×10^{8} pfu	ID	6	6	6
7	6	No vaccination (control volunteers)					6	6

Table 1. *MAL 034 Groups 1-5, 7. Volunteers underwent malaria challenge after completing the vaccination regimen or after no vaccination (controls). The time interval between vaccination with ChAd63 ME-TRAP and MVA ME-TRAP was eight weeks. One Group 1 volunteer completed study procedures to the Day 63 timepoint (seven days post – MVA ME-TRAP vaccination) but discontinued participation in the study (withdrawal of consent) prior to malaria challenge. The time intervals between final vaccination and malaria challenge were: Group 1: 20 days (n=3), 21 days (n=3), 12 days (n=1), 13 days (n=1); Group 2: 28 days (n=4), 29 days (n=4), 22 days (n=1), 23 days (n=1); Group 4: 85-92 days; Group 5: 21 days (n=1), 23 days (n=4), 24 days (n=1);*

Group	Number of volunteers receiving first dose of mixture vaccination*	Time interval to second vaccination	Number of volunteers receiving second dose of mixture vaccination*	Time interval to third vaccination	Number of volunteers receiving third dose of mixture vaccination*	Number of volunteers undergoing challenge	Number of volunteers completing follow up
8	5	8 weeks	4	8 weeks	4	4	5
9	4	8 weeks	4	-	-	4	4
10	4	4 weeks	4	4 weeks	4	3	4

Table 2. *MAL034 Groups 8-10. Volunteers underwent malaria challenge after completing the vaccination regimen. *Mixture vaccination consisted of intramuscular administration of mixture formulation of ChAd63 ME-TRAP 5×10^{10} vp and MVA ME-TRAP 2×10^{8} pfu. One volunteer was withdrawn from Group 8 following the first vaccination, when it was determined that there was a past history of a medical condition that constituted an exclusion criterion. One volunteer was withdrawn from Group 10 following the third vaccination due to withdrawal of consent. The time intervals between final vaccination and malaria challenge were: Group 8: 20-21 days; Group 9: 20-22 days; Group 10: 29-30 days.*

Three malaria challenge experiments were conducted. In the first malaria challenge experiment, conducted on 09/06/2010 – 10/06/2010, volunteers from Groups 1, 2 and 3 underwent challenge. Two volunteers from Group 1 were sterilely protected (malaria challenge at 21 and 20 days post MVA ME-TRAP), and these volunteers were entered in to Group 6 (Table 3, below). In the second malaria challenge, conducted on 02/03/2010 – 03/02/2010, volunteers from Groups 4,5,7,8,9,10 underwent challenge, and the two Group 6 volunteers underwent rechallenge. One volunteer from Group 5 was sterilely protected (malaria challenge at 21 days post-MVA ME-TRAP). This volunteer was entered in to Group 6, and underwent rechallenge on 30/09/2010 in a third malaria challenge experiment. This final malaria challenge experiment was conducted also for the malaria vaccine clinical trial, VAC039.

Group	Number of volunteers	Number of volunteers undergoing malaria rechallenge	Number of volunteers completing follow up
6	3	3	3

Table 3. *MAL034 Group 6. Three volunteers (two from Group 1 and one from Group 5) were sterilely protected at first challenge. These volunteers received no further vaccinations and were entered in to Group 6 and underwent rechallenge. The time intervals between final vaccination and rechallenge for these three volunteers were between 258-260 days.*

Inclusion and Exclusion Criteria

Volunteers were required to meet all of the inclusion criteria and none of the exclusion criteria to be eligible to enter in the study.

Inclusion criteria

- Healthy adult aged 18 to 50 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
- For females only: willingness to practise effective contraception throughout the study
- Agreement to refrain from blood donation during the course of the study
- Written informed consent

Exclusion criteria

- Participation in another research study involving an investigational product in the 30 days preceding enrolment, or planned use during the study period.
- Prior receipt of an investigational malaria vaccine encoding ME-TRAP or any other investigational vaccine likely to impact on interpretation of the trial data
- Administration of immunoglobulins and/or any blood products within the three months preceding the planned administration of the vaccine candidate

- Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days) immunosuppressant medication within the past 6 months (inhaled and topical steroids are allowed)
- Pregnancy, lactation or intention to become pregnant during the study
- Contraindication to both anti-malarial drugs (Riamet® and chloroquine)
    - concomitant use with other drugs known to cause QT-interval prolongation, (e.g. macrolides, quinolones, amiodarone etc)
- An estimated, ten year risk of fatal cardiovascular disease of ≥5%, as estimated by the Systematic Coronary Risk Evaluation (SCORE) system
- History of arrhythmia or prolonged QT interval;
- positive family history for sudden cardiac death
- History of allergic disease or reactions likely to be exacerbated by any component of the vaccine, e.g. egg products, Kathon.
- History of clinically significant contact dermatitis
- Any history of anaphylaxis in reaction to vaccination
- History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ)
- History of serious psychiatric condition
- Any other serious chronic illness requiring hospital specialist supervision
- Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 42 units every week
- Suspected or known injecting drug abuse
- Seropositive for hepatitis B surface antigen (HBsAg)
- Seropositive for hepatitis C virus (antibodies to HCV)
- Any other significant disease, disorder or finding, which, in the opinion of the Investigator, may either put the volunteer at risk because of participation in the study, or may influence the result of the study, or the volunteer's ability to participate in the study.
- History of clinical *P. falciparum* malaria
- Travel to a malaria endemic region during the study period or within the previous six months
- Any clinically significant abnormal finding on screening biochemistry or haematology blood tests or urinalysis
- Any other finding which in the opinion of the investigators would significantly increase the risk of having an adverse outcome from participating in the protocol or impair interpretation of the study data.

The following exclusion criterion was in place during recruitment of volunteers for Groups 1,2 and 3. This exclusion criterion was removed (in accordance with Substantial Amendment 4) prior to commencement of recruitment of volunteers for Groups 4, 5, 7, 8, 9 and 10.

- Seropositive for simian adenovirus 63 (antibodies to ChAd63) at a titre > 1: 200 (except control volunteers)

Volunteer Information and Consent

There were separate Patient Information Sheets (PIS) for Control volunteers, vaccinees undergoing malaria challenge for the first time, and vaccinees undergoing rechallenge. Revised versions of these documents were made according to the amendments to the study detailed above. Volunteers provided written informed consent to participate in the study prior to being screened for eligibility. The Consent Form underwent revisions according to the amendments to the study detailed above. Volunteers signed two copies of the consent form: one for the Investigators' records, and one for the volunteer to keep.

Protocol Deviations

Protocol deviations are listed below:

- Six volunteers had a single study visit (Group 5 Day 58, n=3; Group 10 Day 58, n=3) conducted by telephone, rather than at the study centre, due to adverse weather conditions. There were no scheduled blood tests for these visits.

- One Group 8 volunteer attended the D70 visit three days later than that permitted by the time window; the safety and immunology blood tests were done at this visit.

- One Group 10 volunteer did not attend the D2 visit, and the visit was conducted by telephone, 8 days late; there were no scheduled blood tests for these visits.

- Random plasma glucose testing was omitted at the screening visit for four volunteers.

- Three volunteers received a dose of MVA ME-TRAP (2 x 10^{8}pfu) mixed with ChAd63 ME-TRAP (5 x 10^{10} pfu) as prescribed by the MAL034 protocol, however the doses of MVA ME-TRAP were taken from vials allocated and labelled for the clinical trial, VAC033. The MVA ME-TRAP vaccine used in VAC033 was identical to that intended for use in MAL034 with identical IMP name, route of administration, dose concentration, volume, expiry date and lot number.

These protocol deviations caused no significant impact on the safety of the volunteers, nor on the scientific integrity of the study.

Vaccines

Vials of ChAd63 ME-TRAP batch 01 contained a concentration of 1.3 x 10^{11} vp/ml 10 mM Histidine, 35 mM NaCl. The dose of ChAd63 ME-TRAP used was 5 x 10^{10} vp, given intramuscularly in a volume of about 385µl.

MVA ME-TRAP batch 051204 was provided in vials of 300 μL volume at a concentration of 5×10^8 pfu/mL in 10 mM Tris buffer. The dose of MVA ME-TRAP used was 2×10^8 pfu, given intradermally in a volume of about 400 μL.

In accordance with Substantial Amendment 5, MVA ME-TRAP lot 0060109 was provided in vials of 200 μL volume at a concentration of 8.6×10^8 pfu/mL in 10 mM Tris buffer. The dose of MVA ME-TRAP used was 2×10^8 pfu, given intradermally or intramuscularly in a volume of about 233 μL.

Study vaccines were manufactured under Good Manufacturing Practice conditions by the Clinical Biomanufacturing Facility, University of Oxford (ChAd63), and IDT Biologika, Rosslau, Germany (MVA ME-TRAP).

7. Results

Efficacy

Volunteers underwent malaria sporozoite challenge by five infectious *A. stephensi* mosquito bites in a standard procedure as described in the clinical trial protocol. The TRAP antigen in the challenge strain (3D7) differs by 37 amino acids (approximately 6.5%) from the vaccine strain (T9/96), thereby constituting a heterologous challenge[1]. Figures 1-4 illustrate the results of the three challenge experiments. All control volunteers (Groups 3 and 7, Figures 1 and 2, respectively) developed patent malaria infection, as has been the case with all control volunteers in our malaria challenge studies to date. Sterile efficacy was seen in volunteers receiving ChAd63 ME-TRAP / MVA ME-TRAP prime-boost immunisation (Groups 1 and 5, Figures 1 and 2, respectively), except those undergoing delayed challenge after vaccination (Group 4, Figure 2). Sterile efficacy was not seen in volunteers receiving ChAd63 ME-TRAP alone (Group 2, Figure 1) or in volunteers receiving vaccination with the mixture formulation (Groups 8-10, Figures 2 and 3).

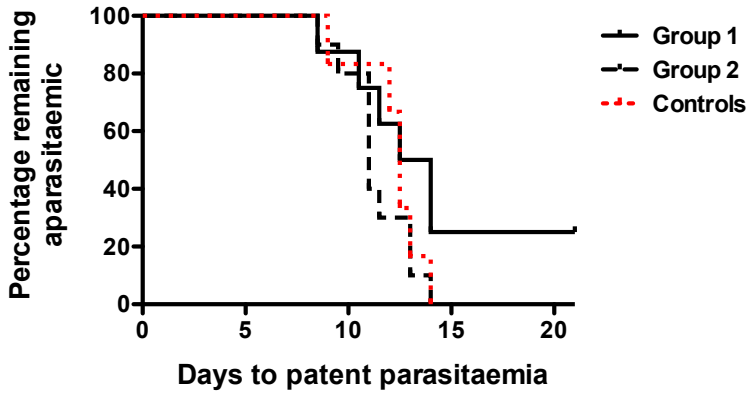


Figure 1: *First malaria challenge experiment: Kaplan-Meier survival curve of days to patent parasitaemia following malaria challenge. ChAd63 ME-TRAP alone did not protect any volunteers. Two ChAd63 ME-TRAP / MVA ME-TRAP prime-boost vaccinated volunteers in group 1 (25% of those challenged) were sterilely protected against malaria infection.*
Group 1: ChAd63 ME-TRAP 5 x 10^{10}vp boosted with MVA ME-TRAP 2 x 10^{8}pfu.
Group 2: ChAd63 ME-TRAP alone.
Group 3: controls

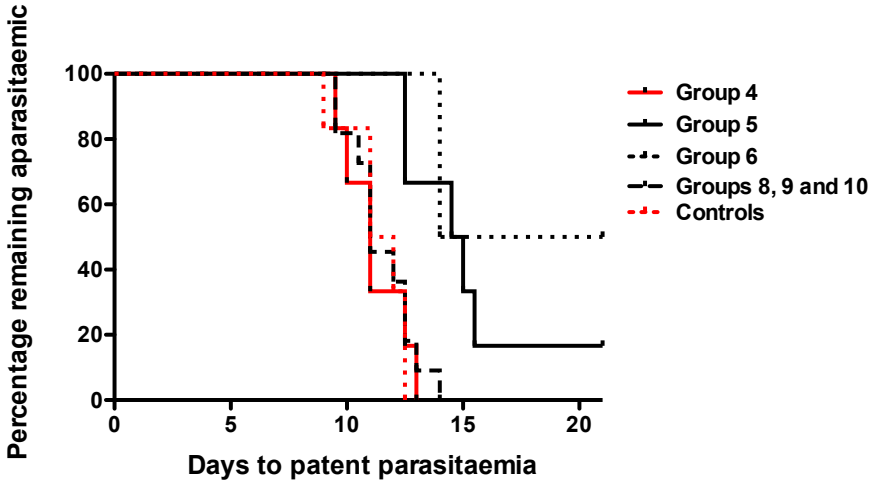


***Figure 2**: Second malaria challenge experiment: Kaplan-Meier curve of days to patent parasitaemia following malaria challenge. One of 6 ChAd63 ME-TRAP / MVA ME-TRAP prime-boost vaccinated volunteers in group 5 was sterilely protected against malaria infection. There was no delay to parasitaemia in Group 4 volunteers (delayed challenge after Ad-MVA prime boost immunization) or in volunteers receiving mixture regimens (Groups 8-10). One of the 2 volunteers from Group 1 protected at first challenge was again protected at rechallenge, and the other had a delay to patency (Group 6).*

Group 5: ChAd63 ME-TRAP 5 x 10^{10}vp boosted with MVA ME-TRAP 2 x 10^{8}pfu.
Group 4: ChAd63 ME-TRAP 5 x 10^{10}vp boosted with MVA ME-TRAP 2 x 10^{8}pfu, with a delayed challenge.
Groups 8,9 and 10 volunteers ("All Mix groups") are shown collectively.
Group 6 ("Rechallenged"): the two volunteers from Group 1 protected at first challenge who underwent rechallenge in this experiment.

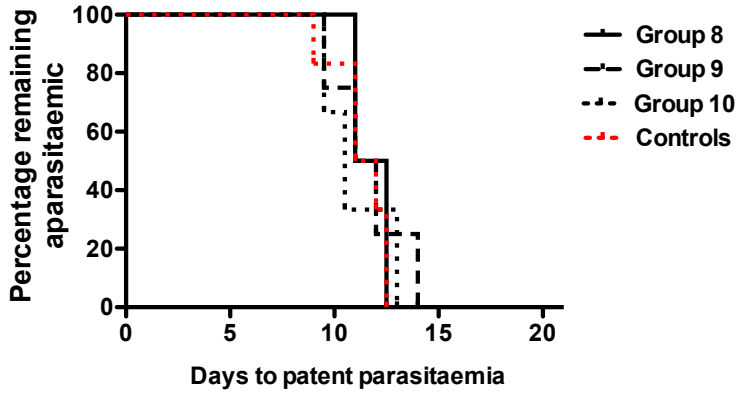


***Figure 3**: Second malaria challenge experiment: Kaplan-Meier curve of days to patent parasitaemia for Groups 8-10, shown individually. For those receiving the mixture formulation, neither the interval between vaccinations nor the number of vaccinations altered the efficacy of vaccination.*

There were no significant differences in time to patency between the first and second challenges for either ChAd63 ME-TRAP / MVA ME-TRAP prime-boost vaccinated volunteers (Group 1 vs Group 5) or control volunteers (Group 3 vs Group 7). Data was pooled from the first and second challenge experiments for the ChAd63 ME-TRAP / MVA ME-TRAP prime-boost vaccinated volunteers (Groups 1 and 5 - excludes Group 4 volunteers who underwent delayed challenge after vaccination) and the control volunteers (Groups 3 and 7).

Figure 4 illustrates the efficacy of vaccination in ChAd63 ME-TRAP / MVA ME-TRAP prime-boost vaccinated volunteers (Groups 1 and 5) compared to the control volunteers (Groups 3 and 7). Overall, ChAd63 ME-TRAP / MVA ME-TRAP prime-boost vaccination had an efficacy of 58% (8 of 14 volunteers having sterile protection or significantly delayed parasitaemia). Three of fourteen (21%) volunteers who underwent prime-boost vaccination were sterilely protected against malaria challenge- two volunteers from Group 1 and one volunteer from Group 5. In addition, a further 5 of 14 prime-boost vaccinated volunteers had a significant delay to onset of parasitaemia, at 14 or more days post challenge, a significant delay to patent parasitaemia indicating protective efficacy corresponding to a 96% reduction in liver parasite burden. Based on the 2.8 day difference in mean time to parasitaemia between the control volunteers (11.8 days) and the five delayed vaccinees (14.6 days), at a 12-fold parasite growth rate per 48 hours[2] here is a 27-fold reduction in parasite density emerging from the liver.

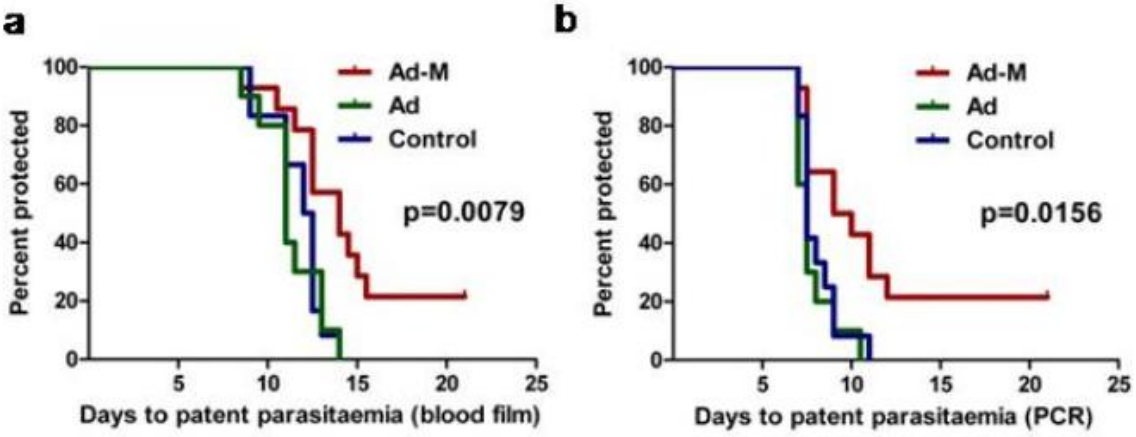


Figure 4: *MAL034: Kaplan-Meier log rank comparison of days to positive blood film (a) and positive PCR (>20 parasites/ml) (b). Kaplan-Meier survival analysis demonstrated significant delay in time to patent parasitaemia in the prime-boost vaccinees compared to the control group as measured by blood film microscopy (p = 0.008, log rank test,) or a real time quantitative PCR assay[3] (to > 20 parasites/ml, p = 0.016, log rank test).*

Ad-M (red line): the 14 volunteers in Groups 1 and 5 who underwent malaria challenge after prime boost vaccination with ChAd63 ME-TRAP and MVA ME-TRAP.
Ad (green line): the 10 volunteers who underwent malaria challenge after vaccination with ChAd63 ME-TRAP alone.
Control (blue line): the fourteen control volunteers in Groups 3 and 7.

Mean days to positivity by blood film: Ad-M 14.6 days (95% CI: 12.3-16.8); Ad 11.3 days (95% CI: 10.2-12.5); Controls 11.8 days (95% CI: 10.8-12.7). Mean days to positivity by PCR: Ad-M 11.6 days (95% CI: 8.5-14.7); Ad 7.8 days (95% CI: 7.0- 8.6); Controls 8.1 days (95% CI: 4-8.8).

At rechallenge of both sterilely- protected vaccinees from Group 1 (Figure 2), one volunteer was sterilely protected again, and one had delay in parasitaemia to day 14 when compared to the controls (p = 0.034, log rank test), indicating maintenance of protective immunity to eight months after last vaccination and sporozoite exposure. In the third malaria challenge experiment, undertaken also for the VAC039 study, the volunteer from MAL034 Group 5 who was sterilely protected at first malaria challenge underwent rechallenge. There was a delay in the time to parasitaemia (Figure 5), with a time to parasitaemia of 14.5 days, compared to a median of 11.5 days in the control volunteers.

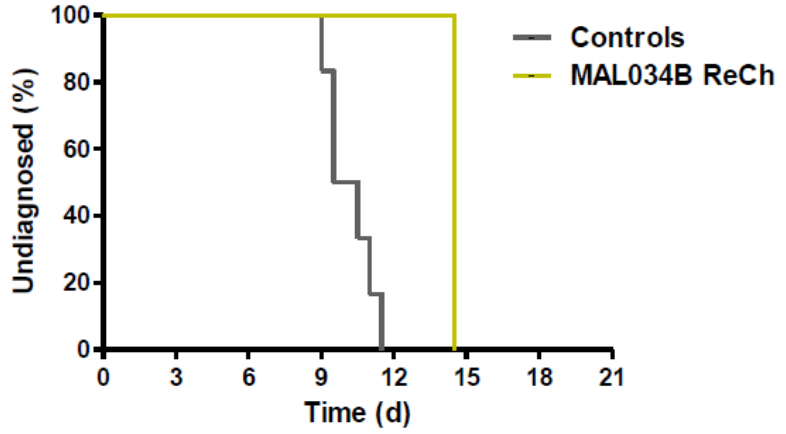


Figure 5: *Third malaria challenge experiment: Kaplan-Meier analysis of days to patent parasitaemia following malaria challenge.*
Grey line: control unvaccinated volunteers
Green line: single volunteer from Group 5 of MAL034 protected at first challenge, who underwent rechallenge in this experiment.

Immunogenicity

Cellular Immunogenicity: ELISPOT

For Group 1 and 5 prime-boost volunteers, T cell responses targeted predominantly the TRAP antigen rather than the ME string. We observed cellular responses to TRAP in 100% of these volunteers and multiple peptide pools (>3/6) were recognised in every case, both at the peak of the response and time of sporozoite challenge. T cell responses to the heterologous 3D7 challenge strain antigen were on average 73% of the response to the vaccine strain antigen.

Figures 6a – 6j illustrate the ELISPOT results for all volunteers.

Figure 6a: MAL 034 Group 1
(AdCh63 ME-TRAP / MVA ME-TRAP prime-boost)

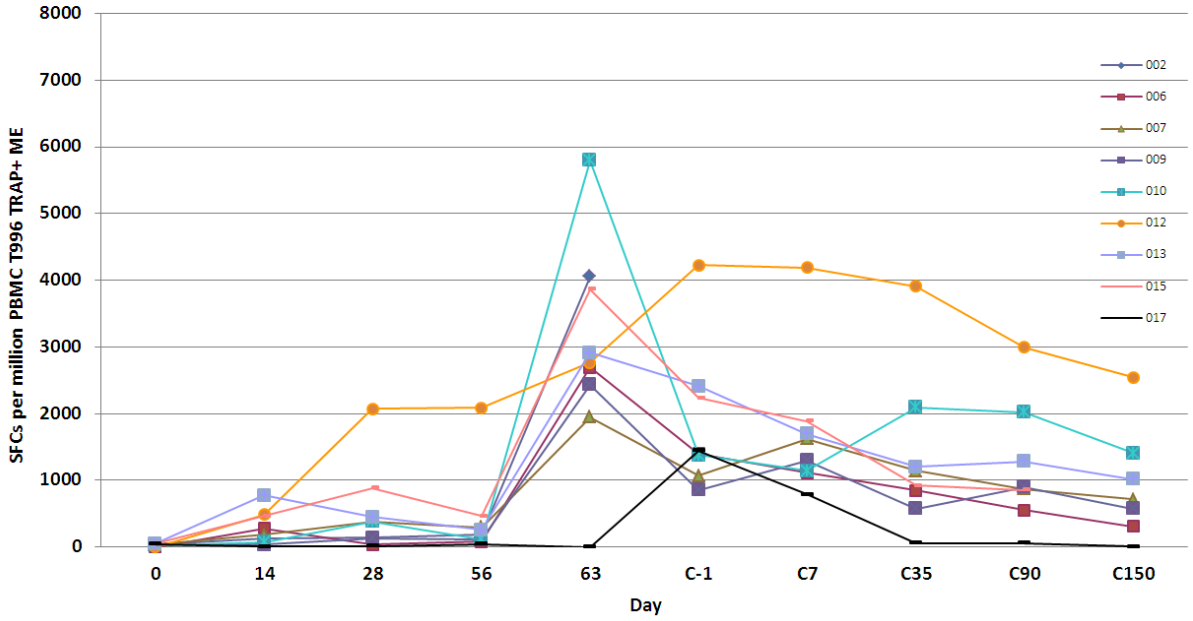


Figure 6b: MAL034 Group 5
(AdCh63 ME-TRAP / MVA ME-TRAP prime-boost

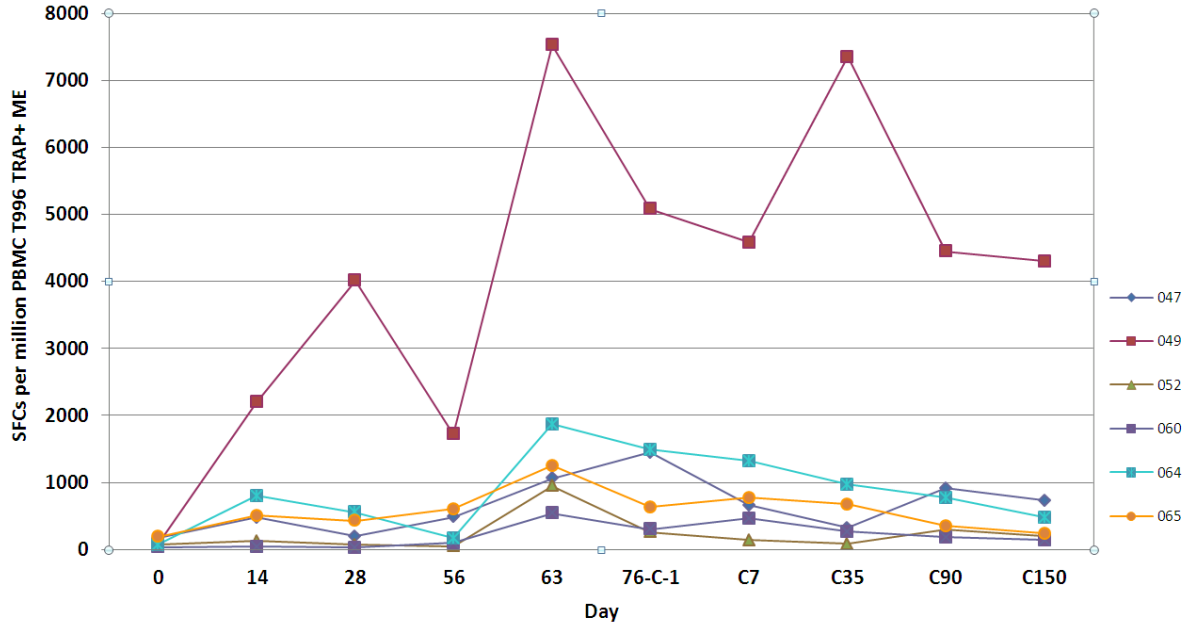


Figure 6c: MAL 034 Group 4
(AdCh63 ME-TRAP / MVA ME-TRAP prime boost

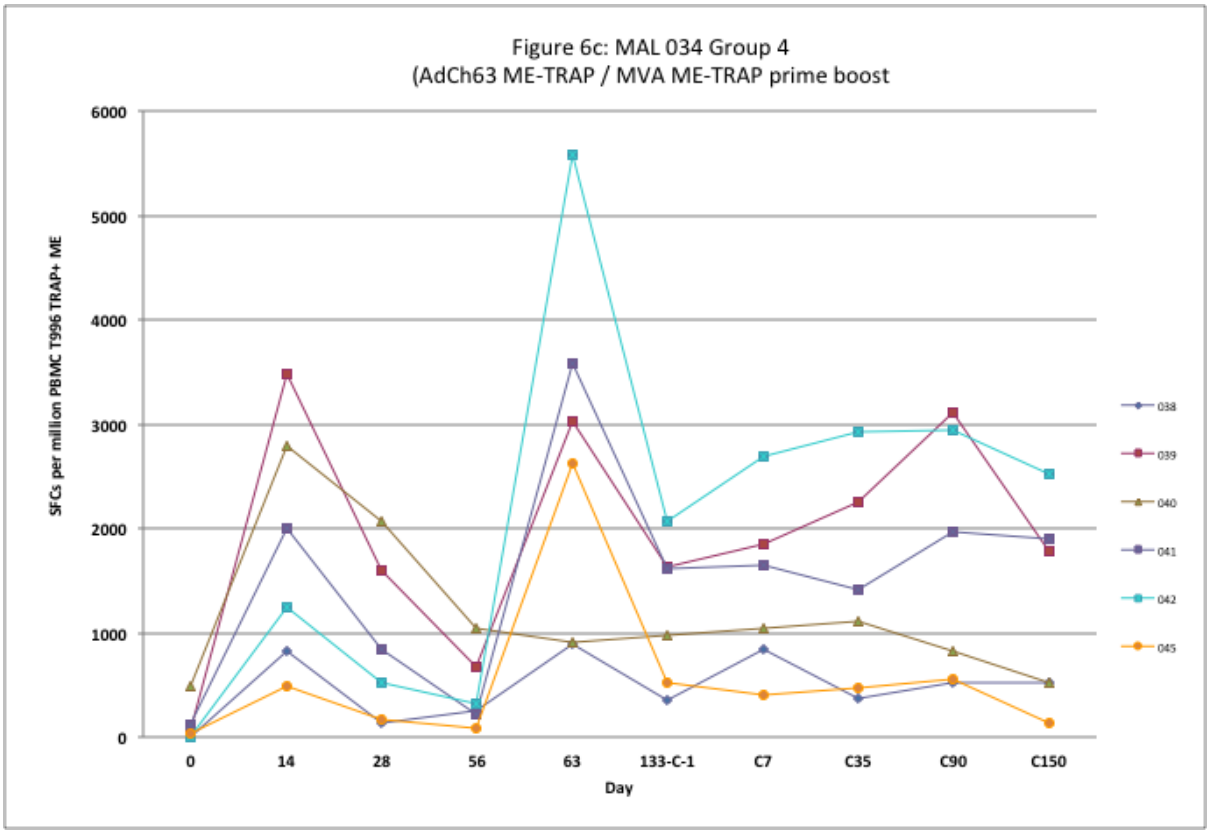
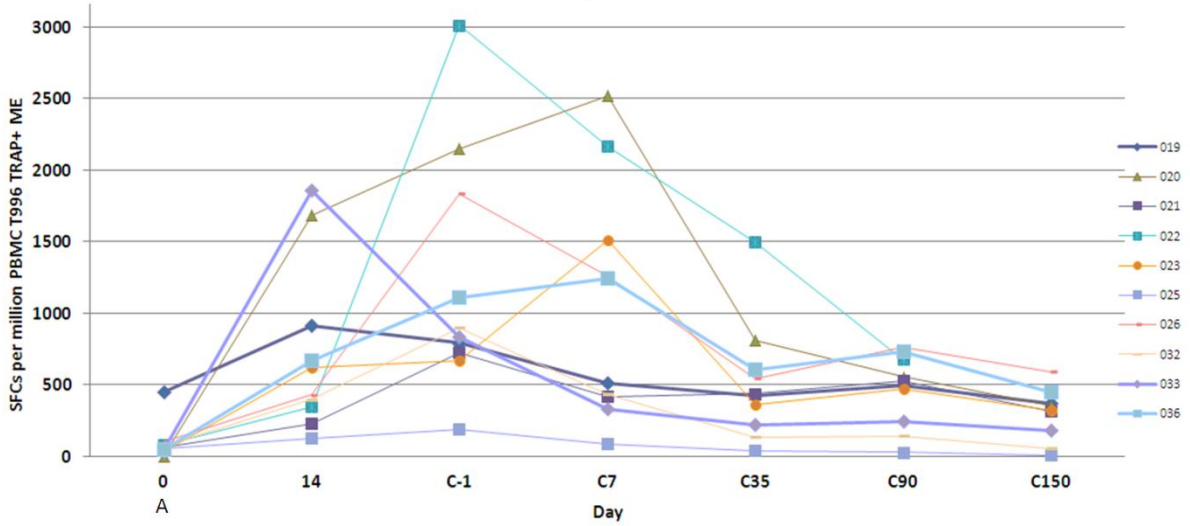


Figure 6d: MAL 034 Group 2
(AdCh63 ME-TRAP)



A

Fig 6e: MAL 034 Group 8
(vaccination with mixture formulation)

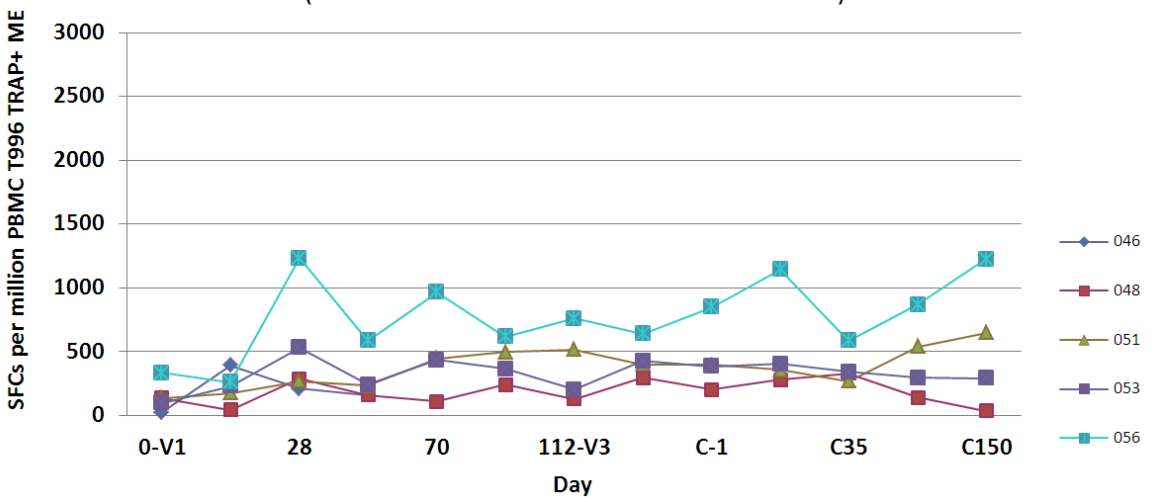


Fig 6f: MAL 034 Group 9
(vaccination with mixture formulation)

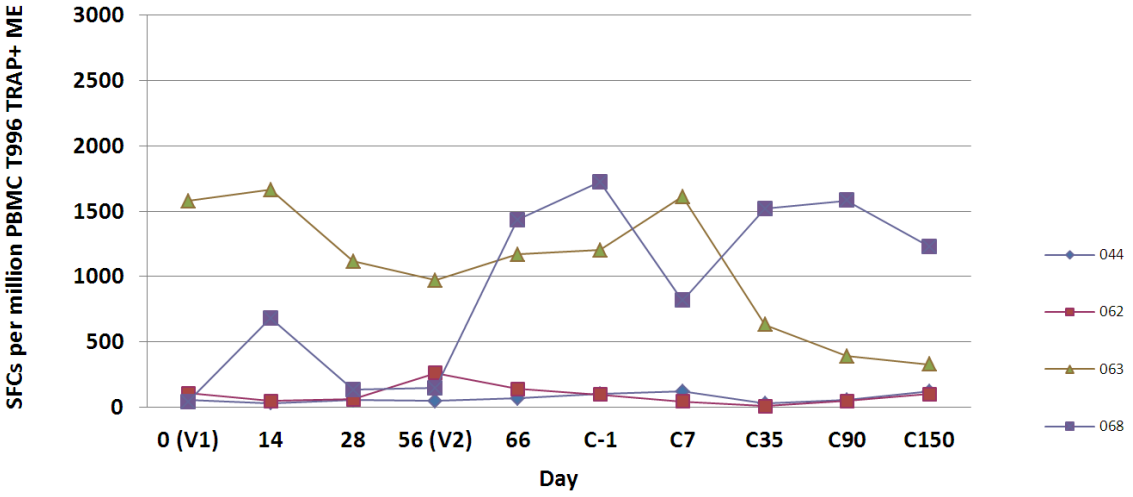


Fig 6g: MAL 034 Group 10
(vaccination with mixture formulation)

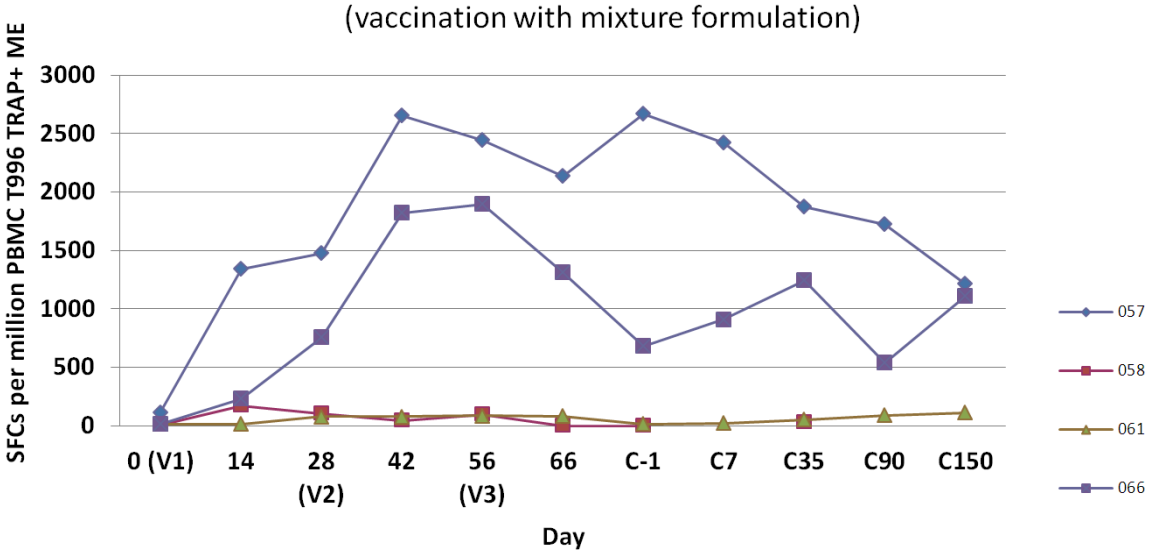


Fig 6h: MAL 034 Group 3 (controls)

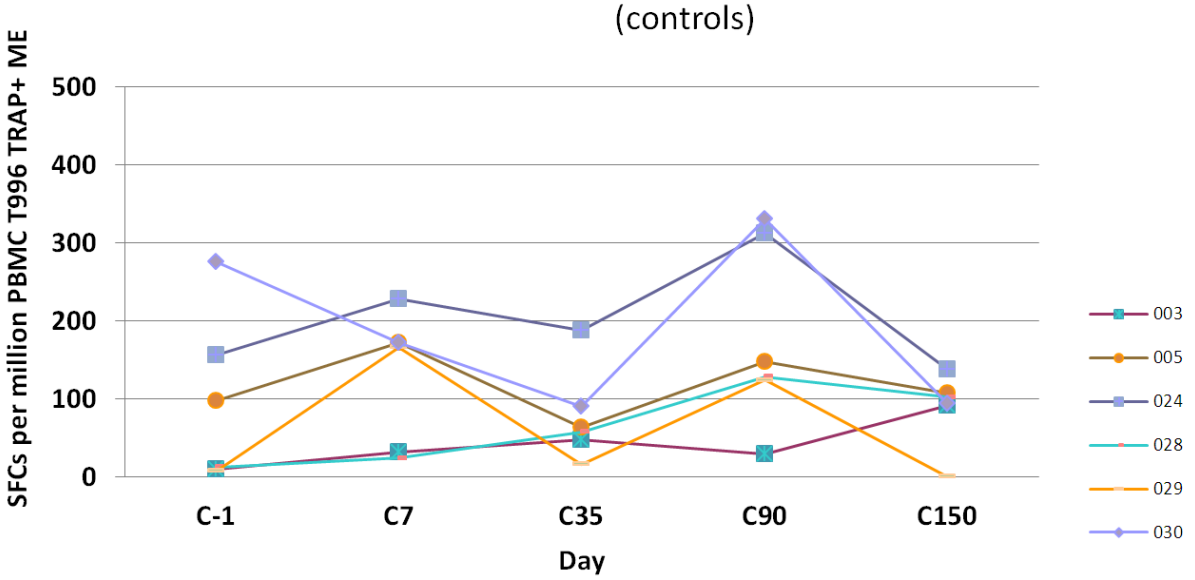
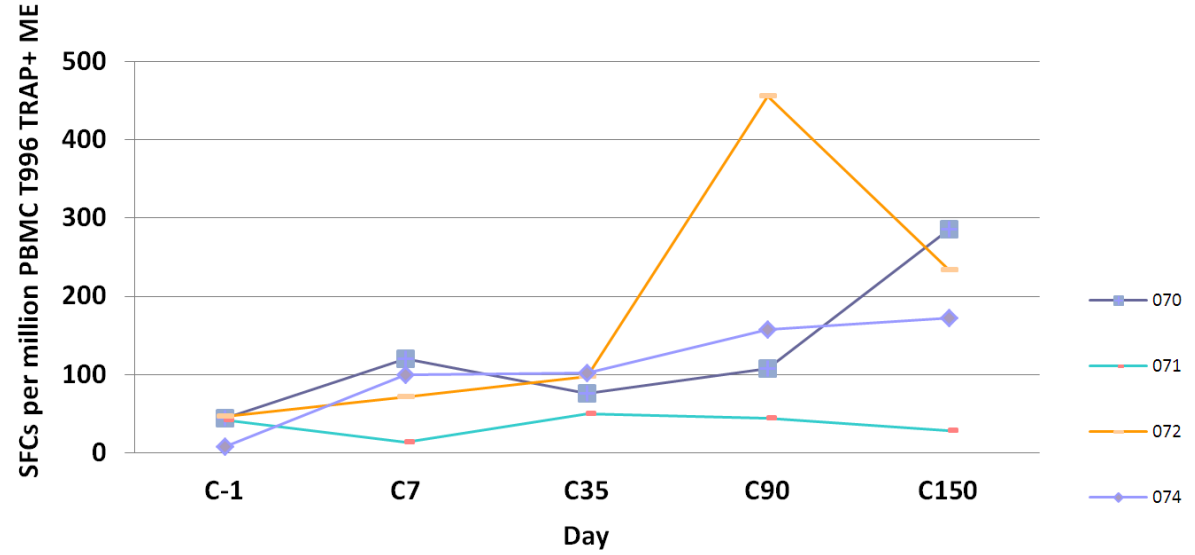


Fig 6i: MAL 034 Group 7 (controls)



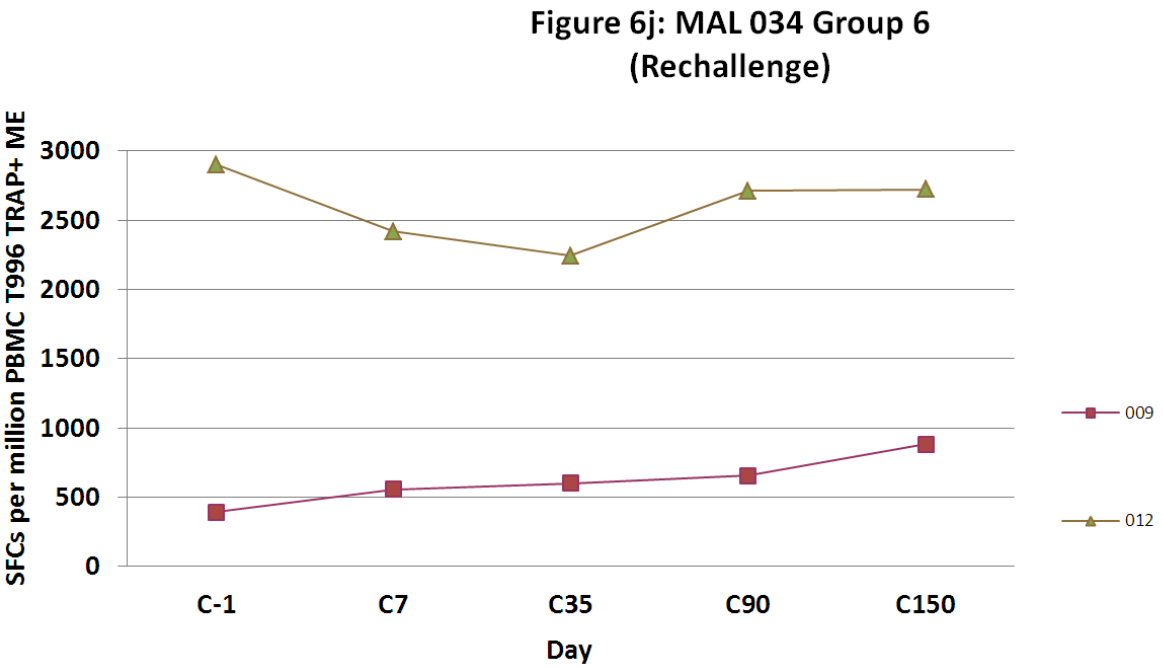


Figure 6: *ELISPOT results, MAL034. Figures 6a-6c: ELISPOT results for volunteers in Groups 1, 5, and 4, who received vaccination with ChAd63 ME-TRAP ("A") at Day 0 and vaccination with MVA ME-TRAP at Day 56 ("M"). Figure 6d: ELISPOT results for volunteers in Group 2, who received vaccination with ChAd63 ME-TRAP ("A") at Day 0. Figures 6e-6g: ELISPOT results for volunteers in Groups 8-10, who received vaccinations with the mixture formulation of ChAd63 ME-TRAP and MVA ME-TRAP ("V1"/"V2"/"V3") at the timepoints indicated. Figures 6h and 6i: ELISPOT results in control volunteers (study groups 3 and 7). "C" denotes the day of malaria challenge.*

Figure 6j: ELISPOT results at repeat malaria challenge ("C" denotes rechallenge) for volunteers from Group 1 with sterile efficacy at first malaria challenge, who were entered in to Group 6 and underwent rechallenge.

Figure 7 shows the median ELISPOT results for the fourteen prime-boost vaccinated volunteers from Groups 1 and 5 who underwent malaria challenge and the 10 Group 2 volunteers. Immune responses to ME-TRAP in these prime-boost vaccinated volunteers peaked one week after boosting at a median of 2436 (inter-quartile range [IQR] 1064-3862) spot-forming cells (SFC) per million peripheral blood mononuclear cells (PBMC) in an *ex-vivo* interferon-gamma enzyme-linked immunospot (ELISPOT) assay, compared with a median of 864 SFC/10^6 PBMC (IQR 710-1910) in the prime-only group (group 2) (ChAd63), p = 0.04. Boosting with MVA significantly increased the breadth of the response (p = 0.012 Mann Whitney U test, Figure 1b), and the magnitude of the ELISPOT response to TRAP after adenovirus priming was strongly associated with the subsequent response to MVA (r_s = 0.70, p = 0.005).

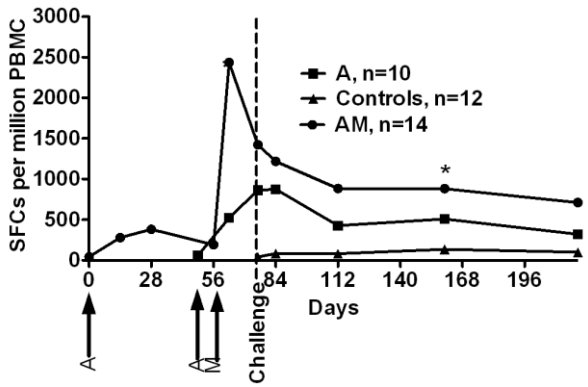


Figure 7: *MAL034: Median ME-TRAP IFN-gamma ELISPOT responses, in spot forming cells per million peripheral blood mononuclear cells).*
A: the ten Group 2 volunteers receiving single vaccination with ChAd63 Me-TRAP
AM: The fourteen prime-boost volunteers from Groups 1 and 5 who underwent malaria challenge.

Figure 8 demonstrates ELISPOT measurements for MAL-034 Group 1,2,5 vaccinees on the day prior to challenge.

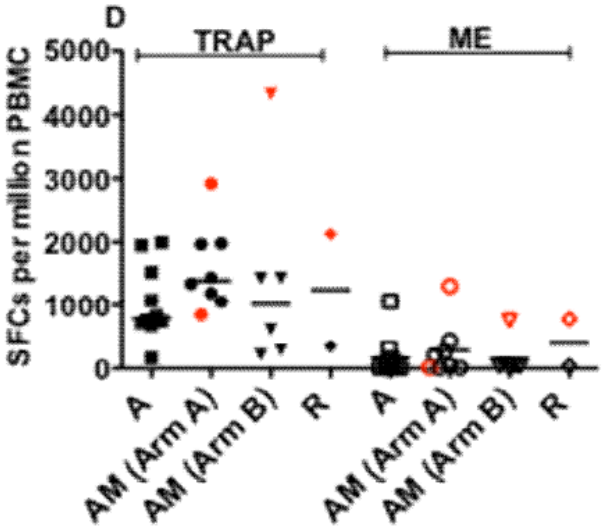


Figure 8. *MAL034 Immunogenicity: Individual responses, by ELISPOT, to TRAP and ME at time of sporozoite challenge. "AM (Arm A)" refers to the eight prime-boost volunteers in Group 1 who underwent malaria challenge in the first challenge experiment. "AM (Arm B)" refers to the six prime-boost volunteers in Group 5 who underwent malaria challenge in the second challenge experiment. "A" refers to the ten volunteers in Group 2 who received ChAd63 ME-TRAP only and underwent challenge. "R" refers to the two volunteers from Group 1 with sterile protection on first malaria challenge, who underwent rechallenge. Volunteers with sterile protection against malaria are shown in red.*

Follow-up of Group 1 vaccinees to day 150 post-challenge showed good maintenance of effector T cell frequencies with responses 712 SFC/10^6 PBMC (IQR 310-1412) representing 50% of the response at the time of challenge.

Cellular Immunogenicity: Flow Cytometry

Analysis of responses by flow cytometry showed that vaccination induced high frequencies of antigen-specific $CD4^+$ and $CD8^+$ T cells, containing IFNγ, interleukin-2 (IL-2), tumour necrosis factor-alpha (TNFα) or displaying CD107a, a marker of the capacity of T cells for cytotoxic degranulation. T cells induced by the ChAd63 ME-TRAP / MVA ME-TRAP prime-boost immunisation were more polyfunctional than those induced by vaccination with ChAd63 ME-TRAP alone. The median frequency of polyfunctional $CD4^+$ T cells containing IFNγ, IL-2 or TNFα simultaneously at the time of challenge for Group 1 and 5 volunteers was 0.1% of antigen-specific $CD4^+$ T cells (IQR 0.02-0.24) compared with 0.04% (IQR 0.02-0.25) for Group 2. Frequencies of polyfunctional $CD8^+$ T cells were also higher in these Group 1 and 5 volunteers (median 0.03%, IQR 0-0.24) compared with 0.01% (IQR 0-0.03) in Group 2. However, the total percentage of $CD8^+$ T cells containing IFNγ in Group 1 and 5 volunteers was much higher (median 0.12%, [IQR 0.05-0.7] mean 0.36% [SEM 0.12]) representing exceptionally strong immunogenicity.

Antivector Immunity

For Group 1, 2 and 5 volunteers, neutralising antibody titres to the adenovirus vector measured pre-vaccination were generally low as expected for a simian adenovirus (median titre 74, IQR 0-168) and these antibody levels did not correlate negatively with the magnitude of vaccine induced T cell or antibody responses to TRAP, but a trend (r_s = 0.79 p = 0.057) toward higher induced peak T cell responses to the insert was noted in those with antibodies to the vector (Figure 9).

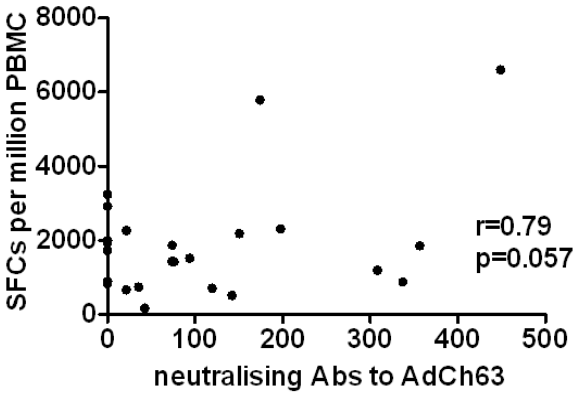


Figure 9. *MAL034: Spearman's correlation of pre-existing antibodies to ChAd63 with peak vaccine-induced ELISPOT responses to TRAP.*

Following the removal of the exclusion criterion of nAb titre >200 for vaccinated volunteers, we observed no attenuation of immunogenicity or increase in reactogenicity (data not shown). There was no correlation between neutralising antibody titre and time to patency, r_s=0.134, p=0.53 (figure 10).

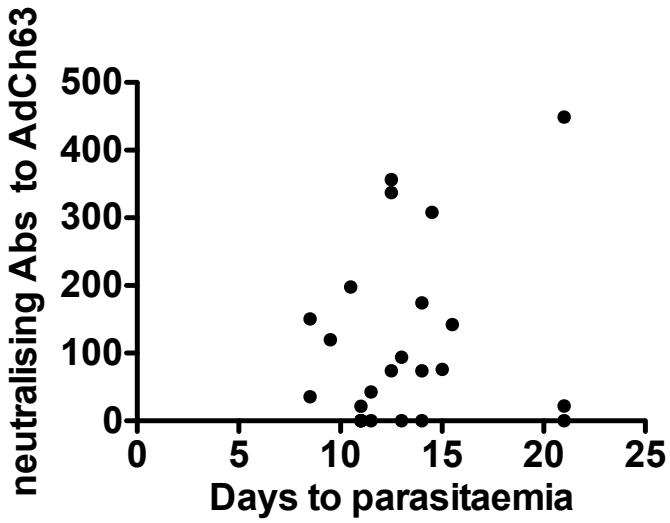
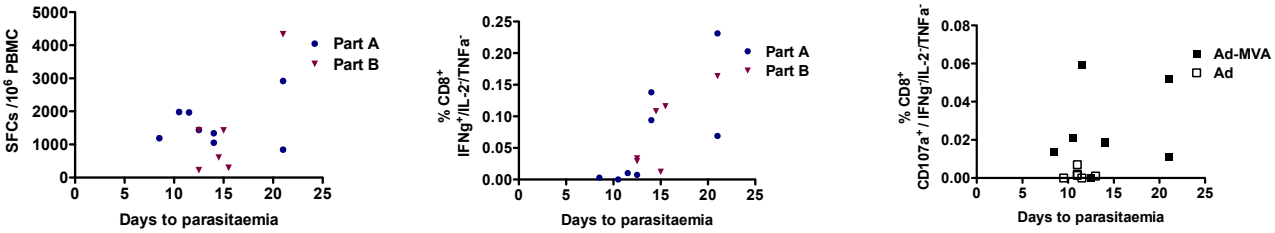


***Figure 10**. Effect of anti-vector antibody titre on vaccine efficacy. Lack of correlation between neutralizing antibodies to the ChAd63 vector and days to patent parasitaemia, Spearman's $r_s = 0.134$, $p = 0.53$.*

Immunological Correlates of Protective Immunity

To search for likely immune mechanisms implicated in the protective efficacy of prime-boost vaccination, both cellular and antibody responses at the time of challenge were studied for their association with delay to patent parasitaemia (Ewer K, et al, submitted). *Ex vivo* IFNγ ELISPOT responses to TRAP (summed across the pools of peptides representing the T9/96 strain antigen) showed no statistically significant association with efficacy (Figure 11a). Cultured ELISPOT assays used to measure a central memory T cell population showed very strong responses at the time of sporozoite challenge but again did not significantly correlate with protection. Antibodies to TRAP were measured by ELISA for Group 1 and 2 volunteers and did not correlate with vaccine efficacy.



a b c

***Figure 11. Correlates of protective efficacy.** (a) Correlation of time to parasitaemia with ex vivo ELISPOT responses for the 14 prime-boost vaccinees in Groups 1 and 5 who underwent malaria challenge, p = 0.97 (b) Correlation of time to parasitaemia with frequency of $CD8^+$ $IFN\gamma^+/IL\text{-}2^-/TNF\alpha^-$ for the 14 prime-boost vaccinees in Groups 1 and 5 who underwent malaria challenge p = 0.0005. For these volunteers plus Group 2 vaccinees (n=24), correlation of time to parasitaemia with frequency of $CD8^+$ $IFN\gamma^+/IL\text{-}2^-/TNF\alpha^-$, $r_s=0.61$, $p=0.002$. Both (a) and (b) were assessed at time of sporozoite challenge. (c) Correlation of time to parasitaemia with frequency of $CD107a^+/IFN\gamma^-/IL\text{-}2^-/TNF\alpha^-$ $CD8^+$ T cells at day 150 post-challenge in the first challenge, p = 0.02.*

Further analysis of immune correlates of protective efficacy included measures of polyfunctional as well as monofunctional $CD4^+$ and $CD8^+$ T cells and mean fluorescence intensity (geometric and

integrated). In the first challenge, we analysed combined data from Groups 1 and 2, and identified the frequency of $CD8^+$ T cells secreting IFNγ but not IL-2 or TNFα (r_s = 0.63, p = 0.005), as the strongest correlate of vaccine efficacy. In Group 1, the association was also strong despite small numbers (r_s = 0.84, p = 0.011). However cytokine-secretion on a per cell basis was not associated with protection, suggesting that the quantity of cytokine secreted alone was not the protective factor. Analysis of $CD107a^+$ expression at a later time point (150 days after the first challenge) showed that the frequency of these lytic $CD8^+$ T cells also correlated with efficacy (r_s = 0.61, p = 0.02, Figure 11c). We then reassessed and confirmed the association between protective efficacy and $CD8^+$ T cells secreting IFNγ alone in Group 5 volunteers (n=6) (r_s = 0.64, p = 0.018). Analysis of the combined data from the first and second challenges showed a very clear correlation (r_s = 0.81, p = 0.0005, Figure 11b). A secondary analysis of subgroups showed that gamma-interferon secreting $CD8^+$ T cells were also significantly higher in vaccinees showing either partial or sterile protection than in non-protected vaccinees (figure 12).

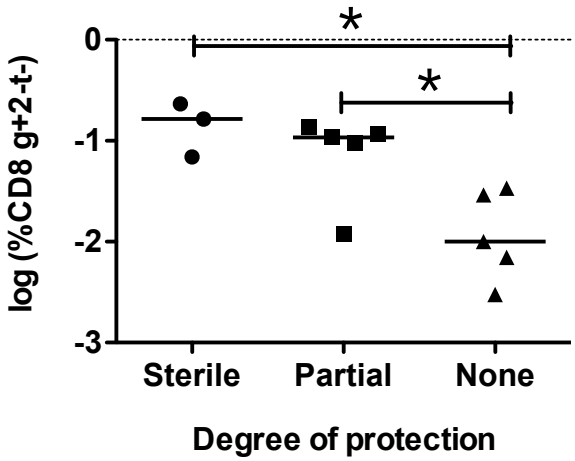


Figure 12. *MAL034: Frequencies of $CD8^+$ $IFN\gamma^+IL2^-TNF\alpha^-$ T cells before challenge, P=0.009 across groups using 1 way ANOVA. * P<0.05 after Bonferroni multiple comparison test for comparison between groups.*

8. Safety Evaluation

No serious adverse events occurred in this study. Adverse events related to vaccination are detailed below.

Adverse events related to ChAd63 ME-TRAP

ChAd63 ME-TRAP was administered to 31 volunteers in Groups 1, 2, 4 and 5 of MAL034 (this excludes administration of the mixture formulation of ChAd63 ME-TRAP and MVA ME-TRAP). The vast majority of adverse events related to ChAd63 ME-TRAP were mild and transient. The median duration of all adverse events was 48 hours, and all fully resolved without complications. One volunteer experienced a severe migranous-type headache on the day of immunization, which resolved within 24 hours with simple analgesia. The safety profile is illustrated in Figure 13, and Table 4 contains the full AE listings.

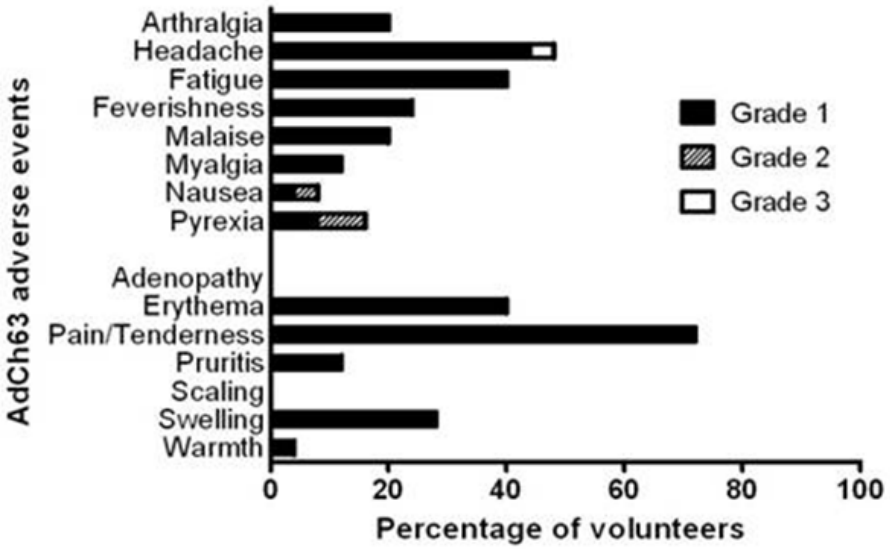


Figure 13. *MAL034, Groups 1,2,4 and 5: Adverse events related to ChAd63 ME-TRAP.*

All adverse events related to ChAd63 ME-TRAP 5 x 10^{10} vp IM (31 volunteers)			
	Mild	Moderate	Severe
Local pain	20	0	0
Local swelling	7	0	0
Local erythema	10	0	0
Local warmth	1	0	0
Local pruritus	3	0	0
Local scaling	0	0	0
Adenopathy	0	0	0
Axillary tenderness	1	0	0
Headache	19	0	1
Malaise	6	0	0
Myalgia	4	0	0
Fatigue	13	0	0
Feverishness	8	0	0
Fever	2	2	0
Arthralgia	5	0	0
Nausea	3	1	0
Coryza	2	0	0
Urticaria	2	0	0
Sore throat	3	0	0
Back pain	1	0	0

Table 4: *MAL034: All solicited and unsolicited local and systemic adverse events considered possibly, probably or definitely related to ChAd63 ME-TRAP. Highest intensity AE per volunteer recorded.*

Adverse events related to MVA ME-TRAP

21 volunteers received MVA ME-TRAP in Groups 1, 4 and 5 of MAL034 (this excludes those who received it in a mixture formulation with ChAd63 ME-TRAP). Safety data is detailed in Table 5. Overall 90% of volunteers experienced one or more systemic AEs and all experienced one or more local reactions following MVA ME-TRAP. Four AEs were considered severe post-MVA ME-TRAP; all were local swelling (defined as swelling of > 5cm in diameter). The mean maximal diameter of severe swelling in these volunteers was 7.45 cm and the mean duration of severe intensity was 3.25 days, with a mean duration of 11.5 days in total. All post MVA adverse events resolved. The mean duration of all systemic adverse events was 48 hours; the mean duration of all local adverse events was 5 days. Moderate erythema (54% of volunteers), swelling (33% of volunteers) and pain (24% of volunteers) were also observed. Three volunteers experienced moderate-grade fever (all < 38.5C) for less than 24 hours. One volunteer also experienced moderate-grade arthralgia for less than 24 hours which resolved with paracetamol.

All adverse events related to MVA ME-TRAP 2 x 10^8 pfu ID (21 volunteers)			
	Mild	Moderate	Severe
Local pain	14	5	0
Local swelling	10	7	4
Local erythema	10	11	0
Local warmth	19	0	0
Local pruritis	18	0	0
Local scaling	12	0	0
Local adenopathy	1 Supraclavicular 3 Axillary	0	0
Axillary tenderness	3	0	0
Headache	12	0	0
Malaise	8	0	0
Myalgia	5	0	0
Fatigue	11	0	0
Feverishness	8	0	0
Fever	3	3	0
Arthralgia	7	1	0
Nausea	5	0	0
Coryza	3	0	0
Urticaria	0	0	0
Sore throat	1	0	0
Back pain	1	0	0
Migraine	1	0	0

Table 5: *MAL034: All solicited and unsolicited local and systemic adverse events considered possibly, probably or definitely related to MVA ME-TRAP (Group 1, 4 and 5 subjects). Highest intensity AE per volunteer recorded.*

Adverse events related to ChAd63 ME-TRAP + MVA ME-TRAP Mixture Formulation

The mixture formulation of MVA ME-TRAP 2 x 10^8 pfu with ChAd63 ME-TRAP 5 x 10^{10} vp was administered intramuscularly in multi-immunisation schedules to volunteers in Groups 8, 9, and 10 of MAL034. Combining groups 8,9 and 10, a total of 13 volunteers received a first dose of the mixture, 12 volunteers received a second dose of the mixture, and 8 volunteers received a third dose of the mixture. A total of 33 doses were administered.

Tables 6 and 7, below, detail the occurrence of local and systemic adverse events. Reactogenicity was higher for the first dose of the mixture, but reduced with subsequent doses. All volunteers experienced at least one systemic and local AE following the first dose. Three volunteers experienced grade 3 injection-site pain that reduced to mild/mod within 24 hours. Following the second dose, no severe local reaction was observed, however one volunteer experienced rigors and fever lasting 12 hours on the evening of vaccination that resulted in a day missed from work. This was graded as severe. Overall 9/12 volunteers experienced at least one systemic and local adverse event with the second dose. There were no severe adverse events after the third dose, with 7/8 vaccinees reporting a local and 6/8 reporting a systemic AE.

Adverse Event	First dose: number(%)	Second dose: number(%)	Third dose number(%)	Total Number (%)
Pain				
Grade 1	5 (38)	7 (58)	5 (63)	17 (52)
Grade 2	5 (38)	0 (0)	0 (0)	5 (15)
Grade 3	3 (23)	0 (0)	0 (0)	3 (9)
Erythema				
Grade 1	8 (62)	5 (42)	2 (25)	15 (46)
Grade 2	0 (0)	0 (0)	0 (0)	0 (0)
Grade 3	0 (0)	0 (0)	0 (0)	0 (0)
Swelling				
Grade 1	3 (23)	3 (25)	1 (13)	6 (18)
Grade 2	0 (0)	0 (0)	0 (0)	0 (0)
Grade 3	0 (0)	0 (0)	0 (0)	0 (0)
Scaling				
Grade 1	0 (0)	0 (0)	0 (0)	0 (0)
Grade 2	0 (0)	0 (0)	0 (0)	0 (0)
Grade 3	0 (0)	0 (0)	0 (0)	0 (0)
Pruritus				
Grade 1	0 (0)	0 (0)	2 (25)	2 (6)
Grade 2	0 (0)	0 (0)	0 (0)	0 (0)
Grade 3	0 (0)	0 (0)	0 (0)	0 (0)
Warmth				
Grade 1	5 (38)	2 (17)	1 (13)	8 (24)
Grade 2	0 (0)	0 (0)	0 (0)	0 (0)
Grade 3	0 (0)	0 (0)	0 (0)	0 (0)
Other				
Grade 1	0 (0)	0 (0)	0 (0)	0 (0)
Grade 2	0 (0)	0 (0)	0 (0)	0 (0)
Grade 3	0 (0)	0 (0)	0 (0)	0 (0)

Table 6*. Local adverse events following mixture administration of ChAd63 ME-TRAP and MVA ME-TRAP in groups 8, 9 and 10 of MAL034. Local adverse events reported are those considered possibly, probably, or definitely related to vaccination.*

Adverse Event	First dose: number(%)	Second dose: number(%)	Third dose number(%)	Total Number (%)
Fever				
Grade 1	1 (8)	0 (0)	0 (0)	1 (3)
Grade 2	2 (15)	1 (8)	0 (0)	3 (9)
Grade 3	0 (0)	1 (8)	0 (0)	1 (3)
Feverish				
Grade 1	10 (77)	3 (25)	1 (13)	14 (42)
Grade 2	0 (0)	0 (0)	0 (0)	0 (0)
Grade 3	0 (0)	0 (0)	0 (0)	0 (0)
Arthralgia				
Grade 1	6 (46)	3 (25)	3 (38)	11 (33)
Grade 2	0 (0)	0 (0)	0 (0)	0 (0)
Grade 3	0 (0)	0 (0)	0 (0)	0 (0)
Myalgia				
Grade 1	7 (54)	5 (42)	4 (50)	16 (49)
Grade 2	0 (0)	0 (0)	0 (0)	0 (0)
Grade 3	0 (0)	0 (0)	0 (0)	0 (0)
Malaise				
Grade 1	11 (85)	3 (25)	3 (38)	16 49)
Grade 2	0 (0)	1 (8)	0 (0)	1 (3)
Grade 3	0 (0)	0 (0)	0 (0)	0 (0)
Fatigue				
Grade 1	12 (92)	8 (67)	4 (50)	24 (73)
Grade 2	0 (0)	0 (0)	0 (0)	0 (0)
Grade 3	0 (0)	0 (0)	0 (0)	0 (0)
Headache				
Grade 1	9 (69)	5 (42)	3 (38)	17 (52)
Grade 2	0 (0)	0 (0)	0 (0)	0 (0)
Grade 3	0 (0)	0 (0)	0 (0)	0 (0)
Nausea/vomiting				
Grade 1	2 (15)	0 (0)	0 (0)	2 (6)
Grade 2	0 (0)	0 (0)	0 (0)	0 (0)
Grade 3	0 (0)	0 (0)	0 (0)	0 (0)
Other				
Grade 1	1 (8)	6 (50)	1 (13)	8 (24)
Grade 2	0 (0)	0 (0)	0 (0)	0 (0)
Grade 3	0 (0)	1 (8)	0 (0)	1 (3)

Table 7*. Systemic adverse events following mixture administration of ChAd63 ME-TRAP and MVA ME-TRAP in groups 8, 9 and 10 of MAL034. Local adverse events reported are those considered possibly, probably, or definitely related to vaccination.*
Grade 1 "Other" systemic adverse events were: vasovagal event (n=2), left shoulder stiffness (n=1), coryza (n=2), low back pain (n=1), sore throat (n=1), loss of appetite (n=1).
Grade 3 "Other" systemic adverse events were: rigor (n=1).

Laboratory abnormalities

Laboratory abnormalities that were considered adverse events related to any study procedures are described below.

At seven days following MVA ME-TRAP boost vaccination, one volunteer was found to have mild neutropaenia, which was considered probably related to vaccination with MVA ME-TRAP. No action was taken, and the abnormality had resolved on repeat testing 24 days later.

Following sporozoite challenge, three volunteers experienced grade 1 neutropaenia (one of whom had received ChAd63 ME-TRAP only, one of whom had received ChAd63 ME-TRAP and MVA ME-TRAP, and one control volunteer) and one control volunteer experienced grade 1 thrombocytopaenia. These abnormalities were considered related to sporozoite challenge, and resolved on repeat testing.

One Group 4 volunteer was found to have a Haemoglobin level of 10.5 g/dL on Day 56 (immediately prior to MVA ME-TRAP vaccination). This volunteer was diagnosed as having mild iron deficiency anaemia, which was considered possibly related to blood donation for the study (not considered related to vaccination). Iron supplementation was prescribed for the volunteer by their General Practitioner. The abnormality had resolved no repeat testing 31 days later.

Safety Conclusions

This study raised no specific safety concerns related to study procedures. Vaccinations were generally well tolerated, all adverse events resolved, and there were no SAEs or SUSARs. The vaccines ChAd63 ME-TRAP and MVA ME-TRAP have since undergone evaluation in further clinical trials.

9. Discussion and Conclusions

Heterologous prime-boost immunisation with ChAd63 ME-TRAP followed eight weeks later by MVA ME-TRAP was shown to have durable partial efficacy against malaria infection by malaria sporozoite challenge. Efficacy was not demonstrated with vaccination with ChAd63 ME-TRAP alone or with regimens using the Mixture Formulation of ChAd63 ME-TRAP + MVA ME-TRAP. The efficacy (sterile protection or delay to patency) was 8/14 volunteers undergoing early challenge (Groups 1 and 5; excludes Group 4 undergoing delayed challenge) post ChAd63 ME-TRAP / MVA ME-TRAP prime-boost vaccination. This compares with 9/38 individuals (24%, p = 0.02, chi square test) with earlier DNA-MVA and FP9-MVA regimes[1,4,5].

ChAd63 ME-TRAP / MVA E-TRAP prime-boost immunisation induced the most potent CD8+ T cell responses to date for any vaccination approach in clinical trials for malaria. The $CD8^+$ T cell levels were comparable to those achieved in macaque pre-clinical models[6]. The overall level of T cell response induced was 5-10 fold higher than with previous prime-boost regimes using the same antigenic insert[1,4,5], with a CD4:CD8 ratio of close to 1:1. The levels of $CD8^+$ response associated with efficacy, though substantial, are much lower than the extremely high $CD8^+$ T cell levels required for efficacy in murine malaria models[7,8], possibly in part related to the longer duration of the liver stage, typically 7 days vs 2 days, with *P. falciparum* than rodent *Plasmodia*.

The safety findings indicate that ChAd63 ME-TRAP / MVA ME-TRAP prime-boost immunisation is well tolerated by vaccinees, and support the excellent safety track record to date of ChAd63 ME-TRAP and MVA ME-TRAP and other vaccines utilising MVA and adenoviral vectors.

These findings support the extension of clinical development of this approach to the assessment of efficacy against natural malaria infection in malaria-endemic areas, and age-deescalation testing towards infants, the major age group in malaria-endemic areas that would benefit from a malaria vaccine targeting the preerythrocytic stage of *P. falciparum*. Further development of this approach could include use of novel schedules of vaccine administration or novel adjuvants. The identification here of an immunological correlate of efficacy could inform the assessment of potential efficacy of such novel approaches based on immunogencity results in Phase I clinical studies.

10. References

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