Clinical Study Report

A Phase I/IIa Study of the Safety, Immunogenicity and Parasite Growth Inhibitory Activity of AMA1-C1/Alhydrogel® + CPG 7909, an Asexual Blood Stage Vaccine for Plasmodium falciparum Malaria

VAC035

OXREC A: 07/H0604/137
Eudra CT number: 2007-005389-11
CTA number: 21584/0250/001-0001

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

Trial Clinician & Report Author

Signed: Date: ____/____/_____

Print name: Dr A. Lawrie

Project Manager

2. OVERVIEW

Study title:	A Phase I/IIa Study of the Safety, Immunogenicity and Parasite Growth Inhibitory Activity of AMA1-C1/Alhydrogel® + CPG 7909, an Asexual Blood Stage Vaccine for *Plasmodium falciparum* Malaria
Trial code:	VAC035
Study description:	Open label Phase I/IIa study
Test IMPs:	AMA1-C1/Alhydrogel® + CPG 7909
Indication studied:	Safety, immunogenicity & efficacy
Sponsor:	University of Oxford
Chief Investigator:	Professor A.V.S. Hill, University of Oxford, UK
Co-Investigator:	Dr Ruth Ellis, Laboratory of Malaria Imunology and Vaccinology (LMIV), NIAID/NIH, USA.
Co-Investigator:	Dr Saul Faust, Wellcome Trust Clinical Research Facility University of Southampton, UK
Study centres:	Centre for Clinical Vaccinology and Tropical Medicine Churchill Hospital Old Road Headington Oxford OX3 7LJ Wellcome Trust Clinical Research Facility Southampton General Hospital C Level, West Wing Mailpoint 218 Tremona Road SO16 6YD
Clinical Phase:	I/IIa
Study dates:	26th January 2010 - 28th September 2010
Enrolment:	Completed
Publication:	Duncan C.J.A et al. Impact on Malaria Parasite Multiplication Rates in Infected Volunteers of the Protein-in-Adjuvant Vaccine AMA1-C1/Alhydrogel + CPG 7909. PLoS One. 2011;6(7):e22271. Epub 2011 Jul 22.

GCP Statement: This study was performed in compliance with ICH Good Clinical Practise (GCP) including the archiving of essential documents.

3. PROTOCOL SYNOPSIS

Objectives	Primary Objective: To demonstrate a correlation between *in-vitro* growth inhibition assay (GIA) and parasite multiplication rate (PMR) *in-vivo*. Secondary Objective: To detect differences in the parasite multiplication rate responses between unvaccinated control subjects and volunteers vaccinated with AMA1-C1/Alhydrogel® + CPG 7909. Tertiary Objective: To assess the safety, reactogenicity and immunogenicity of the AMA1-C1/Alhydrogel® + CPG 7909 vaccine.
Trial design	Non randomised, un-blinded phase I/IIa trial in healthy, malaria naïve adults.
Sample Size	Group 1 (n=7) 2 doses of AMA1-C1/Alhydrogel® + CPG 7909 ((80 µg AMA1-C1, 800 µg Alhydrogel, 564 µg CPG 7909) intramuscularly at 0 and 8 weeks followed 2 weeks later by intravenous inoculation with *p. falciparum* infected erythrocytes. Group 2 (n=3) Intravenous inoculation with *P. falciparum* infected erythrocytes. Total: 10 volunteers The primary endpoint sample size calculation estimated that with 5 control subjects and 12 vaccinated subjects there was greater than 80% power to demonstrate a correlation coefficient > 0.5 if the true correlation coefficient was 0.7. The study was not specifically powered to assess the secondary endpoint. Although the planned sample size included 12 vaccinated volunteers and 5 controls (with a total 6 controls and 15 vaccinated volunteers to allow for drop-outs), recruitment was halted after interim primary endpoint analysis on the vaccinated volunteers (unadjusted for interim monitoring), which demonstrated that the primary endpoint had been reached after the initial challenge of 5 vaccinated volunteers and 3 controls.
Main criteria for inclusion	• Subject is willing and able to give informed consent for participation in the study • Healthy, non-pregnant adult aged 18 - 50 years • Resident in or near Oxford for the duration of the challenge study

	• Seropositive for Cytomegalovirus and Epstein Barr virus • Female subjects of child bearing potential must be willing to ensure that they practice effective contraception during the study • Males must be willing to use barrier contraception from day of first vaccination onwards until 3 months after the second vaccination • Able (in the Investigator's opinion) and willing to comply with all study requirements • Willing to allow his or her General Practitioner and consultant, if appropriate, to be notified of participation in the study • Agreement to permanently refrain from blood donation.
Duration of treatment	7 volunteers received two vaccinations with AMA1-C1/Alhydrogel® + CPG 7909 intramuscularly. All volunteers underwent intravenous inoculation with *P. falciparum* infected erythrocytes ('Blood-stage' challenge).
Criteria for Evaluation of Objectives	Primary Objective: Correlation between growth inhibitory assay results and parasite multiplication rates. Secondary Objective: Comparison of parasite multiplication rates between vaccinees and controls. Tertiary Objectives: Actively and passively collected data on adverse events and markers of humoral and cell-mediated immunity.
Statistical methods	Data was classified as skewed (ELISA, ELIspot, qPCR, ICS) or not (GIA, PMR), and the former log-transformed in Pearson's correlation coefficient (r). Groups were summarised by arithmetic (non-skewed or log transformed skewed data) or geometric means (skewed) with 95% confidence intervals (CI) or medians with inter-quartile ranges (IQR), and these were compared by t test (two sample, non-skewed), Mann-Whitney test (two sample, skewed), or Wilcoxon signed rank test (paired, skewed). Safety data analysis was mainly descriptive; however the Fisher's exact test was calculated to determine the significance of any difference in the proportions of volunteers with specific adverse events following the first and second vaccinations. All tests were two-tailed with $P < 0.05$ considered significant, using STATA Release 11.0 and Graph-Pad Prism 5.0. Time to positive qPCR or blood film was assessed using survival methods (Kaplan-

	Meier, log-rank test).
Blinding	Non-Blinded
Controls	3 non-vaccinated infectivity controls.
Randomisation	Non Randomised

4. ETHICS AND REGULATORY APPROVAL

INDEPENDENT ETHICS COMMITTEE APPROVAL

The study protocol and related documents were reviewed and approved by the Oxford Research Ethics Committee A. The initial ethical approval for the trial was given on 3rd December 2007, 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: 21584/0250/001-0001
- Eudra CT number: 2007-005389-11

The study was approved by the MHRA on 15th July 2009. Where appropriate, all subsequent substantial amendments were submitted to the MHRA for approval prior to implementation.

5. INVESTIGATORS

Title	Name and affiliation
Chief Investigator & Principal Investigator - Oxford	Professor A.V.S. Hill – University of Oxford
Principal Investigator – Southampton	Dr S. Faust – Wellcome Trust Clinical Research Facility, Southampton
Trial Clinicians	Dr C. Duncan – University of Oxford Dr S. Sheehy – University of Oxford Dr T. Havelock – Wellcome Trust Clinical Research Facility, Southampton
Project Manager	Dr A. Lawrie – University of Oxford
Monitor	Appledown Monitoring
Laboratory Investigators	Dr K. Ewer – University of Oxford Dr S. Draper – University of Oxford Dr A. Douglas – University of Oxford Ms K. Collins – University of Oxford Mr S. Elias – University of Oxford Ms F. Halstead – University of Oxford

6. DESCRIPTION OF INVESTIGATIONAL PRODUCTS

AMA1-C1/Alhydrogel® is protein-adjuvant vaccine targeting the *P. falciparum* blood-stage antigen AMA1. It contains protein that corresponds to the ectodomain of the mature AMA1 protein from the FVO and 3D7 strains of *P. falciparum* respectively. It is supplied as a slightly turbid suspension in single dose vials for IM administration. Each 2.0 mL vial contains 1.0 mL. 0.5 mL of vaccine contains 800 µg Alhydrogel® (up to the equivalent of 400 µg of aluminium) onto which 80 µg of recombinant AMA1-C1 has been bound. AMA1-C1/Alhydrogel® was manufactured at the WRAIR Bioproduction Facility (Silver Spring, Maryland) according to cGMP. The Drug Substances were prepared in unbuffered, isotonic saline without preservatives. The product conforms to the US Code of Federal Regulations for endotoxin, sterility, and general safety. Aluminium hydroxide gel (Alhydrogel®, HCI Biosector, Denmark) has been extensively used as an adjuvant in many licensed human vaccines. Aluminium-containing adjuvants are in routine human use and contained in many licensed human vaccines.

CPG 7909 is a short synthetic oligodeoxynucleotide adjuvant. It is supplied in sterile single dose vials of 0.08 mL at 10 mg/mL in saline. CPG 7909 is manufactured according to cGMP standards and conforms to the US Code of Federal Regulations for endotoxin, sterility, and general safety.

7. STUDY POPULATION

Participant flow is summarised in Figure 1. Recruitment began in July 2009 and continued until March 2010. The first volunteers were vaccinated in January 2010 and the challenge occurred in April 2010. The final study visits occurred in September 2010. All volunteers were recruited, screened, enrolled and followed up at the Oxford site.

Seventy-five potential participants were screened for inclusion. Fifty-four volunteers did not meet inclusion criteria; 52 of these had negative IgG antibody to either Epstein Barr Virus (EBV) or Cytomegalovirus (CMV). Six volunteers were vaccinated initially. One volunteer withdrew consent after the first vaccination after moving out of the study area, and was replaced. This replacement volunteer (V5) therefore received both vaccines with a four-week interval, whilst other volunteers received two vaccines with an eight-week interval. Both intervals were immunogenic in previous phase I trials, and this represented the only deviation from the study protocol during the study (Table 1). Another vaccinated volunteer withdrew from the study prior to the challenge, again because they left the study area.

In total five vaccinated volunteers were challenged together with three unvaccinated control volunteers. All volunteers gave written informed consent for leftover blood samples to be *"stored for up to 15 years and used in further studies of the body's immune response to malaria vaccination and malaria"* (A Study of Exploratory Immunological Assays to Provide a Laboratory Based Correlate of Protection From Malaria; OXREC Number: 06/Q1606/123).

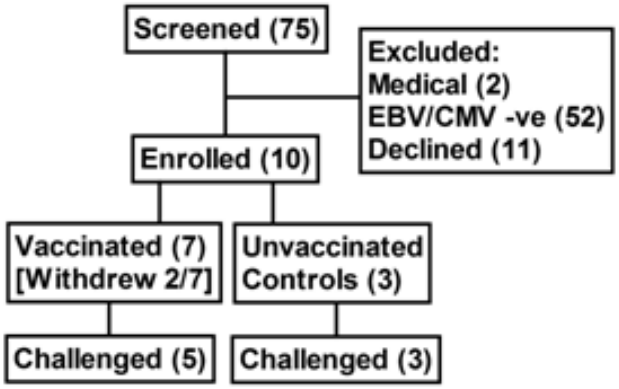


Figure 1: Participant Flow

8. PROTOCOL DEVIATIONS

Deviation	*Site: Oxford*	*Site: Southampton*
Entry criteria	0	0
Withdrawal criteria	0	0
Dosing regimen	1*	0
Concomitant medication	0	0
Other*	0	0

Table 1: *Protocol deviations.* See section 7.*

9. RESULTS

9.1 DEMOGRAPHICS OF STUDY POPULATION

Characteristics	**Vaccine Group** (n = 7)	**Control Group** (n = 3)
Male (%)[*]	4 (57)	1 (33)
Median age (range) [†]	28 (22-45)	27 (25-29)
Median time to inoculation in minutes (IQR) [†µ]	4 (0.5-7.5)	4 (0-4)

Table 2: *Demographics of volunteers. No significant differences were identified between the groups using *P = 1.0, Fisher's exact test and †P = 0.83, Mann-Whitney test. µApplies only to the 5/7 vaccinated volunteers who underwent challenge. IQR = interquartile range.*

The baseline demographic details of the participants in the vaccine and control groups are included in Table 2. The groups were similar in distribution for both age (vaccinees median 28 (range: 22-45), controls median 27 (range 25-29) and gender (male: female ratio in vaccinees 4: 3; controls 1: 2). Inoculum viability was 25% of the pre-freeze parasitemia, therefore the actual inoculum delivered was 250 viable parasites per volunteer.

9.2 PRIMARY OUTCOME – Correlation between GIA & PMR

Only three positive qPCR data points were available for one control volunteer (C1), insufficient for accurate estimation of PMR for this individual. This volunteer is therefore excluded from primary and secondary outcome analysis. There was a significant inverse correlation between PMR and homologous-strain (3D7) GIA on day of challenge (day 70) in the vaccine recipients (r = -0.93 [95% CI: -1.0, -0.27] P = 0.02, Figure 2). This was also observed for log10 ELISA against homologous-strain (3D7) AMA1 on day of challenge (day 70) (r = -0.93 [95% CI: -1.0, -0.25] P = 0.02, Figure 2). When immunised and control volunteers were analysed together the GIA correlation became non-significant (r = -0.61 [95% CI: -0.94, 0.27] P = 0.15, Figure 2). No correlation was observed with PMR and heterologous-strain (FVO) GIA and log10 ELISA responses (data not shown).

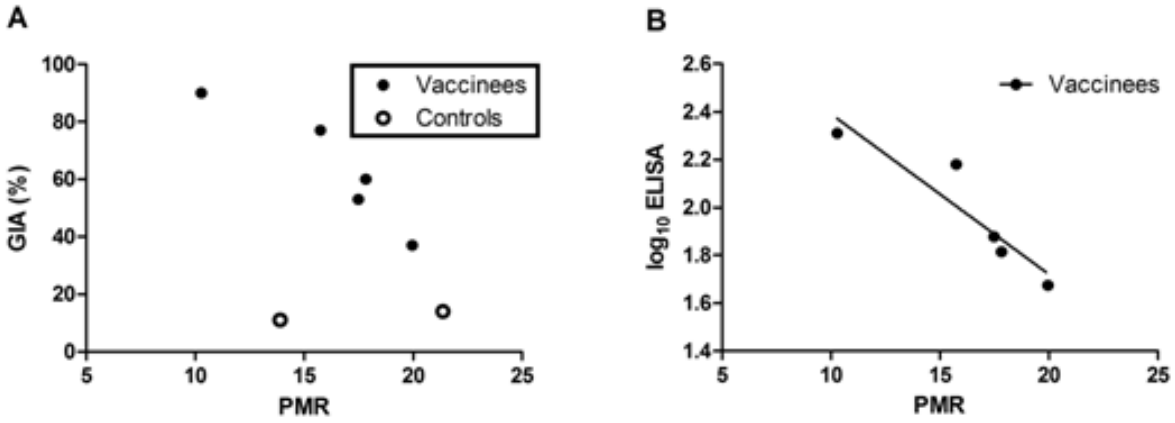
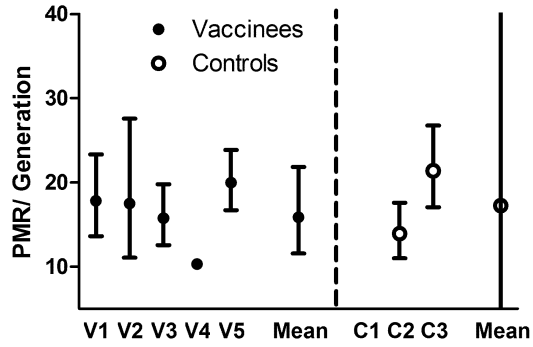


Figure 2. Vaccine-Induced in vitro Growth Inhibitory Activity (GIA) and Antibody Titre Correlates with in vivo Parasite Multiplication Rate (PMR). *(●) Represents immunised volunteers and (○) represents control volunteers. All analyses are two-tailed Pearson correlation coefficients. Assays of GIA and ELISA (both 3D7-AMA1) were performed once in triplicate on day of challenge samples. GIA is expressed as percent inhibition calculated as follows: 100 – [(O.D.650 of infected RBCs with tested IgG – O.D.650 of normal RBCs only)/(O.D.650 of infected RBCs without any IgG – O.D.650 of normal RBCs only) x_100]. ELISA units are log10 µg/ml. Parasite multiplication rate per 48-hours was modelled from qPCR data.A. Correlation between vaccine-induced GIA (% of infected RBCs without IgG) on day of challenge and 48-hour PMR (r = -0.93 [95% CI: -1.0, -0.27] P = 0.02). When all volunteers (vaccinated and control) were examined together there was a trend towards an association (r = -0.61 [95% CI: -0.94, 0.27] P = 0.15).B. Correlation between log10 transformed 3D7-AMA1 ELISA (µg/ml) and 48-hour PMR (r = -0.93 [95% CI: -0.99, -0.25] P = 0.02).*

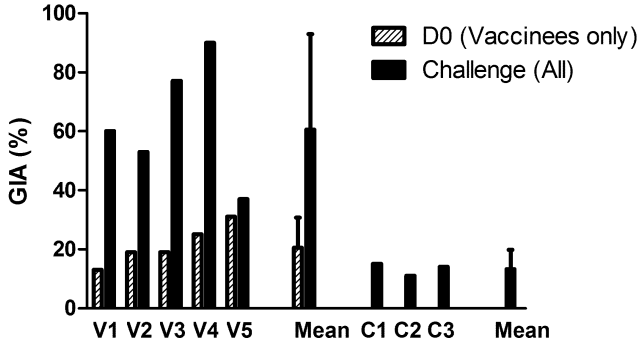
9.2 SECONDARY OUTCOME – Comparison of PMR between vaccinees & controls

Assessment of secondary outcome was limited by having only two control volunteers in which PMR could be accurately modelled. No significant difference was observed in mean PMR between the vaccine and control groups (vaccine group 16-fold [95% CI: 12-22], control group 17-fold [95% CI: 0-65], P = 0.70 t test, Figure 3A). The parasite multiplication rates in 48 hours (with 95% CIs) for individual vaccinated volunteers were: V1: 17.8 fold (13.6-23.3); V2: 17.5 fold (11.1-27.6); V3: 15.7 fold (12.5-19.8); V4: 10.3 fold (10.1-10.4); V5: 20.0 fold (16.7-23.9). For individual control volunteers the PMR was C2: 13.9 fold (11.0-17.6), and C3: 21.4 fold (17.1-26.8), Figure 3A. The modelling strategy fitted the data well. The mean R2 value was 0.93 for vaccinated subjects and 0.92 for controls with a mean of 6.6 PCR data points per volunteer.

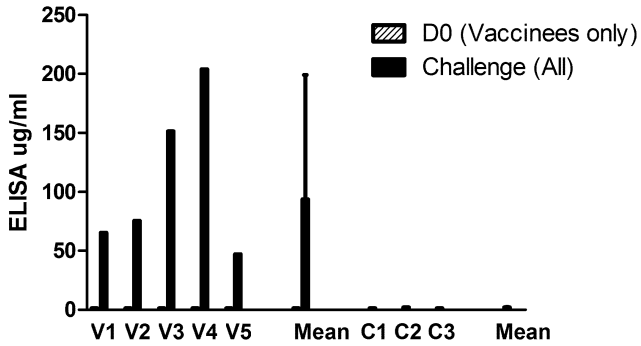
A


Vaccinees
Controls
PMR/ Generation
40
30
20
10
V1 V2 V3 V4 V5 Mean C1 C2 C3 Mean

B


D0 (Vaccinees only)
Challenge (All)
GIA (%)
100
80
60
40
20
0
V1 V2 V3 V4 V5 Mean C1 C2 C3 Mean

C


D0 (Vaccinees only)
Challenge (All)
ELISA ug/ml
250
200
150
100
50
0
V1 V2 V3 V4 V5 Mean C1 C2 C3 Mean

D

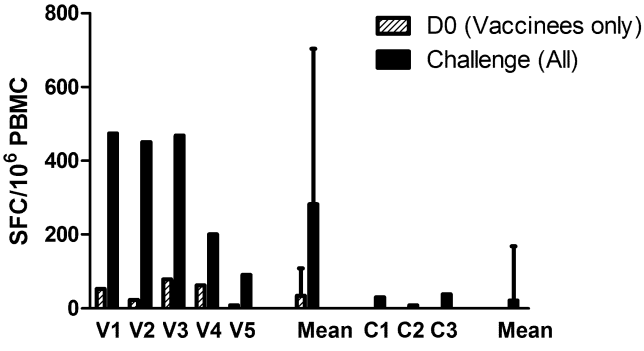

D0 (Vaccinees only)
Challenge (All)
SFC/10^6 PBMC
800
600
400
200
0
V1 V2 V3 V4 V5 Mean C1 C2 C3 Mean

Figure 3. Individual Subject Parasite Multiplication Rates and Immunological Measures at Day of Challenge. *(●) Represents immunised volunteers and (○) represents control volunteers. All panels display means and error bars represent 95% confidence intervals. Assays of GIA and ELISA were performed once in triplicate. ELIspot assays were performed once in duplicate. GIA is expressed as percent inhibition calculated as follows: 100 – [(O.D.650 of infected RBCs with tested IgG – O.D.650 of normal RBCs only)/(O.D.650 of infected RBCs without any IgG – O.D.650 of normal RBCs only) x_100]. ELISA units are µg/ml, ELIspot units are IFN-γ spot forming colonies (SFCs) per 106 PBMCs. Parasite multiplication rate per 48-hours was modelled from qPCR data. A. 48-hour parasite multiplication rates (PMR) for individuals and arithmetic mean 48-hour PMR for the group. PMR for volunteer C1 could not be accurately modeled as there were only three qPCR data-points [7]. Arithmetic mean PMRs were not significantly different (vaccine 16-fold [95% CI: 12-22] (n = 5), control 17-fold [95% CI: 0-65] (n = 2) P = 0.70, t test). B. Individual and group mean percentage GIA. There were similar levels of detectable GIA in all volunteers at enrolment (d0 for immunised group, day of challenge for control group); mean vaccine group 21% [95% CI: 13-30] (n = 5), control group 13% [95% CI: 8-19] (n = 3) P = 0.10, t test.C. Geometric mean antibody ELISA (µg/ml) and D. geometric mean T cell ELIspot responses (IFN-γ SFC/106 PMBC) to 3D7-AMA1 at day 0 (immunised group) and day of challenge (all groups, the first assessment for controls was day of challenge). All immunology endpoints were significantly higher in vaccinees than controls at challenge (GIA P < 0.01 t test; ELIspot P = 0.04 Mann-Whitney; ELISA P = 0.04 Mann-Whitney).*

9.3 TERTIARY OUTCOMES

ADVERSE EVENTS

No unexpected or serious adverse events (AEs) occurred. Vaccine-related AEs occurred at a similar frequency following both vaccinations (Figure 4 & Table 3). The majority of AEs were grade 1 in severity. Overall, the median duration of all injection-site AEs was 3 days (IQR: 2 - 3.75). There was no significant difference in the duration of local or systemic AEs between the first or second doses (local P = 0.14; systemic P = 0.12, Mann Whitney test). The only vaccine-related laboratory abnormality was transient grade 1 leucopenia in a single subject following the first vaccination, which is expected with CPG 7909. Double-stranded DNA antibodies were not observed in any volunteers.

At the end of the study, the only on-going AE, grade 1 systolic hypertension, was deemed unrelated to vaccination. With the volunteer's permission the GP was informed and agreed to arrange appropriate investigation and follow-up of this AE.

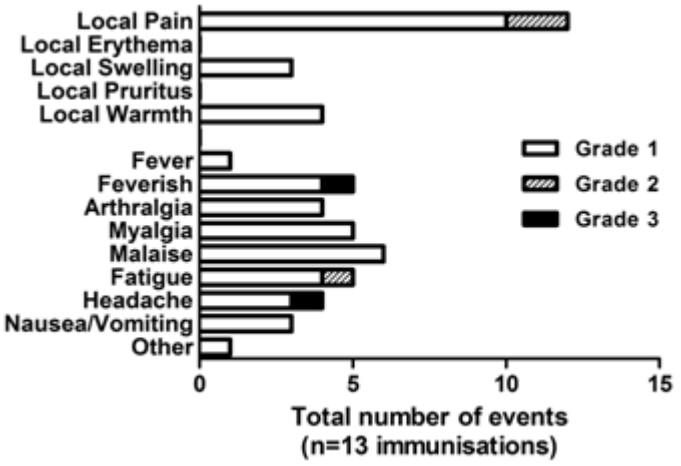


Figure 4. Adverse Events. *All solicited and unsolicited adverse events post-vaccination considered possibly, probably or definitely vaccine-related up to day 140. One volunteer experienced a grade 3 headache and rigors on the evening of the first dose (day 0) which required oral analgesia and resulted in a missed day of work. The rigors resolved within several hours and the headache reduced in intensity within 24 hours, and resolved on day 2. 'Other' refers to transient injection-site discomfort relating to minor trauma 5 days after vaccination.*

Adverse Event	**Dose 1 (n = 7)** N. of volunteers reporting by severity			**Dose 2 (n = 6)** N. of volunteers reporting by severity			**All (n = 13 doses)** N. (%) of volunteers reporting AE at least once by severity		
	Severity (grade)			*Severity* (grade)			*Severity* (grade)		
	1	2	3	1	2	3	1	2	3
Erythema	0	0	0	0	0	0	0	0	0
Pain	6	0	0	2	2	0	4 (57)	2 (29)	0
Swelling	1	0	0	1	0	0	2 (29)	0	0
Warmth	1	0	0	3	0	0	4 (57)	0	0
Arthralgia	2	0	0	2	0	0	2 (29)	0	0
Fatigue	1	1	0	3	0	0	2 (29)	1 (14)	0
Feverishness	2	0	1*	2	0	0	2 (29)	0	1 (14)
Headache	1	0	1*	2	0	0	1 (14)	0	1 (14)
Malaise	3	0	0	3	0	0	4 (57)	0	0
Myalgia	3	0	0	2	0	0	3 (43)	0	0
Nausea	1	0	0	1	0	0	1 (14)	0	0
Pyrexia	0	0	0	1	0	0	1 (14)	0	0
Other†	1	0	0	0	0	0	1 (14)	0	0

Table 3. *Solicited and unsolicited adverse events (AEs) post-vaccination with AMA1/C1/Alhydrogel + CPG 7909. The maximum severity of any AE experienced by the volunteer is recorded. Overall percentage of AEs experienced by volunteers after either dose is summarized in final column. Some AEs were reported by the same volunteer after both immunisations. *Both grade 3 systemic AEs occurred simultaneously in the same volunteer. †Recurrent minor transient discomfort at injection-site day 5 following dose 1. No significant differences in proportion of volunteers experiencing AEs between dose 1 and dose 2 were identified by Fischer's exact test.*

IMMUNOGENICITY - HUMORAL

On the day of challenge, mean percentage vaccine-induced GIA was significantly greater in the vaccine group (63% [95% CI: 38 - 90]) than the control group (13% [95% CI: 9 - 19] $P < 0.01$, t test Figure 3B). GIA was maintained but not boosted at 28 days post-challenge (61% [95% CI: 17-100]), and did not increase significantly post-challenge in the control group.

A significant increase in geometric mean 3D7-strain antibody response measured by ELISA (µg/mL) was observed after the first immunization (n = 7) (D28: 8.3 [95% CI: 4.0 – 17.5] vs D0: 1.27 [range 1.27 – 1.27], $P = 0.02$ Wilcoxon signed rank test, Figure 4A). Responses were non-significantly boosted by the second immunisation in challenged volunteers (n = 5) (D63: 117.4 [95% CI: 58.9 – 233.9] vs D28, $P = 0.06$ Wilcoxon signed rank test, Figure 4A), and were maintained but not boosted by challenge. ELISA titre was significantly higher in vaccinated volunteers at day of challenge (geometric mean vaccine group 93.6 µg/ml [95% CI: 43.9 – 199.3], control group 1.5 µg/ml [95% CI: 0.8 – 2.8] $P = 0.04$, Mann-Whitney test, Figure 3C). No significant increase in antibody titre was identified in the control volunteers (n = 3) post-challenge.

As we have previously observed, 3D7 GIA and 3D7 $\log_{10}$ ELISA titers correlated strongly (r = 0.97, $P < 0.01$), as did 3D7 and FVO $\log_{10}$ ELISA titers (r = 0.90, $P = 0.03$).

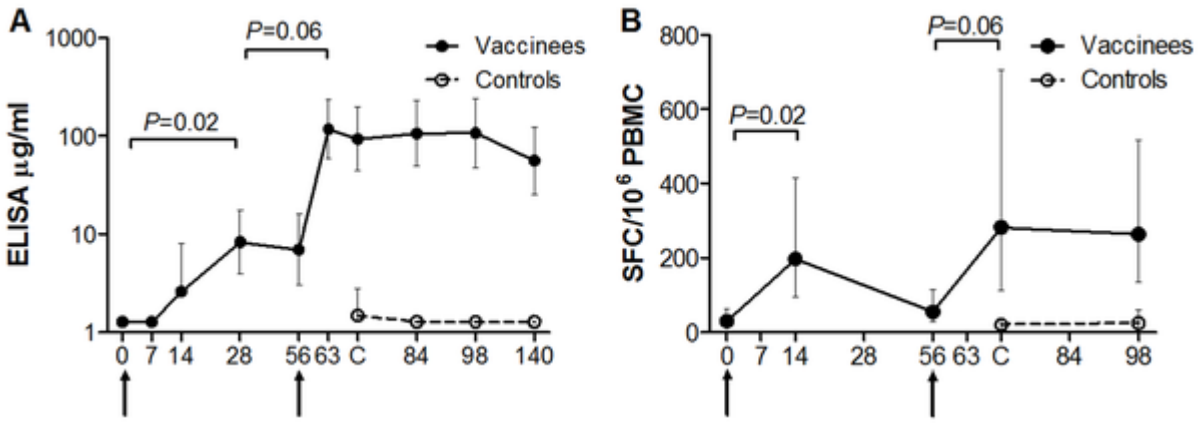


Figure 4. Timecourse of Homologous Strain Antibody and T Cell Responses. *(●) Represents immunised volunteers and (○) represents control volunteers. Numbers on 'x' axes represent days of follow-up. Arrows represent immunisations. Geometric mean 3D7-strain AMA1 antibody responses by ELISA (µg/ml) (A) and ex vivo 3D7-strain IFN-γ ELIspot (SFC/106 PMBC) (B) for immunised volunteers (n = 7) and controls (n = 3) are presented. ELISA assays were performed once in triplicate. ELIspot assays were performed once in duplicate. Statistical comparison with two-tailed Wilcoxon signed rank test. A. A significant increase in 3D7-strain AMA1 antibody responses by ELISA (µg/ml) was observed for first immunisation (n = 7). Responses were non-significantly boosted by the second immunisation in challenged volunteers (n = 5) and were maintained at day 140. No significant increase in detectable response was identified in the control volunteers post-challenge. B. A significant increase in ex vivo 3D7-strain IFN-γ ELIspot (SFC/106 PMBC) response was observed following the first immunisation in all*

volunteers (n = 7). Responses were non-significantly boosted by the second immunisation in challenged volunteers (n = 5). No significant increase in detectable response was observed in control volunteers post-challenge.

IMMUNOGENICITY – CELLULAR

A significant increase in geometric mean T cell response measured by *ex vivo* IFN-γ ELIspot spot-forming colonies (SFC) per 10^6 PBMCs was observed following the first immunisation in all volunteers (n = 7) (D14: 197.8 [95% CI: 94.2 – 415.7]) vs D0: 30.1 [95% CI: 14.3 – 62.0], *P* = 0.02 Wilcoxon signed rank test, Figure 4B). These responses contracted non-significantly by day 56 after the first immunisation (D56 geometric mean 63.6 IFNγ SFC/10^6 PBMCs [95% CI: 27.3 – 147.9]; P = 0.06, Wilcoxon signed-rank test), and were non-significantly boosted by the second immunisation in challenged volunteers (n = 5) (D70 geometric mean 282.4 IFNγ SFC/10^6 PBMCs [95% CI: 113.3 – 704.3] *P* = 0.06 Wilcoxon signed rank test, Figure 4B). T cell responses by ELIspot were significantly higher in vaccinated volunteers at day of challenge (geometric mean vaccine group 282.4 SFC/10^6 PBMCs [95% CI: 113.3 – 704.3], control group 20.9 SFC/10^6 PBMCs [95% CI: 8.0 – 38.0] *P* = 0.04, Mann-Whitney test, Figure 3D). No significant increase in detectable response was observed in control volunteers post-challenge, Figure 4B.

The phenotype of the vaccine-induced T cell responses was predominantly $CD4^+$ by flow cytometry (data not shown). On the day of challenge (dCH) AMA1-C1 antigen-stimulated Th1 responses were detected in all vaccinated volunteers (n = 5) and multifunctional responses were detected. There was a non-significant trend to an increase in the frequency of live $CD3^+$ $CD4^+$ T cells positive for TNF-α, IFN-γ and IL-2 after vaccination (TNF-α d0: 0.003% [range 0.002 - 0.014], dCH: 0.009% [range 0.0 – 0.035], *P* = 0.31; IFN-γ d0: 0.0% [range 0.0 – 0.020], dCH: 0.010% [0.0 – 0.069], *P* = 0.58; IL-2 d0: 0.036% [range 0.022 – 0.061], dCH: 0.051% [range 0.035 – 0.072], *P* = 0.13 Wilcoxon signed rank test, Figure 5). There was no significant correlation between T cell responses on the day of challenge, measured by either ELIspot or ICS, and *in vivo* parasite multiplication (data not shown).

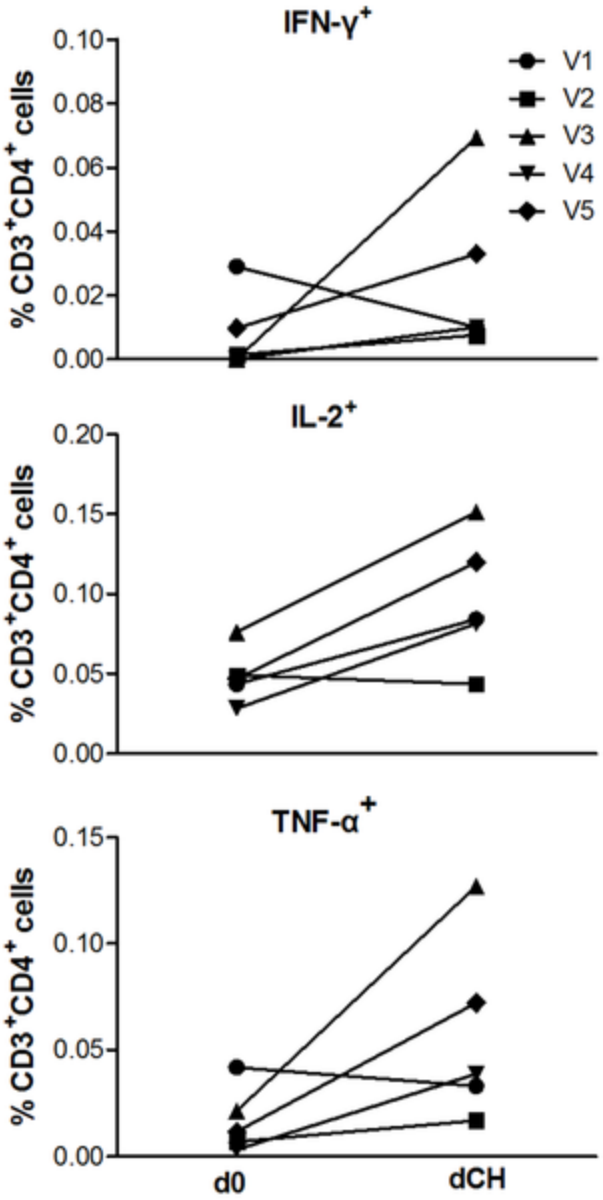


Figure 5. Intracellular Cytokine Staining. *AMA1-C1 protein-stimulated live CD3+ CD4+ T cells positive for the Th1 cytokines IFN-γ TNF-α and IL-2 assayed on cryopreserved PBMCs obtained on the day of enrollment (d0) and day of challenge (dCH) from vaccinated and challenged volunteers (n = 5). There was a non significant increase in median percentage of live CD3+ CD4+ cells positive for TNF-α, IFN-γ and IL-2 (TNF-α d0: 0.003% [range 0.002 - 0.014], dCH: 0.009% [range 0.0 – 0.035], P = 0.31; IFN-γ d0: 0.0% [range 0.0 – 0.020], dCH: 0.010% [0.0 – 0.069], P = 0.58; IL-2 d0: 0.036% [range 0.022 – 0.061], dCH: 0.051% [range 0.035 – 0.072], P = 0.13 Wilcoxon signed rank test).*

9.4 ANCILLARY ANALYSES – CHALLENGE ENDPOINTS

All volunteers were inoculated within 40 minutes of inocula preparation. All volunteers developed microscopy positive parasitaemias on thick-film by day 9 post-challenge (range 7-9). Although not surprising for a small sample there was no significant difference in pre-patent period between the vaccine and control groups by survival analysis (vaccine group median 8.5 days (range 7.5 – 9), control group median 9 days (range 7 – 9) P = 0.45 log-rank test, see Figure 6A). There was also no significant difference in days to first positive PCR between the vaccine and control groups by survival analysis (vaccine group median 5.5 days (range 5 – 5.5), control group median 5.5 days (range 5 – 6.5) P = 0.40 log-rank test, see Figure 6B). Individual volunteer qPCR values are displayed in Figure 7A (vaccine group) and Figure 7B (control group). Parasite density at diagnosis (vaccine group median 4602 p/ml [IQR: 1472-19632], control group median 3613 p/ml [IQR: 543-11402]) was not significantly different (P = 0.79 Mann-Whitney test, Figure 7C). Only 2/8 volunteers developed a malaria symptom (grade 1 myalgia in 2/8, feverishness in 1/8) at the time of blood-film diagnosis. After treatment initiation six volunteers developed minor grade 1 malaria symptoms. None had objective fever. There were no challenge-related laboratory abnormalities.

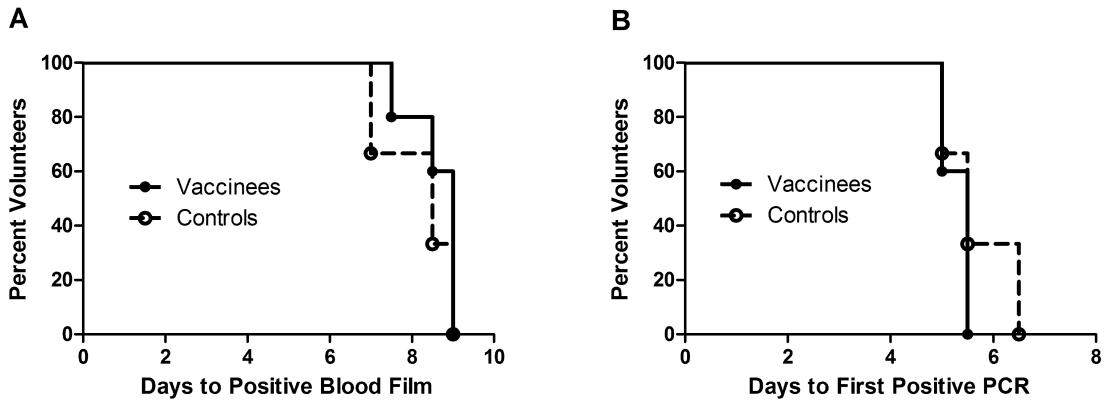


Figure 6. Kaplan-Meier Survival Analysis of Time to Parasitaemia. *Survival analysis of A. time to parasitaemia by thick blood film microscopy (P = 0.45 log-rank test), vaccine group (bold line) mean days 8.6 (95% CI: 7.8-9.4), control group (hatched line) mean days 8.2 (95% CI: 5.2-10.8). B. Time to first positive qPCR value (P = 0.40 log-rank test). Vaccine group mean days 5.3 (95% CI: 5.0-5.6), control group mean days 5.7 (95% CI: 3.8-7.6).*

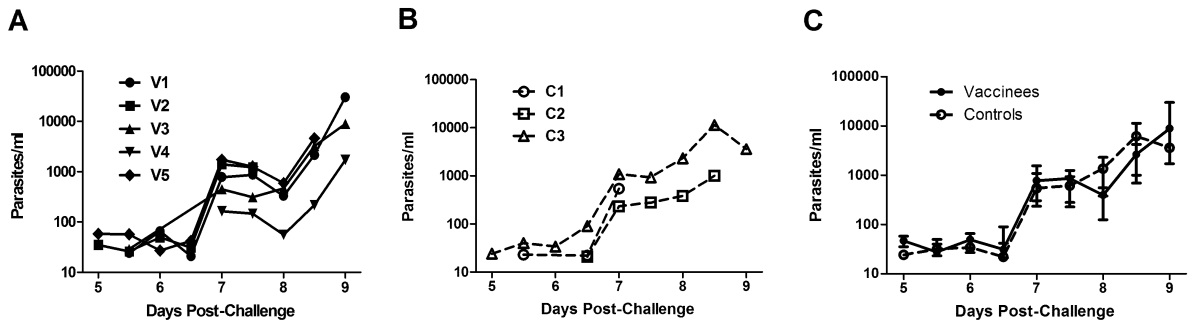


Figure 7. Quantitative PCR. *Individual qPCR data for **A**. immunised and **B**. control volunteers. **C**. Median qPCR data (with interquartile ranges) for vaccine and control groups. No significant differences were observed between the groups at any time-point (data not shown). qPCR was performed once in triplicate at each timepoint.*

10. CONCLUSION & DISCUSSION

We observed a significant inverse relationship between vaccine-induced GIA and parasite multiplication rate in vaccinated subjects. This is an important observation in a small sample and needs to be confirmed in larger studies. However, there was no evidence to suggest that the magnitude of the vaccine-induced effect was sufficient to impact overall PMR in the vaccinated volunteers, and there was consequently no effect on pre-patent period. However the small sample size, particularly that of the control group, limited the statistical power to assess differences in PMR between the groups.

GIA induced in most vaccinated volunteers may have been insufficient to significantly reduce PMR in comparison to unvaccinated controls. Although the volunteer with 90% in vitro growth-inhibitory activity had the lowest parasite multiplication rate (10-fold), in most volunteers the GIA did not reach this level. Other adjuvanted AMA1 vaccines have induced similar levels of GIA without a significant impact on estimated PMR, albeit after sporozoite challenge.[1] Modelled estimates suggest much greater antibody levels are required to control in vivo parasite growth.[2] Non-human primate studies have shown that very high antibody titers and in vitro growth inhibition of >70% (using serum, not purified IgG) are needed for protection with AMA1.[3] Similar results in primates have been reported for another blood-stage antigen, MSP1.[4] A recent study indicates the pre-patent PMR in semi-immune Gambian adults is significantly lower than has ever been achieved by a blood-stage vaccine candidate in humans.[5]

As well as antibodies,[6] other immune responses are likely to be involved in determining *in vivo* PMR including cytokines,[7] T effector[8] and T regulatory cells[7] and antibody-dependent cellular inhibition by monocytes[9,10] and neutrophils.[11] We observed a clear relationship between homologous-strain log10 ELISA titre and PMR in vaccinees but there was no relationship between PMR and the modest vaccine-induced CD4+ T cell responses measured by ex-vivo IFN-γ ELIspot or ICS. This may reflect the insufficient magnitude of the T cell response, lack of statistical power to detect such a relationship, analysis of the non-protective T cell phenotype, or the absence of an association.

The association between GIA and PMR in vaccinees reported in this study provides some support for a protective role of very high levels of GIA-inducing antibodies, but this result need not imply causation. A recent meta-analysis of prospective sero-epidemiological studies demonstrated an association between IgG to merozoite proteins and reduced clinical malaria.[6] Fewer studies have prospectively assessed functional antibody responses such as GIA.[6,12] Most of these studies suggest that antibody demonstrating inhibitory activity *in vitro* contributes to a reduced risk of clinical malaria,[13-15] although some do not[16] (reviewed in[12]). The data are similarly conflicting in numerous animal models,[3,4,16] suggesting that multiple potential immune effector mechanisms may operate in humans (reviewed in[12]). Data from a sporozoite challenge trial of an AMA1-containing multi-stage virosomal vaccine demonstrated significant reduction in PMR without detectable GIA or cellular responses.[17] However, there was also a trend to reduced liver-emerging parasites. A similar significant reduction in liver-emerging parasites was observed with the adjuvanted AMA1 vaccine discussed above,[1] which may have also induced strain-specific efficacy in a field trial in Malian children [Ouattara A., Takala-Harrison S. et al. Allele-Specific Efficacy of the Monovalent Apical Membrane Antigen 1 (AMA1) Malaria Vaccine FMP2.1/AS02A, American Society of Tropical Medicine and Hygiene, November 2010, Abstract #803]. In these studies it is therefore difficult to rule-out the possibility that PMR could have been influenced by immune

responses to sporozoites, liver-stage parasites or liver-emerging merozoites, all of which express AMA1.[1] An inherent limitation of the blood-stage challenge model is thus an inability to detect any pre-erythrocytic vaccine efficacy. Another limitation specific to this inoculum is the requirement for volunteer EBV and CMV seropositivity, which adversely impacted recruitment in this study.

There was an unexpectedly low frequency of clinical malaria symptoms pre-diagnosis (2/8 subjects) in this study in comparison to published data on the clinical features of experimental malaria in healthy volunteers following sporozoite challenge.[18] Parasite density at microscopic diagnosis (geometric-mean 4012 p/ml) was similar to that recorded in sporozoite-challenged control volunteers in a recent study (geometric-mean 4030 p/ml), 10/12 of whom were symptomatic at diagnosis [Ewer K, O'Hara GA, Duncan CJA et al., submitted], suggesting potential attenuated pathogenicity of the blood-stage challenge parasites. Differential expression and rate of switching of expressed var genes by parasites in this inoculum after blood-stage passage has been reported, which could explain the reduction in clinical symptoms observed.[19]

This is the first trial in humans to explore the relationship between vaccine-induced inhibitory antibodies and *in vivo* parasite growth rates following experimental blood-stage malaria infection. It increases by more than a quarter the total number of volunteers who have been experimentally infected with this inoculum, and is only the second vaccine efficacy study using this model. As the first Phase IIa challenge trial of AMA1-C1/Alhydrogel + CPG 7909, it contributes further safety and immunogenicity data on this protein-adjuvant combination. However, there is insufficient evidence to support future phase IIb clinical trials of this vaccine formulation in the immunisation regimen assessed here. While the blood-stage challenge model has limitations, the use of challenge studies (both blood-stage and sporozoite) should greatly speed the clinical development of blood-stage vaccines, allowing early demonstration of possible benefit and rational down-selection of vaccine candidates prior to field trials.

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