Clinical Study Report

A phase I/IIa study to assess the safety and immunogenicity of new malaria vaccine candidates AdCh63 MSP1 alone and with MVA MSP1

VAC037

GTAC: 166
Eudra CT number: 2009-012591-27
CTA number: 24037/38830/15/497

CONFIDENTIAL

CONTENTS

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: ____/____/_____

Print name: Professor A.V.S. Hill

Chief Investigator

Signed: Date: ____/____/_____

Print name: Dr S. Sheehy

Lead Clinician & Report Author

Signed: Date: ____/____/_____

Print name: Dr A. Lawrie

Project Manager

2. OVERVIEW

Study title:	A phase I/IIa study to assess the safety and immunogenicity of new malaria vaccine candidates; AdCh63 MSP1 alone and with MVA MSP1
Trial code:	VAC037
Study description:	Open label observational challenge study.
Test IMPs:	AdCh63 MSP1 & MVA MSP1
Indication studied:	Safety, immunogenicity and efficacy
Sponsor:	University of Oxford
Chief Investigator:	Professor A.V.S. Hill
Study centres:	Centre for Clinical Vaccinology and Tropical Medicine Churchill Hospital Old Road Headington Oxford OX3 7LJ University College London Clinical Research Facility c/o Rayne Building 5 University Street London WC1E 6JJ 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:	20th July 2009 – 20th July 2010
Study dates actual:	12th November 2009 – 22nd September 2010
Enrolment:	Completed
Publication:	Submitted
GCP Statement:	This study was performed in compliance with ICH Good Clinical Practise (GCP) including the archiving of essential documents.

3. PROTOCOL SYNOPSIS

Objectives	<u>Primary Objective:</u> To assess the safety of new candidate malaria vaccines; AdCh63 MSP1 administered alone and with MVA MSP1 in a prime-boost regime to healthy volunteers. Also to assess the safety of the prime-boost vaccine strategy in healthy volunteers following malaria sporozoite challenge. <u>Secondary Objective:</u> To assess the humoral and cellular immune responses generated by AdCh63 MSP1 when administered to healthy volunteers alone, with MVA MSP1, and following sporozoite challenge. <u>Tertiary Objective:</u> To assess the efficacy of AdCh63 MSP1 and MVA MSP1 against malaria sporozoite challenge.
Trial design	Non randomised, un-blinded dose escalation phase I/IIa trial in healthy adults.
Sample Size	<u>Group 1</u> - Subgroup A (1A): 2 volunteers, 1 dose of AdCh63 MSP1 5 x 10^9 vp intramuscularly - Subgroup B (1B): 4 volunteers,1 dose of AdCh63 MSP1 5 x 10^9 vp intramuscularly and 1 dose MVA MSP1 5 x 10^8 pfu 8 weeks later intramuscularly <u>Group 2</u> - Subgroup A (2A): 2 volunteers, 1 dose of AdCh63 MSP1 5 x 10^{10} vp intramuscularly - Subgroup B (2B): 5 volunteers, 1 dose of AdCh63 MSP1 5 x 10^{10} vp intramuscularly and 1 dose MVA MSP1 5 x 10^8 pfu 8 weeks later intramuscularly - Subgroup C (2C): 3 volunteers, 1 dose of AdCh63 MSP1 5 x 10^{10} vp intramuscularly and 1 dose MVA MSP1 5 x 10^8 pfu 8 weeks later intramuscularly with malaria sporozoite challenge 12-28 days following MVA MSP1 administration. Total: 16 volunteers NB: 3 vaccinees in this trial underwent challenge at the same time as 6 unvaccinated “infectivity controls” in another study (MAL034: Eudract Number: 2008-006804-46), in order to confirm infectivity of challenge procedure.

Diagnosis and main criteria for inclusion	• Healthy adults 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 practice continuous effective contraception during the study and a negative pregnancy test on the day(s) of vaccination and/or challenge • For males only, willingness to use barrier contraception until 3 months after last vaccination • Agreement to refrain from blood donation during the course of the study • Written informed consent
Duration of treatment	All volunteers received one vaccination with AdCh63 MSP1 intramuscularly. 12 volunteers also received one vaccination with MVA MSP1 intramuscularly 8 weeks later. 3 of these volunteers underwent sporozoite challenge 12-28 days later (experimental malaria infection).
Criteria for Evaluation of Objectives	Primary Objective: Actively and passively collected data on adverse events. Secondary Objective: Markers of humoral and cell-mediated immunity. Tertiary Objective: Time to malaria diagnosis as defined by positive thick film microscopy & PCR.
Statistical methods	Descriptive analysis of safety and immunology data. Kaplan Meier analysis of efficacy data.
Blinding	Non-Blinded
Controls	Controlled
Randomisation	Non Randomised

4. ETHICS AND REGULATORY APPROVAL

INDEPENDENT ETHICS COMMITTEE APPROVAL

The study protocol and related documents were reviewed and approved by the Gene Therapy Advisory Committee. The initial ethical approval for the trial was given on 25th September 2009, and where appropriate, all subsequent substantial amendments were approved by this committee prior to implementation.

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 Practise (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 performed in compliance with the requirements of the Medicines and Healthcare products Regulatory Agency (MHRA);

- CTA number: 24037/38830/15/497
- Eudra CT number: 2009-012591-27

The study was submitted to the MHRA on 29th July 2009 and was approved on 23rd September 2009. Where appropriate, all subsequent substantial amendments were submitted to the MHRA for approval prior to implementation.

5. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Title	Name and affiliation
Chief Investigator & Principal Investigator - Oxford	Professor A.V.S. Hill – University of Oxford
Principal Investigator – UCL	Dr. J Doherty – University College London
Trial Clinicians	Dr S. Sheehy – University of Oxford Dr C. Duncan – University of Oxford
Project Manager	Dr. A Lawrie – University of Oxford
Monitor	Ms S. Saunders –Appledown Monitoring
Laboratory Investigators	Dr S. Draper – University of Oxford Mr S. Elias – University of Oxford

6. DESCRIPTION OF INVESTIGATIONAL PRODUCTS

The insert encodes a composite sequence from a blood-stage *Plasmodium falciparum* malaria antigen, merozoite surface protein 1 (MSP1). To generate vectored vaccine candidates suitable for clinical assessment in the challenging area of blood-stage vaccine development we included i) the four N-terminal conserved regions (Blocks 1, 3, 5 & 12) to generate T cell responses to more conserved rather than very variable regions of MSP1; ii) two allelic variants of the C-terminus of MSP1 arrayed in tandem in the vectored insert; and iii) recently described point mutations in $MSP1_{19}$, to enhance overall immunogenicity and increase the likelihood of developing protective "inhibitory antibodies" rather than unwanted "blocking" antibodies.

The investigational medicinal product **AdCh63 MSP1** is a replication defective simian adenoviral vector expressing MSP1 administered intramuscularly to prevent malaria. AdCh63 MSP1 was manufactured under Good Manufacturing Practice conditions by the Clinical Biomanufacturing Facility (CBF), Churchill Hospital, Oxford, and presented in glass vials of 0.65 mL. Each vial of AdCh63 MSP1 contains a concentration of 1.4×10^{11} vp / mL formulated in 10mM histidine, 7.5% sucrose, 35mM NaCl, 1mM $MgCl_2$, 0.1% PS80, 0.1mM EDTA, 0.5% ethanol, pH 6.6. Further details relating to batch release and manufacturing can be found in the AdCh63 MSP1 IMP-D.

MVA MSP1 was manufactured under Good Manufacturing Practice conditions by Impfstoffwerk Dessau-Tornau (IDT), Germany. MVA MSP1 is supplied as a liquid, at a target concentration of 9.43×10^8 plaque-forming units (pfu)/mL. The virus suspension is supplied as sterile 0.3 mL aliquots in 2.0 mL clear glass injection vials. Final certification of the final product and associated labelling takes place at the CBF in Oxford.

The vials were stored between –70°C and –90°C, in a locked freezer, at the University of Oxford, Churchill Hospital. All movements of the study vaccines between CBF and the University of Oxford and between the locked freezer and clinic room were fully documented.

7. STUDY POPULATION

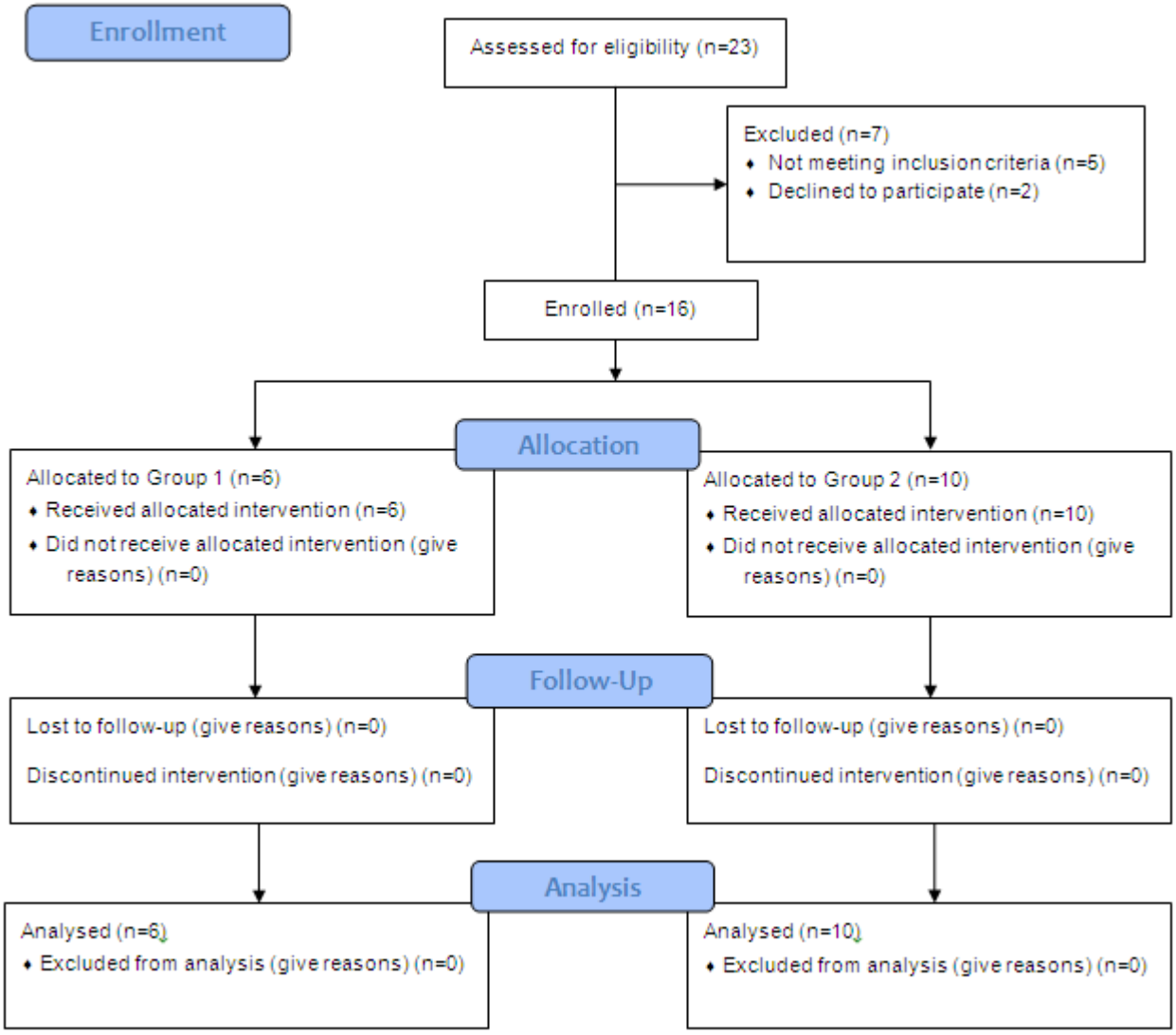

Figure 1: CONSORT diagram of study progress. All volunteers were recruited and enrolled at the Oxford site.

8. PROTOCOL DEVIATIONS

Deviation	*Site: Oxford*	*Site: UCL*
Entry criteria	0	0
Withdrawal criteria	0	0
Incorrect dosing regimen	1	0
Concomitant medication	0	0
Other*	6	0

Table 1: Protocol deviations. 'Other' included; 5 interventions or clinical reviews taking place outside the time window specified in the protocol, and an incorrect volume of blood being drawn at one visit. All volunteers were recruited and enrolled at the Oxford site.

9. RESULTS

9.1 DEMOGRAPHICS OF STUDY POPULATION

Volunteer group	Mean age (range)	Gender (% Male)
1A (n=2)	20 (19-21)	100%
1B (n=4)	26 (22-30)	25%
2A (n=2)	23 (N/A)	0%
2B (n=5)	20.8 (19-23)	20%
2C (n=3)	21 (19-23)	66%

Table 2: Demographics of volunteers.

9.2 ADVERSE EVENTS

There were no serious adverse events during the study.

(a) AdCh63 MSP1

The vast majority of local and systemic adverse events (AEs) following AdCh63 MSP1 were mild in severity (97%) and all resolved completely (Figure 2). A dose response was seen in reactogenicity; 5×10^9 vp AdCh63 MSP1 was less reactogenic both locally and systemically than 5×10^{10} vp. 69% of volunteers experienced one or more local injection-site reactions which were all mild in intensity with the exception of one case of moderate pain (post AdCh63 MSP1 5×10^{10} vp). 94% of volunteers experienced one or more systemic AEs; these were all mild with the exception of one case of headache of moderate severity starting on day of vaccination and lasting 2 days post AdCh63 MSP1 5×10^{10} vp.

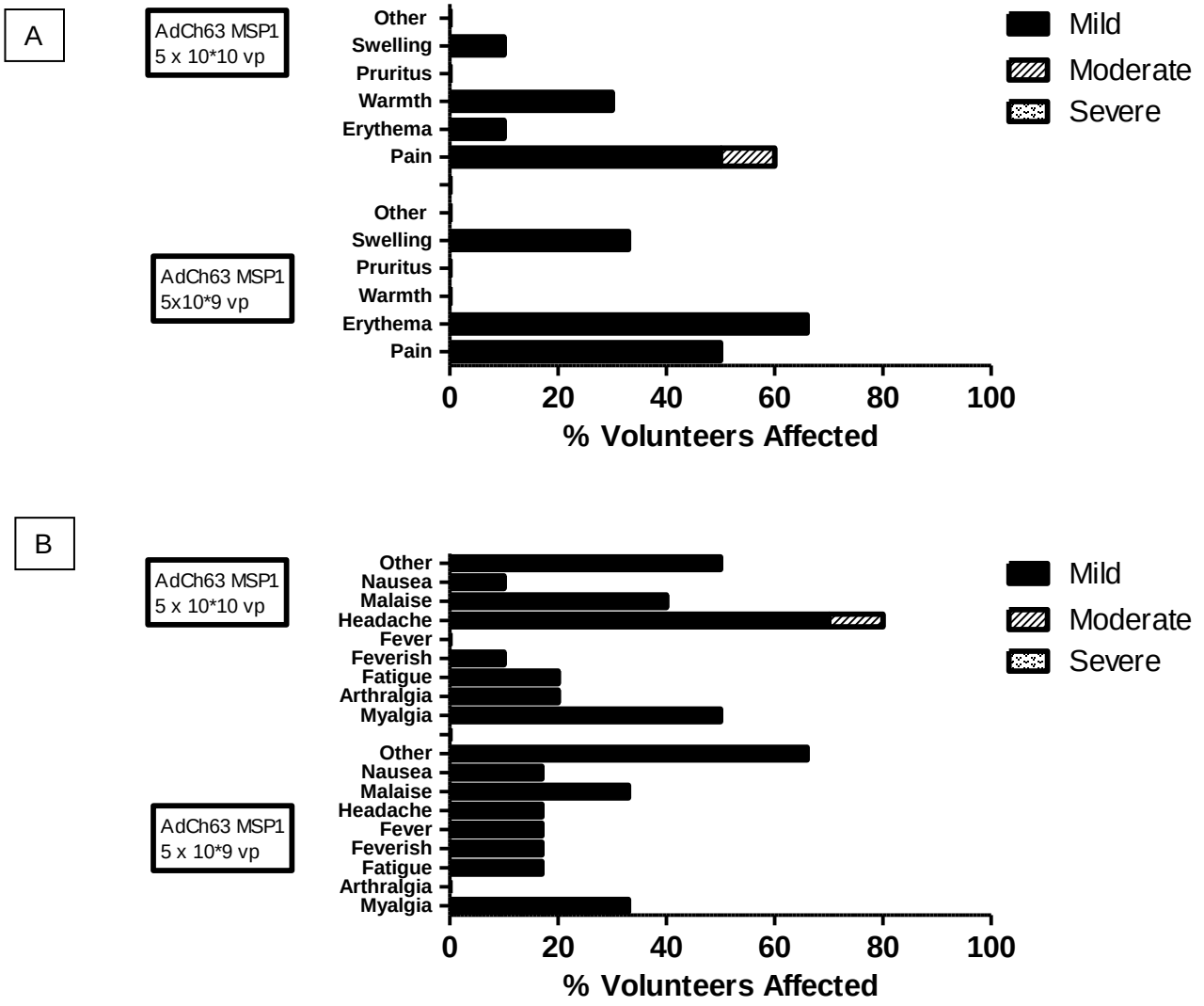

Figure 2: Adverse events deemed possibly, probably or definitely related to AdCh63 MSP1. The highest intensity adverse event per subject is listed. Figure 2a: Local adverse events post AdCh63 MSP1. Figure 2b: Systemic adverse events post AdCh63 MSP1. 'Other' adverse events post 5 x 10^9 vp AdCh63 MSP1 included; grade 1 thrombocytopenia, coryzal symptoms, diarrhoea and hordeolum. 'Other' adverse events post 5 x 10^{10} vp AdCh63 MSP1 included; sore throat, 2 cases of coryzal symptoms, grade 1 thrombocytopenia & elevated ALT (69 IU/L). All 'other' AEs were considered possibly related to vaccination due to a temporal association.

(b) **MVA MSP1**

Ten of the 12 volunteers who received MVA MSP1 had the vaccination administered as one injection. Two volunteers had the vaccination split as two injections of 2.5 x 10^8 pfu in each deltoid.

100% of volunteers experienced one or more local adverse events post MVA MSP1 (Figure 3). 100% of volunteers described injection site pain, which was moderate or severe in intensity in 75% of volunteers. Other local site reactions were mild in severity with the exception of 2 volunteers who had moderate or severe erythema post vaccination. In both cases the erythema was of delayed onset (3-5 days post vaccination) and between 3cm and 8cm distal to, and not including the vaccine site. In both cases, the volunteers were systemically well. This may represent non-specific activation of the inflammatory system post vaccination.

100% of volunteers described one or more systemic adverse events post MVA MSP1 (Figure 3). The majority were mild in severity but 3 volunteers (25%) described a constellation of severe systemic adverse events resulting in one day of absenteeism from work in all 3 cases. These included rigors, malaise, myalgia, fatigue and feverishness which all developed within 24 hours of vaccination and fully resolved within 5 days.

Two volunteers received 5 x 10^8 pfu of MVA MSP1 administered as two separate injections of 2.5 x 10^8 pfu in each deltoid, in order to assess if split dosing reduced local site pain and systemic reactogenicity. Both these volunteers experienced severe systemic adverse events and either moderate or severe local pain and so split dosing of MVA MSP1 was not continued.

In light of the marked reactogenicity with MVA MSP1 seen in this study, in future trials 1-2.5 x 10^8 pfu MVA MSP1 will be used.

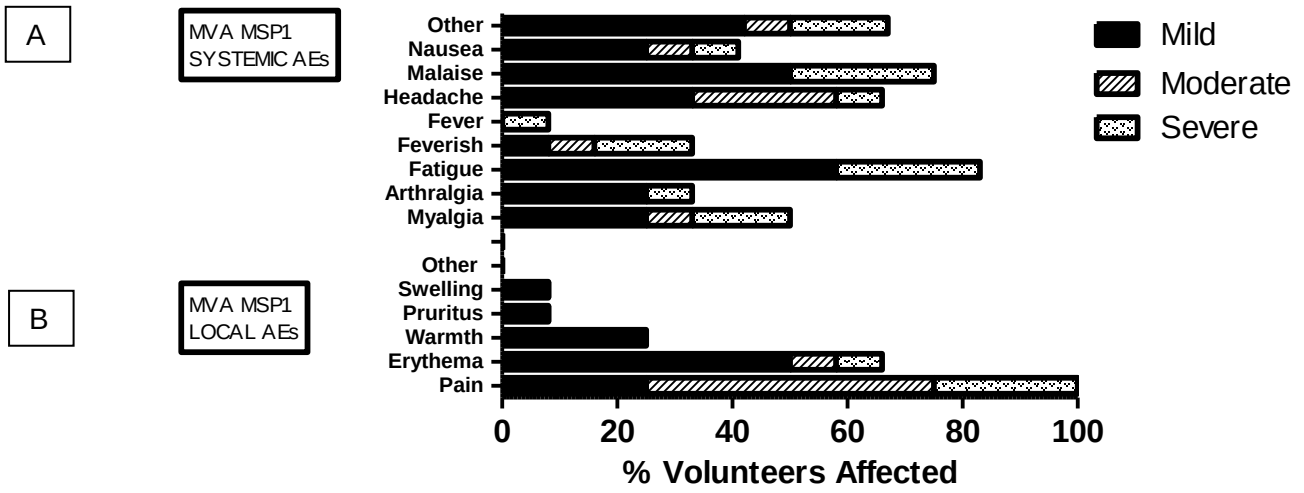

Figure 3: Adverse events deemed possibly, probably or definitely related to MVA MSP1. The highest intensity adverse event per subject is listed. Figure 3a: Local adverse events post MVA MSP1. Figure 3b: Systemic adverse events post MVA MSP1. Mild 'Other' adverse events post MVA MSP1 included; coryzal symptoms, vasovagal, "prickly skin," oesophagitis, tender cervical lymphadenopathy and elevated ALT (61 IU/L). Moderate 'Other' adverse events post MVA MSP1 included bruising at vaccination site. Severe 'Other' adverse events post MVA MSP1 included 2 cases of rigors.

(c) Sporozoite Challenge

The three volunteers in group 2C (and six unvaccinated infectivity controls enrolled in another sporozoite challenge study; MAL034) underwent a pilot sporozoite challenge with the 3D7 strain of *P. falciparum* 13-16 days following MVA MSP1 immunization. This initial small sample size was chosen because of a very small but potential risk of inducing immunopathology on challenge following the induction of high effector T cell levels to a blood-stage antigen that are not found after natural exposure, and for which there is some evidence in murine models. No unexpected adverse events or clinical signs of immunopathology were observed in volunteers post challenge. There was no difference between vaccinees and controls in the duration individuals were symptomatic prior to diagnosis or the number of symptoms present at time of diagnosis (Figure 4).

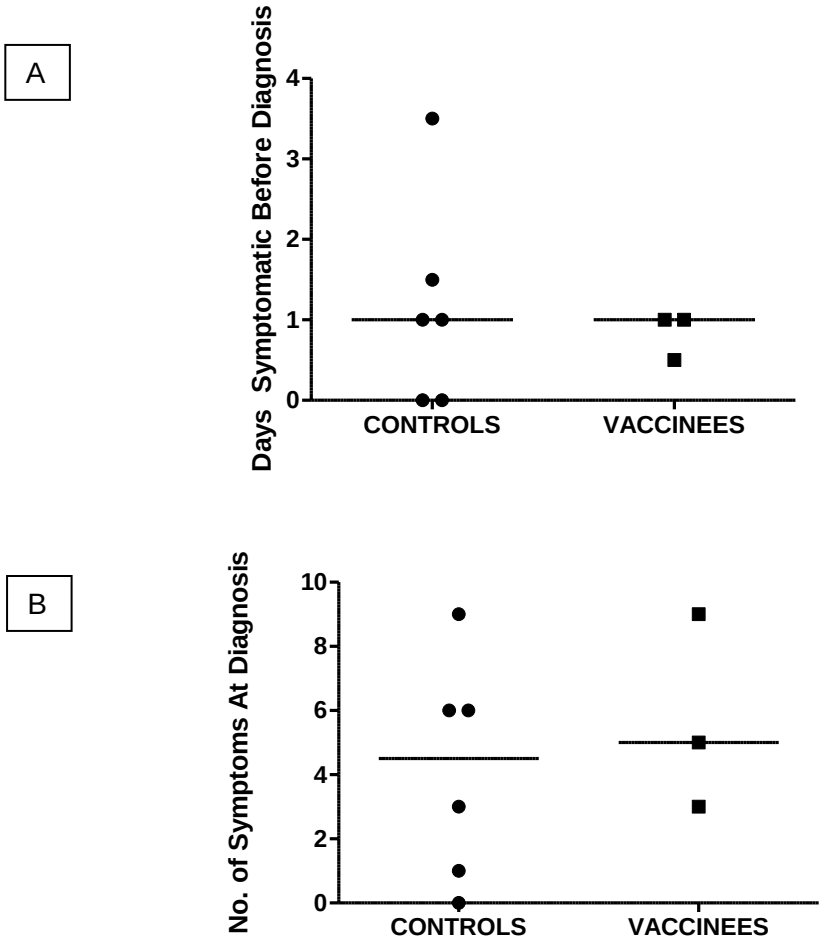

Figure 4. Controls = unvaccinated challenged volunteers (n=6). Vaccinees = Challenged volunteers who received AdCh63-MVA MSP1 (n=3). Median values for each group are indicated. Figure 4A: No. of days each volunteer demonstrated any clinical symptoms consistent with clinical malaria prior to diagnosis (p=0.892). Figure 4B: No. of symptoms consistent with clinical malaria present on day of diagnosis (p=0.694)

9.3 CLINICAL LABORATORY EVALUATION

The following laboratory abnormalities were noted post vaccination (Table 3). All were mild, deemed possibly related to vaccination and resolved fully with no long term sequelae. All laboratory abnormalities resolved by time of next venepuncture* (duration of abnormality is therefore likely to be overestimated, as the abnormality may have resolved prior to retesting).

Laboratory Abnormality	Vaccine	Dose	Onset post vaccination (days)	Duration (days)
Thrombocytopenia	AdCh63 MSP1	$5x10^9$ vp	56	28*
Thrombocytopenia	AdCh63 MSP1	$5x10^{10}$ vp	30	25*
ALT 69 IU/L	AdCh63 MSP1	$5x10^{10}$ vp	14	27*
ALT 61 IU/L	MVA MSP1	$5x10^8$ pfu	8	21*

Table 3: Laboratory abnormalities noted post vaccination. ALT = alanine aminotransferase.

The three challenged vaccinees had daily full blood counts taken between days 7 post challenge and day of diagnosis. Biochemical analysis of these volunteers was also performed on days 7 and 14 days post challenge. The following laboratory abnormalities were noted (Table 4); these findings are consistent with malaria infection and not indicative of immunopathology.

Laboratory Abnormality	Onset Post challenge (days)	Duration (days)
Thrombocytopenia	14	21*
Anaemia	10	3

Table 4: Laboratory abnormalities noted post challenge in vaccinees. ALT = alanine aminotransferase.

9.4 OTHER CLINICAL FINDINGS

No vaccinated volunteer experienced any significant physical abnormality post vaccination. During the challenge phase of the study all volunteers underwent a daily clinical review. Other than clinical findings consistent with malaria infection, all of which fully resolved, no significant findings related to vaccination were noted.

There were no ongoing adverse events at the end of the study.

9.5 IMMUNOLOGY

Cellular Immune Responses Post Vaccination

Vaccination with AdCh63-MVA MSP1 induced T cell responses in all volunteers as measured by ex-vivo IFN-γ ELIspot (Figure 5). There was a trend for stronger median responses in the higher dose group 2 in comparison to group 1, both at day 14 after the prime (median 2785 vs 979 SFU/million respectively, *P*=0.07) and one week after the MVA boost (median 5090 vs 2868 SFU/million respectively, *P*=0.37). This median response in group 2 is, to our knowledge, the highest yet reported following immunization with any vectored vaccine regimen.

T cell responses were induced across the whole antigenic insert in all individuals, indicating no single immuno-dominant region. The median responses in both groups broadly mirrored the composition of the vaccine antigen when represented either as the magnitude or % total ELIspot response.

Similar to that seen in rhesus macaques immunized with an AdCh63-MVA regime, MSP1-specific $CD3^+$ T cells assessed at the peak of the response consisted of a mixed $CD4^+$ and $CD8^+$ phenotype – with sub-populations of multi-functional cells displaying the degranulation marker CD107a and/or producing the cytokines IFN-γ, TNFα and IL-2 (Figure 5). In agreement with the ELIspot data, $CD4^+$ and $CD8^+$ T cell responses tended to be stronger in group 2 in comparison to group 1 (data not shown). $CD8^+$ T cells tended to produce CD107a, IFN-γ and TNFα and less IL-2. CD4+ T cells produced IFN-γ, TNFα and IL-2, but not CD107a as expected (the latter is a marker of cytotoxic T cell degranulation).

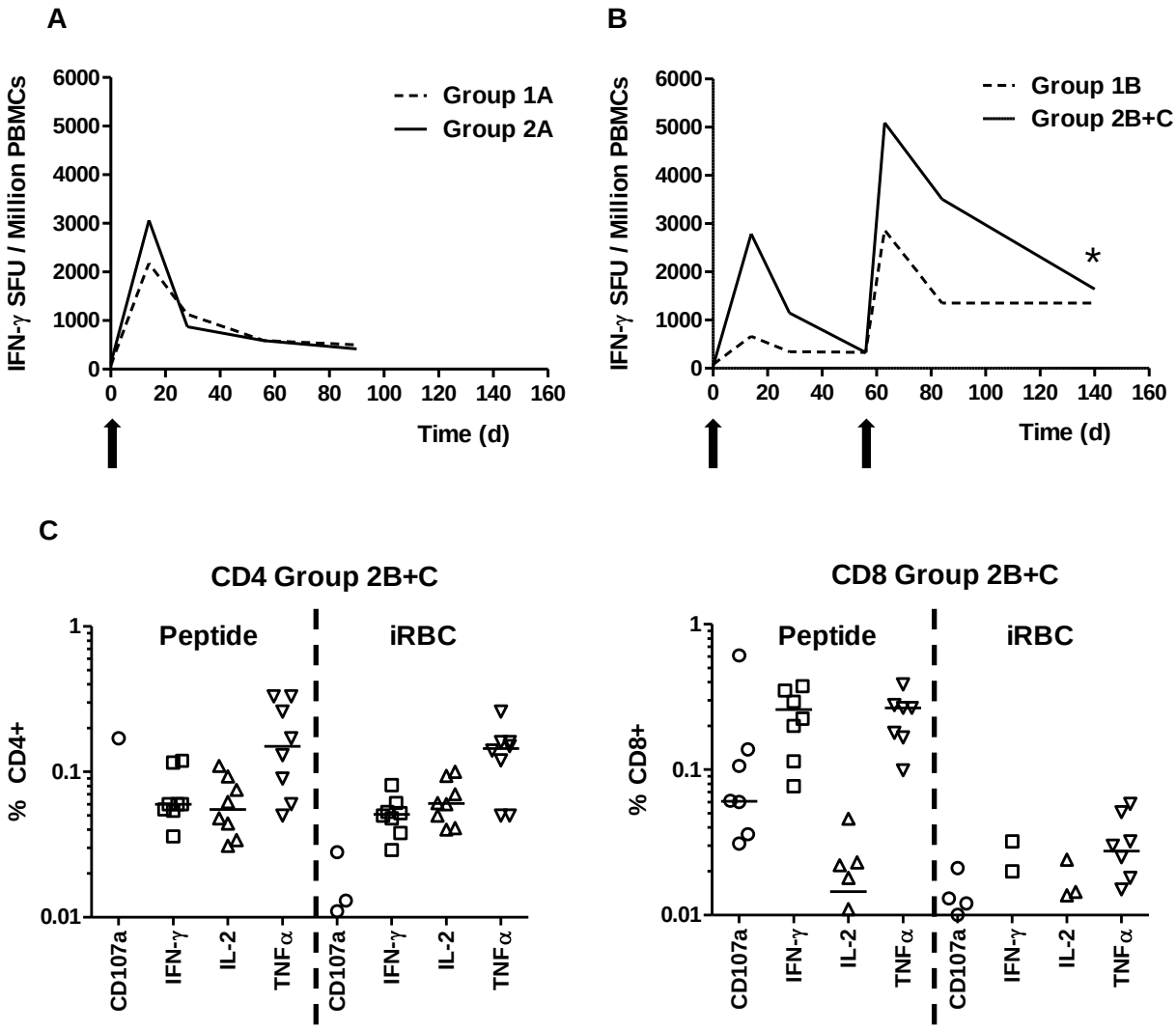

Figure 5: Immunogenicity post AdCh63-MVA MSP1. Figures 5A & 5B: Median ex-vivo IFN-γ ELISPOT responses in PBMCs to the MSP1 insert (summed response across all the individual peptide pools) are shown over time for each group. Figure 5C: Multi-functionality of the CD3$^+$ T cell responses was assessed by polychromatic flow cytometry and ICS following AdCh63-MVA immunization at d84 or dC-1 and re-stimulation of frozen PBMCs with either MSP1 peptides or red blood cells infected with P. falciparum parasites (iRBC). Individual data points and the median are shown for the % CD4$^+$ and CD8$^+$ T cells positive for CD107a, IFN-γ, IL-2 or TNFα. Responses <0.01% are not shown.

Humoral Immune Responses Post Vaccination

AdCh63 MSP1 primed a dose-dependent IgG antibody response against $MSP1_{19}$ in all volunteers that was boosted considerably by MVA MSP1 (Figure 6). Antibody responses peaked four weeks after boosting with median titres of 39.5µg/mL and 27.3µg/mL anti-$MSP1_{42}$ 3D7 and FVO IgG, respectively, in group 2B+C, – levels comparable to or higher than $MSP1_{42}$ protein vaccines formulated in Alum or AS02, but three- to four-fold lower than Alum+CpG. These data show that antibody titres, comparable to protein-in-adjuvant formulations can be generated in humans by adenovirus-MVA vectors, as predicted by animal models. In agreement with published data for other MSP1-based vaccines, IgG responses of this magnitude did not induce functional growth inhibitory activity (GIA) above baseline against the 3D7 strain of *P. falciparum in vitro* (data not shown).

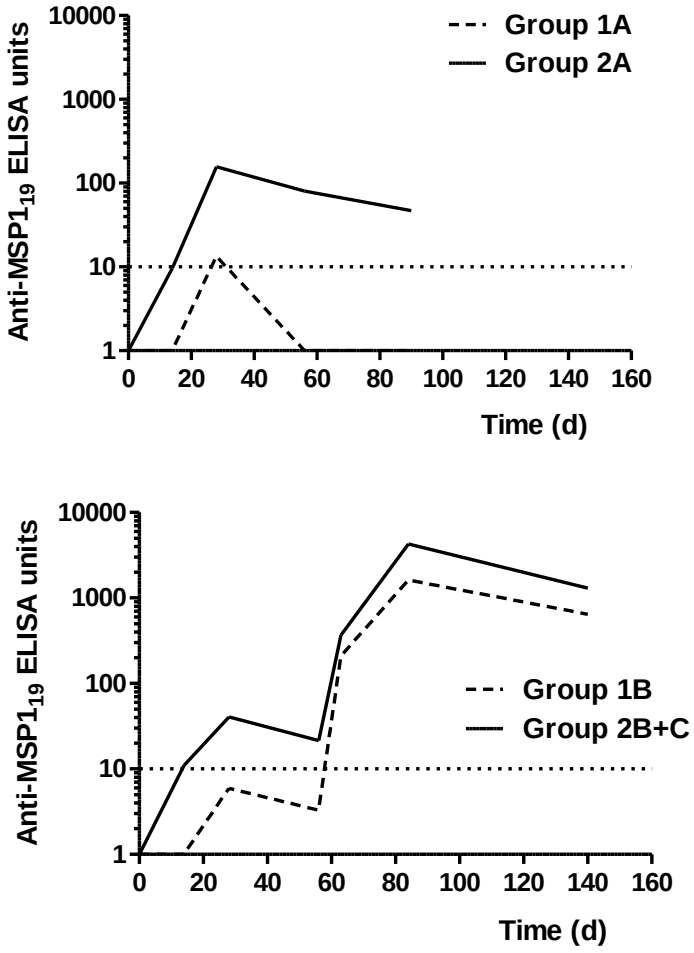

Figure 6. Total IgG ELISA responses against 3D7 $PfMSP1_{19}$ (ETSR / Mad20 allele) as measured in the serum over time following AdCh63-MVA MSP1 immunization. The geometric mean response is shown for each group (analysis includes all applicable data for each group and time-point).

9.6 EFFICACY

All 3 challenged vaccinees and all 6 non-vaccinated control volunteers developed malaria. No unexpected adverse events occurred. Importantly, there was a statistically significant delay in time to diagnosis for the vaccinees compared to control volunteers (*P*=0.032) (Figure 7), a protective effect in terms of clinical outcome only previously observed with vaccines expressing the pre-erythrocytic CSP or TRAP antigens.

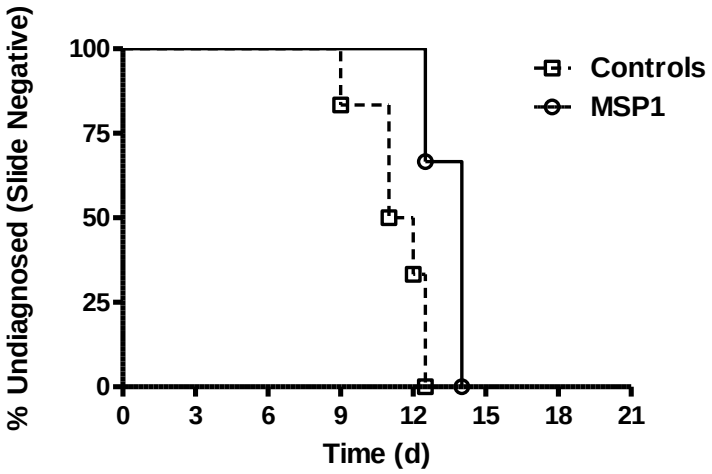

Figure 7. Kaplan-Meier survival analysis of time to patent parasitaemia in days for MSP1 prime-boost vaccinated volunteers (n=3) versus unvaccinated controls (n=6). Median time to patent parasitaemia = 14 days for vaccinees versus 11.5 days for unvaccinated controls (p = 0.032, Log-Rank test).

Vaccinees had significantly higher PCR values at time of diagnosis compared to controls (Figure 8). However, there was no difference between vaccinees and controls in the duration individuals were symptomatic prior to diagnosis or the number of symptoms present at time of diagnosis (Figure 4), arguing against investigator bias.

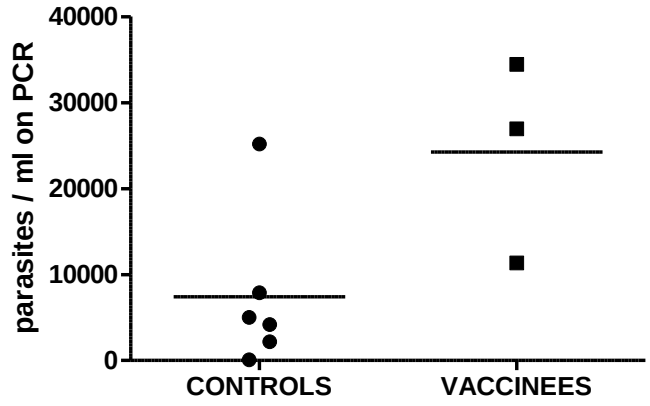

Figure 8. PCR values at time of diagnosis days for MSP1 prime-boost vaccinated volunteers (n=3) versus unvaccinated controls (n=6). Median PCR at diagnosis = 4,598 parasites/ml for controls versus 26,946 parasites/ml for vaccinees (p=0.0476, Mann Whitney U test).

A delay to patency caused by a blood-stage vaccine could be due to reductions in either the liver-to-blood inoculum and/or parasite multiplication rate (PMR). Modelling aims to quantify each of these parameters independently, but no significant differences between vaccinees and controls were observed in either parameter as calculated using both of the standard published models, possibly reflecting the small sample size studied. Following sporozoite challenge, cellular immune responses were maintained and, in agreement with murine malaria models, $MSP1_{19}$-specific IgG responses were induced at low-levels in 5/6 controls, and boosted in the vaccinees.

10. CONCLUSION & DISCUSSION

Heterologous prime-boost with AdCh63-MVA expressing MSP1 administered intramuscularly is safe and immunogenic in healthy malaria naive adults. AdCh63 MSP1 has an excellent safety profile, associated with similar, mild adverse events to those seen with AdCh63 ME-TRAP. In this trial MVA MSP1 was more reactogenic than AdCh63 MSP1 and other MVA vaccines, however the dose of MVA used in this study (5 x 10^8 pfu) was considerably higher than that used for other MVA vectored vaccines (typically 1-2 x 10^8 pfu). In light of this, in future studies, the dose of MVA MSP1 will be reduced to 1-2.5 x 10^8 pfu.

The study has also demonstrated that potent T cell responses induced following AdCh63-MVA MSP1 are safe when exposed to natural antigen, with no evidence of immunopathology. This has important implications for future challenge studies of potent T cell inducing blood-stage vaccines.

This is the first report of statistically significant efficacy in humans induced by a vaccine targeting the MSP1 antigen alone, and provides the first indication, despite a small sample size, that vaccines inducing powerful cellular immunity in conjunction with antibody responses to a blood-stage antigen are safe as well as effective. Trends for reduced liver to blood inoculum and significantly delayed diagnosis in vaccinees (in the absence of reduced parasite multiplication rates or serum growth inhibitory effect) provides preliminary evidence in humans that the classical *P. falciparum* blood-stage antigen MSP1 is in fact a multi-stage antigen and can be targeted in the liver, in agreement with data from chimpanzees, and those in mice showing efficacy of MSP1-specific T cells against liver-stage parasites. A median delay of 2.5 days to patency would equate to >90% reduction in mean liver parasite burden – an effect larger than that reported in mice and possibly reflecting the 6-7 day, rather than 48 hour, liver-stage infection of humans and rodents, respectively. However, early vaccine-induced control of blood-stage parasitaemia that is subsequently lost cannot be ruled out, and larger phase IIa studies will address this issue and better define the overall level of efficacy achievable.

This MSP1-based vaccine, using an insert designed to address target antigen diversity, is intended to be combined with other antigens, such as ME-TRAP (Ewer *et al.*, submitted), in a multi-component multi-stage vectored vaccine strategy, and thus levels of efficacy short of sterile protection are of interest for inclusion in a combination product.

This AdCh63-MVA viral vectored vaccine regimen now provides a safe and clinically-relevant strategy for the development of vaccines against other difficult diseases where strong cellular and/or humoral immune responses are likely to be required for protection.