Study Reference: VAC063

A Phase I/IIa clinical trial to assess the safety, immunogenicity and efficacy of the blood-stage *Plasmodium falciparum* malaria vaccine candidate RH5.1/AS01

Sponsor: University of Oxford

Chief Investigator: Dr Angela M. Minassian

Modification History

Version	Date	Modifications	Author(s)
1.0	2nd June 2016	N/A	Ruth Payne, Simon Draper, Johan Vekemans, David Pattinson
2.0	15th September 2016	Addition of information regarding the use of the informed consent questionnaire Correction of Table 4: Schedule of attendances for Group 3 to reflect actions carried out on day of third vaccination (day 182) which had been incorrectly listed in day 56. Similar correction on page 11, dose section Addition of wording to clarify that stated vaccine and adjuvant doses are nominal and may vary slightly due to dilution, mixing and administration techniques. Blood volumes for Groups 1-4 corrected to include 3 mL Tempus tubes on vaccination days and 1 day post-vaccination visits. Sentence added that an ECG will be done as part of Group 5-6 screening. (correction, as information is already in PIS G5-6) Change in wording of exclusion criterion regarding prior receipt of an investigational malaria vaccine "History of splenectomy" removed as a separate exclusion criterion	Ruth Payne Angela Minassian
3.0	29th May 2017	Selection of dose of RH5.1/AS01 (10 µg) for Group 5 in the Phase IIa study Guy's and St. Thomas' NIHR CRF to continue as a recruitment and vaccination site for the Phase IIa study Removal of Cardiovascular Risk Score and checking of cholesterol levels from the protocol Correction of blood volumes in schedule of attendances for Groups 5 and 6 (Tables 5 & 6) and in groups 1-6 in Table 14 Clarification of vaccination of 2 back-up volunteers in Group 5 (as already explained in the PIS) Clarification of challenge at 2 weeks post final vaccination Blinding of all Investigators (except the Principal Laboratory Investigator) to the malaria qPCR results during the post-challenge phase	Angela Minassian Simon Draper
4.0	11th September 2017	Modification of bleed schedule for Groups 5 and 6. Removal of cholesterol measurement from footer of table 6 Details of biochemistry added to footer of table	Simon Draper Angela Minassian

		5	
5.0	23rd November 2017	Addition of conflicts of interests for Dr AM Minassian and Prof SJ Draper on page 8 Correction of 2 minor typographical errors (and subsequent cumulative volumes) in the bleeding schedule tables for groups 5 and 6 (Tables 5 & 6), and in Table 14 (Groups 5-6)	Angela Minassian
6.0	23rd January 2018	Addition of 3 new groups to assess the durability of the vaccine-induced reduction in PMR (Group 7 = Phase IIa malaria-exposed vaccinees; Group 8 = Phase IIa malaria-exposed controls; Group 9 = newly recruited malaria-naïve controls) Removal of microscopy as a diagnostic tool in the second homologous CHMI, and adjustment of the qPCR diagnostic criteria for commencing anti-malarials Addition of 2 new exclusion criteria (Groups 5-9): G6PD deficiency and abnormal haemoglobinopathy screen Clarification of 2 exclusion criteria (relating to Hepatitis C serology and contraindications relating to use of malarone and riamet)	Angela Minassian Simon Draper
7.0	7th February 2018	Clarification of scientific rationale for re-challenge of healthy volunteers (requested by the MHRA)	Angela Minassian
8.0 (not MHRA approved)	12th July 2018	Addition of further CHMI for groups 8 and 9 to assess immunological response to secondary (Group 9) and tertiary (Group 8) homologous CHMI, and addition of new malaria naïve group (Group 10) as comparative controls undergoing primary challenge. Updates to study group sizes and addition of figure to demonstrate volunteer flow through first, second and third CHMI (Figure 5) Addition of last visit for all vaccinated volunteers (Groups 1-4, 5 and 7) to assess durability of immune responses 1-2 years since their final vaccination.	Yrene Themistocleous Angela Minassian
9.0	10th October 2018	Removal of all aspects added as part of SA008 (not MHRA approved) relating to further CHMI for groups 8 and 9 to assess immunological response to secondary (Group 9) and tertiary (Group 8) homologous CHMI, and addition of new malaria naïve group (Group 10) as comparative controls undergoing primary challenge and related changes to study group sizes and figure to demonstrate volunteer flow through first, second and third CHMI. Extension of period for additional last visit for all vaccination volunteers (Groups 1-5 and 7) to 1-2.5 years and changes relating to tests performed at this visit (removal of all tests except for blood draw for immunological analysis, with urine pregnancy testing replaced by requirement of female volunteers to self-	Yrene Themistocleous Angela Minassian

		report pregnancy status at this visit only). Addition of contact to GP to inform them of the participation in this additional visit (with participant consent).	

A Phase I/IIa clinical trial to assess the safety, immunogenicity and efficacy of the blood-stage *Plasmodium falciparum* malaria vaccine candidate RH5.1/AS01

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Investigator Agreement

"I have read this protocol and agree to abide by all provisions set forth therein.

I agree to comply with the principles of the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice."

Chief Investigator	Investigator Signature	Date 26/10/18
Dr Angela M. Minassian		

"I have read this protocol and agree to abide by all provisions set forth therein.

I agree to comply with the principles of the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice."

Senior Laboratory Investigator	Investigator Signature	Date 26/10/18
Prof Simon J. Draper		

"I have read this protocol and agree to abide by all provisions set forth therein.

I agree to comply with the principles of the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice."

Principal Investigator- Guys and St Thomas	Investigator Signature	Date 26/10/18
Dr Anna L. Goodman		

"I have read this protocol and agree to abide by all provisions set forth therein.

I agree to comply with the principles of the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice."

Principal Investigator- Southampton	Investigator Signature	Date 26/10/18
Prof Saul N. Faust		

Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, the Regulatory Authority and members of the Independent Ethics Committee. This information cannot be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of Dr Angela Minassian or Professor Simon Draper.

Conflict of Interest

1. "According to the Declaration of Helsinki, 2008, I have read this protocol, and declare the following conflict of interest"

Details: I have a family member who is an inventor on patents for RH5-based vaccines.

Chief Investigator	**Investigator**	**Signature**	**Date** 26/10/18
Dr Angela M. Minassian			

2. "According to the Declaration of Helsinki, 2008, I have read this protocol, and declare the following conflict of interest"

Details: I am a named inventor on patents relating to RH5-based vaccines.

Principal Investigator	**Investigator**	**Signature**	**Date** 26/10/18
Professor Simon J. Draper			

3. "According to the Declaration of Helsinki, 2008, I have read this protocol, and declare no conflict of interest"

Details:_____

Principal Investigator	**Investigator**	**Signature**	**Date** 26/10/18
Professor Saul N. Faust			

4. "According to the Declaration of Helsinki, 2008, I have read this protocol, and declare no conflict of interest"

Details:_____

Principal Investigator	**Investigator**	**Signature**	**Date** 26/10/18
Dr Anna L. Goodman			

Table of Contents

1 SYNOPSIS

Title	A Phase I/IIa clinical trial to assess the safety, immunogenicity and efficacy of the blood-stage *Plasmodium falciparum* malaria vaccine candidate RH5.1/AS01
Trial Centres	Centre for Clinical Vaccinology and Tropical Medicine (CCVTM) Churchill Hospital, Old Road, Headington, Oxford, OX3 7LE, UK NIHR Wellcome Trust Clinical Research Facility (NIHR WTCRF) University Hospital Southampton NHS Foundation Trust Southampton, SO16 6YD Guys and St Thomas' NIHR Clinical Research Facility St Thomas Hospital, Westminster Bridge Road, London, SE1 7EN
Clinical Phase	I/IIa
Design	Open-label, multi-centre, first-in-human, dose escalation Phase I/IIa blood-stage *P. falciparum* malaria vaccine trial.
Population	Healthy, malaria naïve males and non-pregnant females aged 18 – 45 years
Sample Size	**Phase I Stage** Group 1: 6 - 12 volunteers Group 2: 6 -12 volunteers Group 3: 12 volunteers Group 4: 12 volunteers **Phase IIa Stage** Group 5: 15 volunteers Group 6: 15 volunteers **Group 7: 5-15 (previously Group 5) volunteers** **Group 8: 5-15 (previously Group 6) volunteers** **Group 9: 5-6 volunteers** **Total: 74 - 84 volunteers**
Follow-up duration	Volunteers will be followed up for a total of approximately 8 months in Groups 1, 2 and 4 and 1 year in Group 3. Follow- up duration will be approximately 8 months for Group 5, 3 months for Group 6, and 3 months for Group 9. Volunteers in Groups 1,2, 3, 4 or 5 who elect to attend for a visit 1-2.5 years after their last vaccination dose will be followed for a further 6 months 2 years. Those Group 5 and 6 volunteers who transition to Groups 7 and 8 will be followed up for approximately a further 6 and 3 months respectively. Volunteers in Group 7 who elect to attend for a visit 1-2.5 years after their last vaccination dose will be followed for a further 9 months – 2 years and 3 months
Planned Trial Period	Approximately 3.5 years, from the Oxford site initiation visit (SIV).
Primary Objectives	To assess the safety of the RH5.1/AS01 vaccine in healthy volunteers at different doses. To establish whether the RH5.1/AS01 vaccine can demonstrate a reduced parasite multiplication rate in vaccinated subjects compared to infectivity controls in a blood-stage controlled human malaria infection model.
Secondary Objectives	To assess the humoral and cellular immunogenicity of RH5.1/AS01 when administered to healthy volunteers at different doses. To assess immunological readouts for association with a reduced parasite multiplication rate. To assess the durability of any reduction in parasite multiplication rate (PMR) in vaccinated (Group 5) subjects by subjecting them to a re-challenge with 3D7 clone parasites approximately 4 months after the primary challenge (Group 7), and comparing the PMR with 1) that of previously challenged Group 6 controls receiving a second challenge (Group 8); and 2) that of newly recruited malaria-naïve controls

	(Group 9) receiving a primary challenge.
Investigational Product	RH5.1 AS01
Form	The RH5.1 is a recombinant protein vaccine in liquid form stored between –70°C and –90°C. It is allowed to thaw to room temperature and then administered within an hour of thawing. It will be mixed with the GSK Adjuvant System AS01 (stored at +2 to +8°C) immediately prior to administration.
Dose	**Group 1:** 3 doses of 2µg RH5.1 in 0.5mL AS01 at days 0, 28 and 56 **Group 2:** 3 doses of 10µg RH5.1 in 0.5mL AS01 at days 0, 28 and 56 **Group 3:** 2 doses of 50µg RH5.1 in 0.5mL AS01 at days 0 and 28, followed by 10µg RH5.1 in 0.5mL AS01 at day 182 **Group 4:** 3 doses of 50µg RH5.1 in 0.5mL AS01 at days 0, 28 and 56 **Group 5:** 3 doses of 10 µg RH5.1 in 0.5mL AS01 at days 0, 28 and 56 Group 7: Group 5 participants receiving 1 further dose of 10 µg RH5.1 in 0.5 mL AS01 approximately 4 months after the last immunisation **Groups 6, 8, 9:** Infectivity controls
Route	Intramuscular (IM) injection in the deltoid region of the non-dominant arm

2 ABBREVIATIONS

AE	Adverse event
ADR	Adverse drug reaction
AID	Autoimmune Disease
AMA1	Apical membrane antigen 1
AR	Adverse reaction
AS01	Adjuvant system 01
CBF	Clinical Bio-manufacturing Facility
CCVTM	Centre for Clinical Vaccinology and Tropical Medicine
ChAd63	Chimpanzee adenovirus 63
ChAd63 RH5	Recombinant chimpanzee adenovirus 63 encoding the *Plasmodium falciparum* reticulocyte-binding protein homologue 5
CHMI	Controlled human malaria infection
CRF	Case Report Form or Clinical Research Facility
CS/CSP	Circumsporozoite protein
CTRG	Clinical Trials Research Governance
DSUR	Development safety update report
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immunospot
FBC	Full blood count
FDA	United States Food and Drug Administration
GCP	Good Clinical Practice
GP	General Practitioner
GSK	GlaxoSmithKline
HCG	Human Chorionic Gonadotrophin
HLA	Human leukocyte antigen
IB	Investigators' Brochure
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IMP	Investigational Medicinal Product
IMP-D	Investigational Medicinal Product Dossier
LSC	Local safety committee
LSM	Local safety monitor
µg	microgram
MHRA	Medicines and Healthcare products Regulatory Agency
MPL	Monophosphoryl lipid A
MSP1	Merozoite surface protein 1
MVA	Modified vaccinia virus Ankara
MVA RH5	Recombinant modified vaccinia virus Ankara encoding the *P. falciparum* reticulocyte-binding protein homologue 5
NHS	National Health Service
NIHR	National Institute for Health Research
pfu	plaque-forming units

PCR	Polymerase chain reaction
PMR	Parasite Multiplication Rate
QA	Quality assurance
QIMR	Queensland Institute of Medical Research
QP	Qualified person
qPCR	Quantitative polymerase chain reaction
QS21	*Quillaja saponaria* saponin molecule fraction 21
REC	Research Ethics Committee
RH5	Reticulocyte-binding protein homologue 5
RUNMC	Radboud University Nijmegen Medical Centre
SAE	Serious adverse event
SAR	Serious Adverse Reaction
SD	Standard deviation
SmPC	Summary of Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected unexpected serious adverse reaction
TMF	Trial master file
USAID	United States Agency for International Development
vp	viral particle
WHO	World Health Organisation
WTCRF	Wellcome Trust Clinical Research Facility

3 BACKGROUND AND RATIONALE

3.1 The need for a vaccine against *Plasmodium falciparum* malaria

Malaria in humans is caused by five species – *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* and *Plasmodium knowlesi*. *P. falciparum* causes the most morbidity and mortality of the *Plasmodium* species, accounting for an estimated 187 million cases of malaria and 438,000 deaths worldwide in 2015. Globally, there are thought to be around 3.2 billion people at risk of malaria, with sub-Saharan African populations at highest risk of acquiring malaria: approximately 90% of deaths are estimated to occur in the World Health Organisation (WHO) African Region. Children under five years of age are the most severely affected, accounting for 70% of deaths from this infection [1].

Since 2000 massive efforts have been made to increase distribution of commodities to prevent malaria across Africa, with a significant reduction in malaria deaths. It is estimated that an estimated 663 (542 – 753) million cases have been averted in Africa since the introduction of control measures in 2000, the most effective of which has been the widespread use of insecticide treated nets (ITNs) which is thought to account for 68 (62 – 73)% of the averted cases [2]. However, challenges to the success of current strategies to combat malaria (such as insecticide-treated nets, indoor residual spraying, and antimalarial drugs) include: the development of resistance of *Anopheles* mosquitoes to certain insecticides; the development of resistance of malaria parasites to chemotherapeutic agents [3]; the absence of a gametocidal drug suitable for mass administration [4]; and the risk of re-importation of malaria into geographic regions previously cleared of malaria using environmental elimination measures.

The Roll Back Malaria (RBM) Partnership was launched in 1998 by the WHO, the United Nations Children's Fund (UNICEF), the United Nations Development Programme (UNDP) and the World Bank. A major goal of the RBM Partnership is to support the development of a vaccine against malaria as a key future strategy for reducing mortality from malaria. The development of an effective vaccine may indeed be necessary for the greater goal of global eradication of malaria [5]. The recently updated Malaria vaccine technology roadmap calls for the development of a vaccine against *P. falciparum* and *P. vivax* by 2030, that will have protective efficacy of at least 75 percent against clinical malaria, suitable for administration to appropriate at-risk groups and development of vaccines to reduce malaria transmission suitable for administration in mass campaigns [6].

3.2 Lifecycle of the *Plasmodium falciparum* malaria parasite

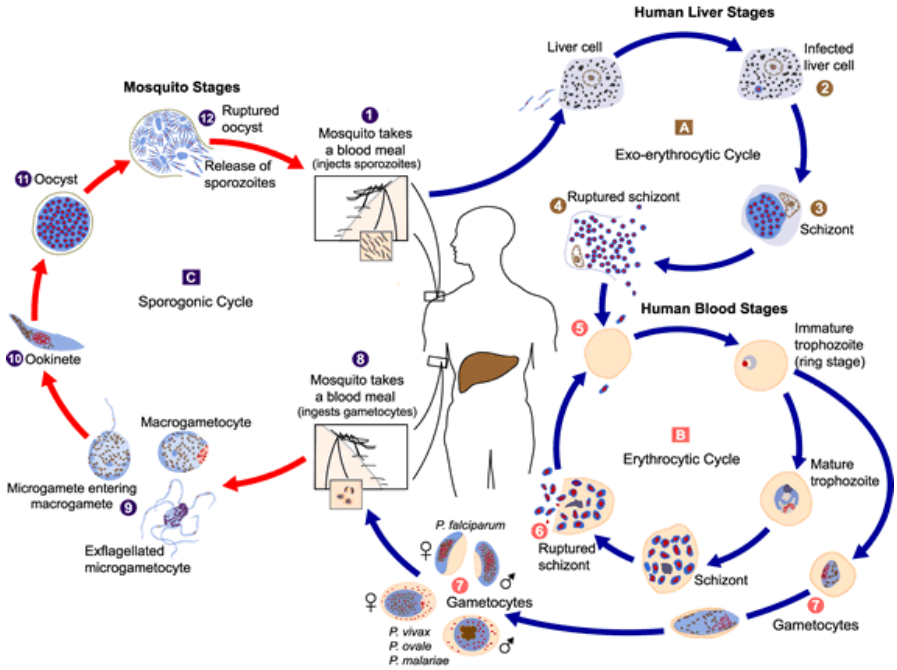

Figure 1: *Lifecycle of Plasmodium falciparum.*

In the pre-erythrocytic stage of human infection, the bite of infected female *Anopheles* mosquitoes transmits malaria sporozoites to the human host where they travel via the bloodstream to the liver and invade hepatocytes (liver-stage). Here they mature into merozoites for 6 to 7 days, after which the hepatocytes rupture releasing thousands of merozoites into the bloodstream. Merozoites then invade erythrocytes where they multiply and after 2 days cause the erythrocyte to rupture, releasing progeny merozoites that in turn invade new erythrocytes (blood-stage). A small percentage of merozoites differentiate into male and female gametocytes. These are ingested by a mosquito when it takes a blood meal and they unite within the mosquito's midgut to form a zygote. The zygote matures and releases sporozoites which migrate to the mosquito's salivary glands and are injected into the human when the mosquito feeds. Infection by sporozoites and the liver-stage of malaria is asymptomatic. It is the blood-stage of infection that is associated with symptoms and potentially severe or fatal complications.

3.3 Rationale for a blood-stage vaccine against *Plasmodium falciparum* malaria

Immunity to malaria develops naturally over time following frequent exposure. No single overriding correlate of protection has been described, however the cumulative acquisition of protective antibodies against blood-stage antigens is well documented and arguably essential for natural immunity [7]. The immunity that develops confers protection against severe disease such as cerebral malaria, hypoglycaemia, metabolic acidosis and renal failure. However, this immunity is dependent on repeated boosting by regular infection and is therefore present in adults but not children who remain at risk of severe and often fatal disease [7]. A vaccine targeting the blood-stage of malaria is an attractive proposition as it could generate an immune response which mimics the already proven protective natural immunity seen in adults, or could control parasitaemia by alternative mechanisms but also allowing for induction of other naturally-acquired immune mechanisms in parallel. Ideally a blood-stage vaccine would therefore lead to a reduction in disease severity whilst still allowing exposure to parasites so the opportunity for the host's natural immunity to develop is preserved [8]. This process would not occur with pre-erythrocytic vaccines, meaning that if the effects of the vaccine waned or control measures failed, individuals living in malaria endemic regions would be at risk of rebound of severe malaria disease [9].

There are several lines of evidence supporting the feasibility of a blood-stage vaccine, including:

- Induction of protective immunity with defined antigens in animal models [10,11];
- The ability of adoptively transferred B cells from immune donors to B cell-deficient mice to clear parasitaemia [11];
- Age-related acquisition of immunity against severe clinical malaria in endemic regions [12,13];
- The ability of passively transferred antibodies from immune adults to protect against natural and challenge infections with *P. falciparum* [14-16].

Natural immunity to malaria targets multiple blood-stage antigens and is slowly acquired. PfEMP1 is a potential target antigen but is highly polymorphic and immunity is variant specific [12]. Field studies have shown that more conserved merozoite surface antigens such as merozoite surface protein 1 (MSP1) and apical membrane antigen 1 (AMA1) are also targets of naturally-occurring protective blood-stage immunity [17,18]. However, both of these antigens have been tested in both protein-in-adjuvant formulations and in viral vectored vaccines, with little success. A Phase IIa efficacy trial using mosquito bite controlled human malaria infection (CHMI) in malaria-naïve adults for the viral vectored vaccines developed in Oxford (ChAd63/MVA-AMA1 and ChAd63/MVA-MSP1) showed that the induction of strong cellular immunity did not impact on parasite growth rates [19]. The demonstration of a significant reduction in parasite replication in the blood is deemed essential for a blood-stage vaccine to effectively prevent illness when pre-erythrocytic control measures have failed [8]. The main challenges for blood-stage malaria vaccine development have been the expression of conformationally correct large antigens, insufficient antibody responses and the extensive polymorphism of many candidate blood-stage antigens [20].

The most promising *P. falciparum* blood-stage antigen to date is reticulocyte-binding protein homologue 5 (PfRH5), as it overcomes two major difficulties for blood-stage vaccination:

- Antibodies can block erythrocyte invasion to high efficiency (requiring lower antibody concentrations than previous studied targets – MSP1 and AMA1)[21,22].
- Antibodies cross-inhibit *in vitro* all *P. falciparum* lines and field isolates tested to date [23].

3.4 **RH5 as an antigen**

Plasmodium merozoite invasion of erythrocytes is a complex process. The merozoite reorients after an initial interaction with the erythrocyte, so that its apical end faces the surface of the cell. A tight junction is formed between the merozoite and erythrocyte when the micronemes and rhoptries of the merozoite discharge their contents. The erythrocyte membrane subsequently encases the merozoite, creating a vacuole around the invading parasite [24]. The micronemal parasite ligands (Duffy-binding proteins, DBPs, or erythrocyte binding antigens, EBAs) and rhoptry ligands (reticulocyte-binding proteins, RBPs and their homologues) are two families of antigens that are functionally conserved across *Plasmodium* species and are thought to be involved in the tight committed attachment step between the parasite and new host red blood cell.

P. falciparum reticulocyte-binding protein homologue 5 (PfRH5) is one of the reticulocyte binding-like (RBL or *P. falciparum* RBP homologue (PfRH)) proteins which are involved in parasite invasion of red blood cells. It is expressed in merozoites and localises to the apical complex. PfRH5 is expressed in all *P. falciparum* strains tested so far, and is essential for parasite survival given two reports that the gene cannot be knocked out [25,26]. PfRH5 binds to its receptor basigin, the Ok blood group antigen, and this interaction mediates an essential interaction required for red blood cell invasion by all tested strains of *P. falciparum* [27]. Low-level antibodies to PfRH5 can be found in the pooled serum of humans from malaria-endemic countries, but not from pooled malaria-non-exposed immune sera [28,29]. More recently these responses have been associated with clinical protection in an endemic setting, supporting the theory that these antibodies may play a part in controlling malaria infection [30]. Importantly, although antibodies have been found, it appears that PfRH5 does not come under significant immune pressure. This may account for the limited polymorphism in PfRH5 which was reported from the malaria whole genome sequencing project [31]

(using >220 field isolates from Africa, Asia and Oceania) where only 12 single nucleotide polymorphisms were identified in this 526 amino acid antigen, and of these only 7 showed a frequency of >5% [21]. The basigin binding site on PfRH5 may also be functionally constrained, thus limiting polymorphism, given few amino acid substitutions have been reported to affect basigin recognition and host red blood cell tropism [26,32,33]. Blocking of the PfRH5-basigin interaction has also been shown to be an important contributor of anti-PfRH5 antibody action [34].

Polyclonal antibodies induced by PfRH5 vaccination (or by natural infection) overcome two of the major difficulties for blood-stage vaccination: firstly, antibodies can block erythrocyte invasion to high efficiency (requiring lower antibody concentrations than previously studied targets, such as PfAMA1 and PfMSP1) [30], and secondly, and even more importantly, these antibodies cross-inhibit *in vitro* all *P. falciparum* lines and field isolates tested to date [21,23,35,36]. Importantly, high-level efficacy induced by PfRH5 vaccination against heterologous strain challenge in an *in vivo Aotus* monkey *P. falciparum* challenge model has also been demonstrated [37]. In summary, the full-length PfRH5 antigen is now the highest priority target identified in the blood-stage malaria vaccine field for over a decade [23].

RH5 has entered clinical testing in a Phase Ia trial in Oxford and Southampton (VAC057) using the viral vectored vaccines ChAd63 RH5 and MVA RH5. This trial demonstrated that antigen-specific T cells and IgG were successfully induced by vaccination, and there were no safety concerns. The antibodies induced by vaccination were able to inhibit parasite growth in an assay of growth inhibitory activity (GIA), and demonstrated functional activity across a number of laboratory and field strains. The level of antibodies induced by viral vectored vaccines is typically lower than with protein-in-adjuvant vaccines, so it is anticipated that RH5 given as a soluble protein with a powerful adjuvant will induce higher levels of antibody than the viral vectored vaccines.

3.5 **Rationale for blood-stage controlled human malaria infection**

The blood-stage controlled human malaria infection (CHMI) model, in which volunteers are inoculated with red blood cells infected with 3D7 clone blood-stage parasites, has been used successfully to test drugs [38]. In 2014 a Phase I/IIa trial of the AMA1 vaccine FMP2.1/AS01B was carried out in Oxford to develop the blood-stage CHMI model for testing blood-stage malaria vaccines. The study was powered to detect a 33% decrease in parasite multiplication rate (PMR) with ≥80% power as the primary efficacy endpoint. Although the vaccine demonstrated no efficacy in this trial it did demonstrate the reproducibility of the blood-stage CHMI model. There was no difference in the mean PMRs between the vaccinees (Group 1) and controls (Group 2). The mean PMR for Group 1 was 10.32 (95% confidence interval (CI) 8.97-11.67) and for Group 2 was 10.31 (95% CI 9.00-11.62), *P*=0.99 using unpaired t test[39].. As part of the analyses in this trial, the PMRs in the fifteen infectivity controls were compared to historical data from twenty one unvaccinated infectivity control volunteers from four previous CHMI trials, in which volunteers were exposed to the bites of five mosquitoes infected with the 3D7 clone of *P. falciparum* [40-42]. PMRs were modelled from these datasets and the pooled data showed a similar mean PMR of 10.23 per 48 h, but a significantly larger spread (SD=4.67; *P*=0.008, F test). These data indicate that the blood-stage model provides better power to observe partial vaccine efficacy against blood-stage parasite growth rates and, moreover, showed that each individual PMR can be modelled from the qPCR data with greater confidence (Payne RO *et al.*, submitted). The blood-stage CHMI model is therefore preferable as a means of assessing blood-stage malaria vaccine efficacy as it will allow for the detection of partial efficacy more conclusively than with sporozoite CHMI.

4 OVERVIEW OF PREVIOUS RESEARCH

4.1 Clinical trials

This is a first-in-human study of the soluble protein RH5.1 vaccine with the GSK adjuvant system AS01. The RH5 antigen has previously been administered to healthy volunteers in the viral vectored vaccines ChAd63 RH5 and MVA RH5 in the Phase Ia clinical trial VAC057 (ClinicalTrials.gov identifier: NCT02181088), with no safety concerns. This trial demonstrated that RH5-specific antibodies and T cells can be induced by vaccination, and that the IgG antibodies are able to inhibit growth of all tested *P. falciparum* strains *in vitro*, including laboratory lines and recently culture-adapted field isolates[43]. These data confirmed the RH5 antigen is immunogenic when administered as a vaccine in healthy UK adults. It is anticipated that the RH5.1/AS01 formulation will elicit higher levels of RH5-specific IgG antibodies than the ChAd63-MVA formulation, given previous experience with other antigens such as AMA1 and MSP1 that have also been tested in humans using both subunit vaccine delivery strategies [42,44-46].

4.2 Pre-clinical studies

RH5.1 has been administered to BALB/c mice with the AS01 adjuvant, and was shown to elicit high-levels of growth inhibitory antibodies. Similar (but not identical) RH5-based protein/adjuvant vaccines have been administered to mice, rats, rabbits and *Aotus nancymaae* monkeys and in all cases have been shown to elicit high-levels of strain-transcending neutralising antibodies [35-37,47-49].

In the recent pre-clinical study conducted in Oxford with RH5.1/AS01 in BALB/c mice, mice were immunised with three doses of RH5.1 - either 2 µg, 10 µg or 50 µg in 50 µL AS01 at 4 week intervals (6 mice per group). A group of mice were also vaccinated with three doses of 50 µg RH5.1 in PBS (n=6), and another 6 mice received 50µg RH5.1 in the Addavax adjuvant for comparison (an oil-in-water emulsion similar to MF59 or AS03 adjuvant). Vaccinations were administered intramuscularly and blood was collected at 4, 8 and 10 weeks. Similar levels of anti-RH5 IgG were induced by all doses of RH5.1 in AS01 (Figure 2). Purified IgG from serum demonstrated inhibition of *P. falciparum* growth in a GIA assay, with the lower doses (2 µg and 10 µg) demonstrating the highest levels of inhibition (lowest EC_{50}s) (Figure 3).

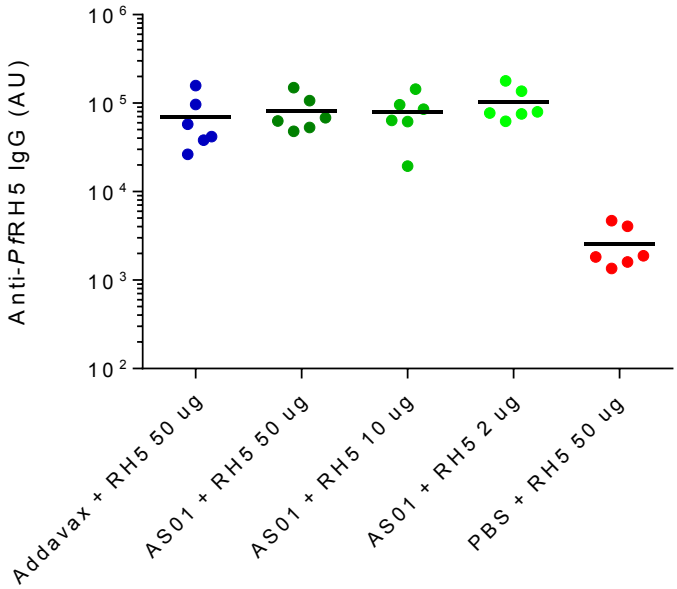

Figure 2: *Anti-RH5 IgG induced by vaccination of BALB/c mice at 0, 4 and 8 weeks. ELISA responses are shown at the peak (week 10).*

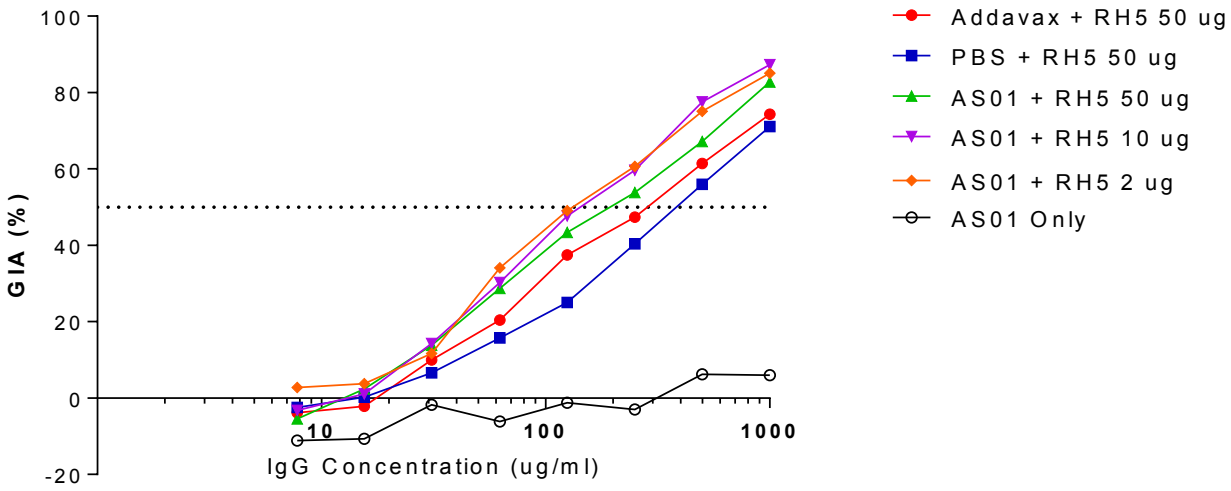

Figure 3: *In vitro GIA of purified IgG from mice vaccinated with RH5.1 in Addavax, AS01 and PBS.*

4.3 Potential immunity conferred by a previous malaria challenge and safety data to support a re-challenge of healthy volunteers

There is no precedent in the modern CHMI era for re-challenge of volunteers who were not sterilely protected and reached thick smear positive on the primary infection. The old neurosyphilis *P. vivax* malaria data [51] showed a reduction in fever episodes and a log reduction in the maximum mean parasite count after a homologous *P. vivax* and *P. falciparum* re-challenge, lending support to the concept of conferred protection from a prior challenge, but with little experience in human blood-stage re-challenge in the modern era, the effect in blood-stage *P. falciparum* remains unknown. Only one previous study has administered a second intravenous *P. falciparum* challenge in humans to date[52]. Five malaria naïve volunteers were repeatedly inoculated with infected erythrocytes at low doses, followed by administration of anti-malarials before development of clinical infection. Three of the four volunteers who completed the study were protected from infection after three rounds of low dose blood-stage challenge and cure, with no demonstrable DNA by qPCR after the fourth inoculation. However, with very small numbers, lack of a control group and detection of residual atovaquone, which may have caused this protective effect, the interpretation of these results is difficult.[52,53]

The reason for the lack of experience of re-challenge in a blood-stage *P. falciparum* challenge model is that until this study, there has never been any protective efficacy shown with a blood-stage vaccine, so there has never been any reason to re-challenge volunteers. Ours is the first study to show any efficacy of a blood-stage vaccine in over 40 years, which is why it is of immense scientific importance to take this further to assess the durability of any vaccine-induced protection, and at the same time answer the fascinating question of whether exposure to a previous challenge (with or without a prior vaccination) may confer immunity against a second challenge.

In the natural exposure setting (i.e., in endemic countries) it is well known that prior exposure to malaria can confer immunity to subsequent exposures, such that individuals can go on to develop asymptomatic infections where they are able to tolerate much higher levels of parasitaemia without symptoms or clinical signs of malaria[7]. Safety of re-challenge is indicated by the natural history of induced protection from previous malaria exposure in endemic areas, as well as the wealth of data from repeated infection administered as malariotherapy, which strongly indicates that exposing a previously malaria-exposed volunteer to a second or third challenge would not confer any additional risk over that of a first challenge. Indeed, it is highly possible that this may in fact confer some protective immunity (and so could result in a

milder infection and delay the time to diagnosis). Alternatively, if there is no immunity from the previous challenge or challenges, then this repeat would be like the prior challenge, with no change in risk.

It is also important (and of particular relevance to a study of healthy volunteers) to clarify that hundreds of healthy volunteers (i.e. not those in endemic countries) in other malaria vaccine trials worldwide, have in fact been re-challenged with *P. falciparum* malaria as part of a controlled human infection model using either the mosquito bite[40,54-56] or injected sporozoite[57,58] routes of challenge (and the same 3D7 strain of *P. falciparum* as used here for blood-stage challenge). These include volunteers in the UK, the Netherlands and the USA[40,54-58]. There have been no safety concerns in any of these trials as result of a re-challenge so there is evidence for safety of a re-challenge, both in the experimental and natural settings, and it is well known that the safety of the blood-stage model is no different from that of the mosquito or sporozoite models. While most of these studies did not re-challenge control volunteers (only vaccinees who had either been sterilely protected or not protected) it is important to highlight that interpreting the effects of a re-challenge in a vaccinated group, without a parallel control group can be very difficult, and we believe our study design is ideal to assess this.

We are therefore confident that:

1) We have a strong scientific rationale for re-challenge, with incorporation of crucial control groups (malaria exposed and malaria naïve);

2) There is no additional concern re volunteer safety, given the wealth of safety data on *P. falciparum* re-challenge from both experimental and natural settings; and

3) The scientific output of the study will be greatly enhanced by including these control groups.

5 CONTROLLED HUMAN MALARIA INFECTION STUDIES

5.1 Microbial Challenge Studies of Human Volunteers

The deliberate infection of human volunteers with micro-organisms has contributed uniquely to our understanding of the pathogenesis, immune responses and the treatment and prevention of numerous microbial diseases including influenza, cholera, typhoid and hepatitis [59,60]. A review by the UK Academy of Medical Sciences on microbial challenge studies recognised that such studies are desirable for providing proof of concept for prophylactic and therapeutic interventions and can significantly accelerate progress to Phase III studies [59].

5.2 Controlled Human Malaria Infection (Challenge)

Plasmodium falciparum malaria is a microbe particularly well suited to challenge studies. It has a relatively short asymptomatic period, a well-established diagnostic laboratory test (thick film microscopy), and no long term sequelae or infectious state following appropriate and timely treatment. Studies involving controlled human malaria infection (CHMI) are a powerful tool for investigating malaria vaccine and prophylactic drug efficacy [61]. With an increasing number of candidate malaria vaccines being developed, the number of centres conducting CHMI studies is expanding [61].

Deliberate infection of humans with malaria was first performed in 1917 by Wagner von Jauregg, primarily as a therapy for patients with neurosyphilis [62]. Thousands of patients underwent the treatment (the objective of which was to induce a febrile illness that was thought beneficial for the progress of the disease), administered by the bites of infectious mosquitoes or by intravenous or subcutaneous inoculation of dissected *Plasmodium* sporozoites suspended in media. The practice stopped with the advent of antibiotics effective against syphilis.

Following the development of protocols for the continuous culture of asexual *P. falciparum* in 1976 [63] and for the generation of mature *P. falciparum* gametocytes *in vitro* in 1981 [64], it became possible to produce laboratory-reared infectious mosquitoes, meaning that CHMI trials could be performed more routinely [65].

The first well-documented CHMI study with laboratory-reared infectious mosquitoes was carried out in 1986 at the US Walter Reed Army Institute of Research (WRAIR), the US Naval Medical Research Institute (NMRI) and the US National Institutes of Health (NIH). Six volunteers were infected with *P. falciparum* sporozoites by the bites of infectious *Anopheles freeborni* and *Anopheles stephensi* mosquitoes [66]. The following year, the efficacies of the first recombinant protein and synthetic peptide *P. falciparum* vaccines were tested in experimentally infected volunteers [67,68].

CHMI has now become established as a key tool to assess the efficacy of novel malaria vaccines and drugs [61]. As CHMI trials are carried out in a controlled environment, they allow detailed evaluation of parasite growth and immunological responses, providing key information for vaccine and drug development [61].

Since the late 1980s, the number of institutions carrying out CHMI with *P. falciparum* has been growing. In 2007, data were published from a total of 532 volunteers [69]. Unpublished analysis shows that a total of 1,343 volunteers were experimentally infected with *P. falciparum* between 1985 and 2009 [70].

Having been stopped in the 1980s due to ethical and safety concerns, experimentally induced blood-stage malaria infection (IBSM) was reintroduced in the 1990s. Human challenge with *Plasmodium falciparum* malaria-infected erythrocytes to enable assessment of blood-stage vaccines, drugs and further investigate early events in blood stage infection has been undertaken in over 100 volunteers since 1997 [71,72]. All of these volunteers have been challenged with aliquots of infected erythrocytes taken from a single donor, and the number of infected erythrocytes has varied from 30 to 6000 [71]. Malaria challenge using this method has always resulted in parasitaemia as detected by PCR [71].

There are several advantages of blood-stage challenge over sporozoite challenge. The ability to give a uniform dose of *P. falciparum*-infected erythrocytes results in much more consistent growth curves [38] with a longer pre-patent period, enabling blood-stage vaccines with partial efficacy to be assessed (Figure 4). The method also ensures the timing and dose of parasites into the circulation as it eliminates the variability of the size of liver-to-blood inoculum seen with sporozoite challenge [71,73]. The number of parasites inoculated is approximately 100 times lower than the number released from the liver following sporozoite challenge, and this enables the blood-stage to be followed for approximately 4-6 more days (2-3 replication cycles) [61]. The relative consistency of the course of infection allows blood tests to assess immunogenicity to be taken at similar stages of blood-stage infection, resulting in data with can be treated with a greater degree of confidence. This allows the numbers of participants required to be reduced, making early direct comparisons of potential vaccine candidates more affordable [71].

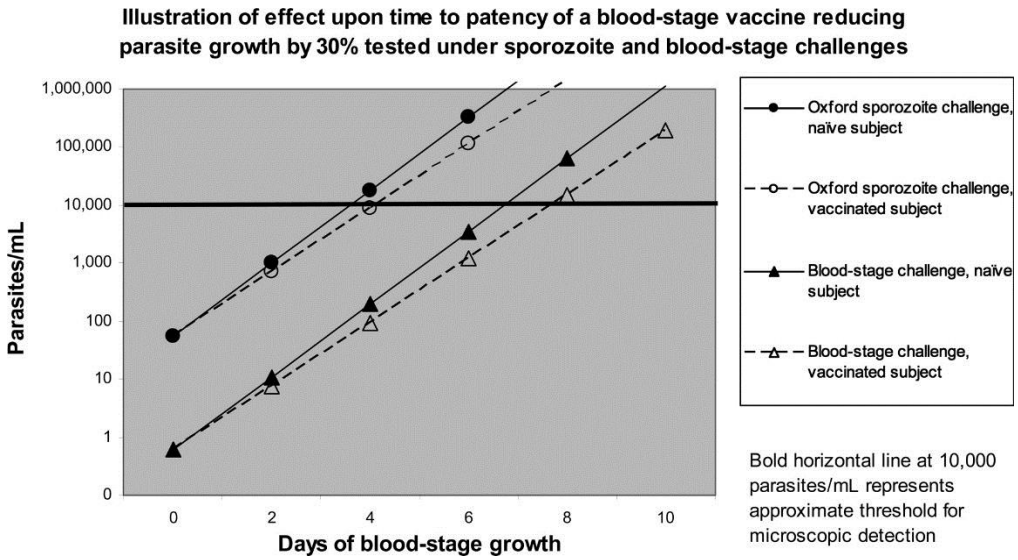

Figure 4: *Schematic of parasite growth after blood-stage and sporozoite challenge with effect of a theoretical blood-stage vaccine reducing parasite multiplication by 30%. Starting levels of parasites are as used in the Oxford pilot study [73]. An 18-fold 48-hour multiplication is assumed (a typical value in vaccine-naïve subjects under both blood-stage and sporozoite challenge).*

Oxford's Experience Conducting CHMI Trials

The University of Oxford has been conducting CHMI studies for the last 12 years. To date, more than 400 volunteers have undergone CHMI in studies conducted by the University of Oxford, including over 120 unvaccinated control volunteers (Table 1). A recent meta-analysis has shown that the symptoms of malaria experienced by volunteers undergoing sporozoite challenge by mosquito bite in Oxford are broadly similar to those experienced by volunteers challenged by mosquito bite at RUNMC and the United States Military Malaria Vaccine Program (USMMVP) [74].

In Oxford, four volunteers have required admission to hospital for observation following challenge (SAE related to CHMI).

- Patient 1: VAC013: Vaccinated Volunteer: Admitted for 48 hours due to vomiting and received intra-venous fluids and anti-emetics as additional therapy.
- Patient 2: VAC022: Vaccinated Volunteer: Admitted for 12 hours following the development of mild eyelid oedema following chloroquine treatment. This resolved spontaneously and was considered related to the treatment with chloroquine.
- Patient 3: VAC039: Unvaccinated Control volunteer: Admitted to hospital for 24 hours for intravenous fluid rehydration and observation following a pre-syncopal episode and moderately severe symptoms of uncomplicated malaria.

- Patient 4: VAC045: Vaccinated Volunteer: Admitted to hospital with lassitude and vomiting due to malaria and malarone treatment. Treated with anti-emetics, intravenous fluid rehydration, cessation of malarone, and antimalarial treatment with Riamet. Discharged from hospital the following day.

Trial (Reference)	Year	Type of Challenge	No. Vaccinees Challenged	No. Controls Challenged	No. SAEs Related to Challenge
VAC004	2000	Sporozoite	12	6	0
VAC007	2000	Sporozoite	9	5	0
VAC013	2001	Sporozoite	14	11	1
VAC015 [75]	2001/3	Sporozoite	16	5	0
VAC017 [75]	2002	Sporozoite	16	4	0
VAC018 [54]	2002/3	Sporozoite	5	5	0
VAC021 [76]	2003/4	Sporozoite	16	6	0
VAC022 [77]	2003	Sporozoite	11	6	1
VAC023 [78]	2003/4	Sporozoite	15	6	0
BSV 1	2005	Blood	0	6	0
VAC030	2005/6	Sporozoite	24	6	0
VAC027 [79,80]	2006/7	Sporozoite	15	6	0
MAL034 & VAC037 [45]	2009/10	Sporozoite	43	12	0
VAC035 [81]	2010	Blood	5	3	0
VAC039 [42]	2010	Sporozoite	36	6	1
VAC049 [82]	2011	PfSPZ Challenge	0	18	0
VAC045[41]	2012	Sporozoite	30	6	1
VAC052	2013	Sporozoite	27	6	0
VAC055	2013	Sporozoite	33	6	0
VAC054	2014	Blood	12	15	0
VAC055 rechallenge	2014	Sporozoite	14	5	0
VAC059	2015	Sporozoite	36	4	0
TOTAL			389	153	4

Table 1: CHMI trials conducted to date by the University of Oxford at CCVTM. Sporozoite = mosquito bite. Blood = Blood stage challenge. PfSPZ Challenge = aseptic, cryopreserved *P. falciparum* administered by injection. Control = unvaccinated infectivity controls.

In a Phase I/IIa sporozoite challenge study assessing the efficacy of viral vectored malaria vaccines in Oxford (VAC039; Clinical trials.gov reference: NCT01142765) [42], one volunteer who underwent sporozoite challenge on 1st October 2010 failed to attend his next scheduled study visit on 7th October 2010. The police were immediately informed and began a nationwide search for the individual. All volunteers had been informed at screening that the police would be notified should they go missing following CHMI if they had not completed a full course of an appropriate anti-malarial treatment. The volunteer was found in The Netherlands by the local police 17 days following CHMI. He then had very mild malaria symptoms. He was admitted to a local hospital where he received appropriate treatment for *P. falciparum*. He had no signs of severe malaria but showed an altered mental state considered unrelated to malaria with apparent memory loss and suicidal ideation. He was therefore transferred for in-patient psychiatric assessment and discharged a few days later to his GP's care. He has subsequently been reportedly diagnosed as schizophrenic.

It emerged that from 2nd October 2010, the day after CHMI, the volunteer had experienced an alteration in his expected behaviour following his arrest the previous evening by the police relating to their investigation of a serious crime. This arrest appeared to trigger his leaving home and disappearance and appears relevant to the subsequent finding of memory loss and suicidal ideation. It emerged on subsequent investigation that the volunteer actually had a history of some psychiatric morbidity pre-dating his involvement in the study by many years, which was not disclosed at screening by the volunteer or his GP. Of note, the volunteer had attended 9 clinic visits prior to challenge and appeared a reliable and appropriate volunteer.

This event was extensively discussed with investigators, colleagues and appropriate authorities and non-study related causality agreed. Given the exceptional circumstances relating to this case, it seems unlikely that a similar event would happen again in the future. Management of the event was extensively reviewed by the trial's Sponsor, the MHRA and ethics committee who felt that appropriate and timely action was taken. Follow-up procedures following CHMI have been reviewed locally, and it has been decided that for sporozoite challenge studies volunteers are contacted daily on days 1-5 post-challenge in order to make sure they are contactable and well. The blood-stage challenge model volunteers are seen from Day 1 post-challenge and therefore followed even more closely initially.

5.3 **Experimentally-induced blood-stage malaria infection (IBSM)**

The samples we propose to use as infectious inocula were produced by Drs Gregor Lawrence, Allan Saul and colleagues at QIMR in Brisbane, Australia in 1994 [83]. The protocol for the study was reviewed and approved by the QIMR Ethics Committee and the Healthy Volunteer Studies Research Ethics Subcommittee, Lothian Health Board (Edinburgh). Procedures were designed to minimise the risk of other infectious agents in the cryopreserved samples.

Collection and cryopreservation of inoculum

Laboratory-reared *Anopheles stephensi* mosquitoes were infected with the *P. falciparum* clone 3D7 (a chloroquine-sensitive strain) by membrane feeding on a blood meal containing gametocytes. Ten and fourteen days later, the mosquitoes were fed on two volunteers. Parasitaemia in the volunteers was followed by daily microscopy from day 4 after infection. Blood was taken and frozen in aliquots from the volunteers 6 hours after they developed fever, when both were microscopically parasite positive. The volunteers were treated with chloroquine soon after blood was drawn with complete recovery.

Initial development of the blood inoculum to be used in this study and its use in five volunteers is described by Cheng *et al.* 1997 [83]. The inoculum used for all volunteers comes from one of the donors described above. This is because the second donor (whose blood has not been used) had a much lower parasitaemia [71]. Blood was collected at the Australian Red Cross Blood Bank in an aseptic manner using standard blood bank equipment. The leukocytes were removed with a leukocytic filter. The thawing and washing of the cells reduced the amount of serum transferred with the red cells by a factor of 1000, compared to injecting the same volume of blood. The volume of inoculum to be given to each volunteer contains a very small volume of red blood cells, equivalent to only 1.5 to 4 microlitres of blood.

The red cells were cryopreserved using a protocol from the American Association of Blood Banks Technical Manual that is normally employed for freezing blood from patients and donors with rare blood groups. Blood from both volunteers was group O and Rhesus negative.

Viability of the inoculum was confirmed by culture in a trial in Nijmegen in 2011 [84], and may be reconfirmed prior to initiation of the study. Viability up to the time expected to be required for inoculations (4 hours) may also be confirmed at that time. For each vial of inoculum used a small amount will be cultured after inoculations are complete to confirm viability and the estimated number of parasitised red blood cells inoculated.

Previous Studies

In the study conducted by Cheng *et al.* [83], two volunteers infected with the inoculum were treated based on positive qPCR prior to development of symptoms; the other three were treated when symptomatic. All recovered fully with rapid decline of parasitaemia after treatment with chloroquine.

Oxford University has previously undertaken three CHMI studies of healthy human volunteers using this inoculum. The first was a pilot study, approved in 2003 by the Oxfordshire Research Ethics Committee (ref. C03.061) in which 5 volunteers received the inoculum. As expected, the results showed less variation in rate of rise of parasitaemia than is typically experienced with a liver-stage (or sporozoite) malaria challenge. The trial was successful, with all volunteers being diagnosed with malaria between the morning of day 7 and the morning of day 9 after inoculation. The symptoms of malaria experienced by volunteers at and around the point of diagnosis were as expected. No SAEs were reported. All volunteers were clinically well after antimalarial treatment when reviewed in clinic at day 42 after inoculation. During the trial one volunteer became mildly anaemic but this was transient, with a normal haemoglobin on repeat testing one week later; and one volunteer had a raised bilirubin of 35 µmol/L on two occasions. All volunteers successfully completed follow-up [73].

Following this a Phase IIa study of the Safety, Immunogenicity and Parasite Growth Inhibitory Activity of AMA1-C1/Alhydrogel® + CPG 7909 was conducted (VAC035). This involved 8 healthy volunteers (5 vaccinees and 3 controls) receiving the blood-stage CHMI with the same inoculum. All individuals challenged developed malaria (confirmed by positive blood film and positive qPCR), although only 2 of the 8 subjects developed clinical malaria symptoms pre-diagnosis [81]. A significant inverse relationship between vaccine-induced growth inhibitory activity (GIA) and PMR was seen in vaccinated subjects, but no impact on overall PMRs was observed across the vaccinated and control groups. However, the sample size in this study was very small, limiting the statistical power to assess differences in PMR between the groups [81].

The most recent blood-stage CHMI trial conducted in Oxford was VAC054, which looked at the safety, immunogenicity and efficacy of the AMA1 vaccine candidate FMP2.1 given with the AS01 Adjuvant System. In this trial 12 vaccinated volunteers and 15 infectivity controls received the blood-stage CHMI inoculum, which was homologous to the vaccine. The vaccine induced functional antibodies which were able to inhibit growth *in vitro* in a growth inhibition activity (GIA) assay, but demonstrated no efficacy with all vaccinees and controls developing blood-stage malaria. There was no effect on PMR in vaccinees compared with the infectivity control group. However, this trial demonstrated the reproducibility of the blood-stage CHMI model, with much larger group sizes than used in previous studies. The study also demonstrated its utility for measuring modest reductions in PMR in comparison to mosquito-bite CHMI where analysis of historical data showed a higher dispersion of data in the infectivity controls [39].

The main reason for the small sample size in VAC035 was the requirement for volunteers to be both Epstein Barr virus (EBV) and cytomegalovirus (CMV) seropositive. This resulted in the exclusion of 52 of 75 volunteers screened. Seropositivity was thought necessary as a precaution because the donor of the infected erythrocytes was EBV and CMV seropositive, and there was therefore thought to be a risk to participants of infection with these viruses. However, both of these herpes viruses are leukocyte-associated and the donor blood was leukodepleted and washed before freezing, as well as being extensively washed during preparation of the inoculum. Leukodepletion has been shown to be effective in preventing transmission of both EBV and CMV [85]. PCR analysis of the donor blood for the IBSM has been negative for both EBV and CMV since this technology became available (Prof James McCarthy, QIMR, personal communication) [71]. Following a risk assessment, QIMR (Australia) no longer consider seropositivity to either of these viruses to be necessary for volunteers receiving blood-stage challenge. In the latest Oxford trial (VAC054) EBV and CMV serostatus were checked prior to CHMI but were not used to exclude volunteers. 63% of volunteers were seronegative for CMV and 11% were seronegative for EBV. There were no cases of seroconversion in this trial. We therefore believe it is unnecessary to require potential volunteers to be

seropositive for EBV and/or CMV, and will not check serostatus in this trial. We will, however, inform volunteers that the risk of transmission of infection cannot be completely excluded and this will be mentioned on the consent form volunteers are asked to sign prior to enrolment.

5.4 **Clinical Presentation Post-CHMI**

Individuals who have received the malaria-infected erythrocyte inoculum are typically expected to develop clinical malaria over the next 7-10 days [72]. The most common symptoms are fatigue and headache, and severe symptoms can include headache, fatigue, malaise, chills, myalgia, rigors, nausea and vomiting. Clinical symptoms generally coincide with the detection of blood-stage parasites at densities of 10–20 parasites per μL of blood by microscopy of thick film smears. This corresponds to a parasitaemia of approximately 0.0004% [61,86]. Severe malaria is generally diagnosed when parasitaemia is 3 logs greater than the peak parasitaemia in challenge trials. After the start of malaria treatment, symptoms can temporarily increase in severity but subside quickly with an average duration of approximately 2-3 days [61]. There has been a suggestion of decreased symptomatology with blood-stage challenge compared with sporozoite challenge, independent of peak parasitaemia, parasite multiplication rate or parasite density at diagnosis [72]. This was also apparent in the recent VAC054 study.

Immediate treatment of volunteers at the earliest phase of microscopically detectable blood-stage infection or at a conservative parasitaemia qPCR cut-off ensures that the potential risks of complications associated with severe malaria are minimised to the greatest extent possible.

Human malaria sporozoite challenge infections have been conducted in over 1,340 volunteers challenged with *Plasmodium falciparum* so far [69,86,87]. Recently, safety concerns were raised in a young volunteer who suffered a cardiac event shortly after treatment for diagnosed malaria. This was diagnosed as probable myopericarditis, although ischaemia could not be ruled out. Although a definite relationship between the cardiac event, which resolved fully and rapidly, and the experimental malaria infection was not established [88], it has been generally agreed that volunteers with an increased risk of cardiac disease should be excluded from such trials [70]. A further case of myopericarditis has since been identified in a recent CHMI study, also at the Nijmegen, Netherlands centre, but in this case the individual was also diagnosed with an intercurrent rhinovirus infection so that the relation to malaria infection is again uncertain. There was a brief episode of clinical chest pain and the volunteer made a full recovery.

5.5 **Ethical Considerations of CHMI trials**

Participants in CHMI trials are healthy volunteers who do not obtain direct health benefit from participation. Challenge trial investigators must exercise all possible safeguards for volunteer safety to ensure that trial participation is of minimal risk. Investigators must also ensure that maximal scientific benefit accrues from each challenge trial.

6 INVESTIGATIONAL PRODUCTS

6.1 The RH5.1 Protein (also see Investigator's Brochure [IB])

RH5.1 was manufactured under Good Manufacturing Practice (GMP) by the Clinical Biomanufacturing Facility (CBF) in Oxford in 2015. The doses to be used in this study are 2 µg, 10 µg and 50 µg. These are nominal doses as the actual dose may vary slightly dependent on dilution, mixing and administration.

The Quality Control Standards and Requirements for the vaccine are described in separate Quality Assurance documents (e.g. certificate of analysis) and the required approvals have been obtained. The vaccines are QP certified, labelled and packed according to applicable regulatory requirements at the CBF.

In summary the RH5.1 protein consists of the entire full-length ectodomain of the PfRH5 antigen (amino acids E26 – Q526) with the sequence based on the 3D7 clone of *P. falciparum*. The vaccine was produced from a stable *Drosophila* S2 cell line, and also contained an N-terminal 18 αα BiP insect signal peptide (MKLCILLAVVAFVGLSLG) which is cleaved off as the protein is secreted from the cell, and a C-terminal four amino acid (E-P-E-A) "C-tag" used for affinity purification. The cell line system called ExpreS^2 was provided by ExpreS^{2}ion Biotechnologies in Denmark. The rationale for this vaccine design was based on previous experience with PfRH5 vaccines developed in other systems. All four putative N-linked glycosylation sequons (N-X-S/T) were mutated Thr to Ala – as performed for a previous PfRH5 protein vaccine produced in mammalian HEK293 cells and tested in rabbits[23] and *Aotus* monkeys [37].

6.2 The Adjuvant: AS01 (also see IB)

Adjuvants have been known to increase the immune response against a given antigen for over 80 years [89]. The AS01 adjuvant system has been developed and manufactured by GlaxoSmithKline (GSK) Biologicals and is presented as a liquid solution in a monodose glass vial. AS01 is a liposome-based Adjuvant System with a specific aim to improve cell-mediated immunity [89]. The adjuvant system contains 3-0-desacyl-4' monophosphoryl lipid A (MPL), a TLR4 (toll-like receptor 4) ligand derived from the cell wall lipopolysaccharide (LPS) of the Gram negative *Salmonella minnesota* R595 strain. The LPS is detoxified by hydrolytic treatment and purification to provide a powerful adjuvant without the toxic effects of the parent molecule [89]. The AS01 adjuvant system also contains QS21, a highly purified saponin extract (triterpene glycoside) from the bark of the South American tree *Quillaja saponaria.* QS21 has been shown to impact antigen presentation to antigen presenting cells (APCs) and favours the induction of cytotoxic T lymphocytes [89]. The 0.5 mL final dose of AS01 contains 50 µg of MPL and 50 µg of QS21 [46]. The AS01E adjuvant contains 25 micrograms of MPL and 25 micrograms of Stimulon QS21 in a 0.5mL dose.

AS01 is closely related to AS02 which contains the same immunostimulants MPL and QS21 as AS01. AS02 is an oil-in-water formulation, while AS01 contains liposomes. AS01B, AS02A and AS01E, AS02D are the adult and paediatric formulations of AS01 and AS02, respectively. The clinical evaluation of GSK's leading malaria vaccine candidate antigen RTS,S started with AS02.

In parallel to the continued evaluation of RTS,S/AS02 in endemic countries, the RTS,S antigen was combined with AS01 in a sporozoite challenge trial. Safety and reactogenicity of RTS,S/AS01 was found to be comparable to that of RTS,S/AS02 [90]. Injection site pain was the most frequently reported solicited local adverse event (AE) in both the RTS,S/AS01 and RTS,S/AS02 groups, occurring with a similar frequency in both vaccine groups (following 81.0% and 82.1% of doses, respectively). Grade 3 pain was reported more frequently in recipients of RTS,S/AS02 than of RTS,S/AS01 (following 9.0% and 2.8% of doses, respectively). Fatigue and headache were the most frequently reported solicited general AEs after vaccination in both groups, occurring with a similar frequency in both the RTS,S/AS01 and RTS,S/AS02 groups (fatigue: following 44% and 36% of doses; headache: following 33% and 35% of doses, respectively). Grade 3 solicited general AEs considered to be related to study vaccine were infrequent (following ≤4.2% of doses).

The frequency of causally related unsolicited AEs was similar in the RTS,S/AS01 and RTS,S/AS02 groups (following 19.7% and 15.2% of doses, respectively). Three serious adverse events (SAEs) were reported (2 in RTS,S/AS01 group, 1 in challenge control group); none were considered to be related to vaccination.

A trend towards improved vaccine efficacy (VE) against infection with RTS,S/AS01 compared to RTS,S/AS02 was observed (50.5% [95% CI: 32.9, 67.1] vs 31.8% [95% CI: 17.6, 47.6]). VE against infection was significant in both the RTS,S/AS01 and RTS,S/AS02 groups compared to infectivity controls ($P \leq 0.001$). In infected subjects, the mean pre-patent period (PPP) was similar in the RTS,S/AS01 (14.4 days) and RTS,S/AS02 (13.6 days) groups. The shortest mean time to infection was in the infectivity control group (10.8 days).

The comparable reactogenicity and safety profile of RTS,S/AS01 and RTS,S/AS02 was confirmed in semi-immune adults [91]. Unadjusted VE against infection over 16 weeks in the RTS,S/AS01 and RTS,S/AS02 groups was 29.5% (95% CI: -15.4, 56.9, p=0.164) and 31.7% (95% CI: -11.6, 58.2, p=0.128) respectively. Significantly higher anti-CS antibody geometric mean titres (GMTs) were observed in recipients of RTS,S/AS01 than of RTS,S/AS02 at each post-vaccination time point.

Malaria-048 was a small study of 36 subjects (12 subjects in each of the RTS,S/AS02, RTS,S/AS01 and RTS,S/Saline groups), designed to characterise the immune responses elicited by RTS,S/AS01 and RTS,S/AS02 vaccine formulations in healthy malaria naïve adults as compared to non-adjuvanted RTS,S vaccine, with the aim of demonstrating superiority of the anti-CS antibody responses elicited by RTS,S adjuvanted with either AS01 or AS02 as compared to non-adjuvanted antigen.

Local and general solicited AEs were reported more frequently in the adjuvanted vaccine groups as compared to the non-adjuvanted control, but overall the safety data were assessed as favorable for both adjuvanted vaccine formulations. Both RTS,S/AS02 and RTS,S/AS01 elicited significantly higher anti-CS antibody response than RTS,S/Saline. The primary endpoint of the trial was demonstrated, with the RTS,S/AS01 induced anti-CS response being higher than the RTS,S/AS02 response [92].

The evaluation of RTS,S/AS01E continued in children living in conditions of natural malaria exposure. In total, safety data for the RTS,S/AS01E malaria vaccine candidate have been evaluated following the administration of 19,598 doses to 6756 children aged 5 months to 4 years, and of 13,740 doses to 4698 infants aged 6 to 12 weeks living in malaria-endemic countries. Results from the Phase III pivotal study in children aged 5-17 months showed that in children aged 5-17 months, RTS,S/AS01E reduces clinical episodes of malaria and severe malaria by approximately half during the 12 months after vaccination. The rate of generalised convulsive seizures after RTS,S/AS01 vaccination in children aged 5-17 months, was 1.04 per 1000 doses (95% CI: 0.62, 1.64). RTS,S/AS01E given in co-administration with DTPwHepB/Hib vaccine at 6, 10, 14 weeks of age reduced the risk of malaria by approximately one third. Long term effects on a number of endpoints of public health interest, and the effect of a booster dose at study Month 20 have been characterized. In the older age category, but not in the younger age category, cases of meningitis reported as SAEs were more frequently reported in children vaccinated with RTS,S/AS01 as compared with the control vaccine. Meningitis etiologies were heterogeneous and there was no cluster in post-vaccination time-to-event [93]. EMA evaluation of the risk-benefit balance led to a positive recommendations for RTS,S/AS01E vaccination [94]. WHO recommended pilot introduction studies of RTS,S/AS01E according to a four dose regimen in high and middle endemicity countries in 5-17 months old children [95].

In addition to malaria candidate vaccines, the AS01 adjuvant system has been used in formulation of other candidate vaccines, including HIV and tuberculosis vaccines. No specific safety concern has emerged from the review of safety data from AS01 adjuvanted vaccine candidates in adults. Suspected cases of neuroinflammatory conditions (for example: multiple sclerosis, optic neuritis, and myelitis) and potentially autoimmune adverse events (for example: rheumatoid arthritis, Crohn's disease, thyroiditis, type 1 diabetes mellitus and systemic erythematosis) have been reported with other MPL-containing investigational products. A causal relationship between the products or adjuvant components and these events has not been identified at this time. Evaluation of these and similar events continues.

6.3 **Storage of Vaccines**

The RH5.1 vaccine will be stored between –70°C and –90°C. All movements of the study vaccines will be documented. Vaccine accountability, storage, shipment and handling will be in accordance with local SOPs.

AS01 will be stored between +2 and +8°C in a locked fridge.

The storage conditions will be under the responsibility of the Sponsor.

Any temperature deviation outside the ranges specified above must be reported to the Sponsor as soon as detected. Following an exposure to such a temperature deviation, vaccines will not be used until Sponsor approval has been given.

6.4 **Administration of Vaccines**

The antigen and adjuvant will be mixed in the clinic prior to use to create the RH5.1/AS01 vaccine. This will be carried out as per the Standard Operating Procedure (SOP) MC031.

All vaccines will be administered according to SOP VC002 Vaccination. The vaccine will be administered intramuscularly for all groups in the deltoid muscle of the non-dominant arm preferentially. The vaccinating Investigator will wear gloves. During administration of the vaccines, Advanced Life Support drugs and resuscitation equipment will be immediately available for the management of anaphylaxis. On vaccination day, vaccines will be allowed to thaw to room temperature and administered within 1 hour. Depending on dose and concentration, one or more vials of vaccine may be used.

The injection site will be covered with a sterile dressing and the volunteer will stay in the CCVTM, Southampton NIHR WTCRF or Guys and St Thomas CRF for observation, in case of immediate adverse events. Observations will be taken 30 minutes after vaccination (+/- 5 minutes) and the sterile dressing removed and injection site inspected. Observations will also be taken at 60 minutes (+/- 10 minutes) before the volunteer leaves.

6.5 **Vaccine Supply**

RH5.1 was manufactured at the CBF in Oxford.

AS01 adjuvant system is manufactured by GSK. AS01 is available as a 0.5 ml extractable liquid in a mono-dose glass vial and stored at +2 to +8°C before use. The vaccine and adjuvant doses are nominal and may vary slightly due to dilution, mixing and administration techniques.

AS01 will be supplied to the CBF in Oxford in a refrigerated container with a temperature logger to ensure a cold chain of +2 to +8°C.

The vaccine and adjuvant will be labelled for investigational use only in trial VAC063 by the CBF, who will then transfer the vaccine and adjuvant to the clinical sites. RH5.1 and AS01 will be certified for release for use in this trial by a qualified person (QP) at the CBF in Oxford.

All movements of the study vaccines will be documented. Vaccine accountability, storage, shipment and handling will be in accordance with local SOPs.

The final product for administration will be prepared by mixing of the RH5.1 liquid vaccine with the liquid adjuvant as per SOP MC031.

7 OBJECTIVES AND ENDPOINTS

7.1 Primary objectives

To assess the safety of RH5.1/AS01 in healthy malaria-naïve adults in the UK.

To assess the *in vitro* growth inhibition activity (GIA) against 3D7 clone *P. falciparum* parasites of IgG purified from the serum of vaccinees.

To assess efficacy of the RH5.1/AS01 vaccine by establishing if the vaccine can demonstrate a reduced parasite multiplication rate (PMR) in vaccinated subjects compared to infectivity controls against 3D7 clone parasites in a Phase IIa blood-stage controlled human malaria infection (CHMI) model.

7.2 Primary Outcome Measures

The specific endpoints for safety and reactogenicity will be actively and passively collected data on adverse events.

The specific endpoints for GIA *in vitro* will be assessed from a titration of the purified IgG in the assay.

7.3 Primary efficacy endpoint (Phase IIa stage of the trial, Groups 5 and 6):

PCR-derived parasite multiplication rate (PMR) will be the primary efficacy endpoint for the Phase IIa stage of the trial, and comparison of the endpoint between the Groups 5 and 6 will constitute the primary analysis for efficacy.

The arithmetic mean of the three replicate PCR results obtained for each individual at each timepoint will be used for model-fitting. Negative individual replicates will be handled as specified in the Jenner Institute qPCR SOP. qPCR data points which, based upon the mean of the three replicates, are negative or below the limit of quantification will be handled as specified in the Jenner Institute qPCR SOP . PMR will be calculated using a linear model fitted to $\log_{10}$-transformed qPCR data [96]. As previously, fitted lines will be constrained to pass through the known starting parasitaemia, calculated from the results of a limiting-dilution-based assay of the number of viable parasites in the inoculum [81] and a weight-based estimate of each volunteer's blood volume (70mL/kg) [42].

The distribution of PMRs will be assessed for deviation from normality visually and using a d'Agostino-Pearson test. Standard ladder-of-power transformations may be applied to achieve normality. Equality of variance will be tested with an F test. In the event of deviation from normality, PMRs will be compared by two-tailed Mann-Whitney test; otherwise a two-tailed two-sample t-test will be used, with Welch's correction if variances are non-equal as assessed by F-test.

7.4 Secondary objectives

To assess the humoral and cellular immunogenicity of RH5.1/AS01 using different vaccine doses and vaccination regimens.

To assess the durability of any reduction in parasite multiplication rate (PMR) in vaccinated Group 5) subjects by subjecting them to a re-challenge with 3D7 clone parasites approximately 4 months after the primary challenge (Group 7), and comparing the PMR with 1) that of previously challenged Group 6 controls receiving a second challenge (Group 8); and 2) that of newly recruited malaria-naïve controls (Group 9) receiving a primary challenge. This is using the same Phase IIa blood-stage controlled human malaria infection (CHMI) model.

7.5 Secondary Outcome Measures

P. falciparum RH5-specific immunogenicity will be assessed by a variety of immunological assays.

Other exploratory immunology may be carried out in collaboration with other specialist laboratories, including laboratories outside of Europe. This would involve transfer of serum/plasma and/or peripheral blood mononuclear cells (PBMC), but samples would be anonymised. Volunteers will be consented for this.

7.6 **Secondary efficacy analysis:**

- Time to microscopic patency will be compared between Groups 5 and 6 by Mann-Whitney test.
- A test of the hypothesis that there is a relationship between *in vitro* GIA induced by the RH5.1 vaccine and PMR.
- A test of the hypothesis that there is a relationship between anti-RH5 antibody responses induced by the RH5.1 vaccine and PMR.

7.7 **Secondary efficacy endpoint (Groups 7-9):**

PCR-derived parasite multiplication rate (PMR) will also be the efficacy endpoint for assessing the durability of the vaccine-reduction in PMR. Comparison of this PMR endpoint between Groups 7, 8 and 9 will constitute this secondary analysis for efficacy. PMRs will be calculated from the qPCR data exactly as above (for Groups 5 and 6). The distribution of PMRs will be assessed for deviation from normality visually and using a d'Agostino-Pearson test. PMRs across the 3 groups will be compared by two-tailed Kruskall-Wallis test with Dunn's multiple comparison post-test; PMRs between Groups 7 and 8 will also be compared by two-tailed Mann-Whitney test. Non-parametric analysis is used given the relatively small groups sizes in the re-challenge arm of this trial.

7.8 **Protocol pre-specified analyses for immunological correlates of efficacy (Groups 5 and 6):**

An analysis will be conducted to test for association between 'GIA_{50} titre' (defined as the dilution factor from the serum/plasma IgG concentration to the IgG concentration achieving 50% GIA against 3D7 clone *P. falciparum* parasites using the single-cycle standardised assay SOP at the PATH-MVI GIA Reference Center Laboratory at NIH) and PMR within the RH5-vaccinated group. The relationship between GIA_{50} and PMR will be assessed by Spearman rank correlation. Samples which do not achieve 50% GIA at the highest tested concentration will be ranked based upon the percentage GIA achieved at this highest concentration. Samples achieving <20% GIA at this highest concentration will be treated as tied, on the basis that it is highly unlikely that such weak GIA would achieve an effect upon PMR. The PMRs of volunteers with PMR greater than the control group mean will be treated as tied, on the basis that differences between their PMRs are likely to represent biological noise and are not informative with respect to the relationship between GIA and PMR. GIA and PMR data will be visually inspected. Standard ladder-of-power transformations may be applied if these result in a linear relationship. If a linear relationship appears plausible before or after transformation, a Pearson correlation coefficient will be calculated. If the relationship between GIA and PMR appears non-linear despite transformation, a Spearman rank correlation coefficient and *P* value will be calculated.

A similar analysis will be undertaken to test for association between anti-RH5 antibody response and PMR within the RH5-vaccinated group.

All other analyses will be reported as not pre-specified in the trial protocol. Other analyses may be detailed in the VAC063 laboratory plan. Some assays will be duplicated at different sites. Some of these will involve analysis of frozen samples, and others analysis of fresh samples. Recruitment and Withdrawal of Trial Volunteers

8 RECRUITMENT AND WITHDRAWAL OF TRIAL VOLUNTEERS

8.1 Volunteers

Volunteers may be recruited by use of an advertisement +/- registration form formally approved by the ethics committee and distributed or posted in the following places:

- In public places, including buses and trains, with the agreement of the owner / proprietor.
- In newspapers or other literature for circulation.
- On radio via announcements.
- On a website or social media site operated by our group or with the agreement of the owner or operator (including on-line recruitment through our web-site).
- By e-mail distribution to a group or list only with the express agreement of the network administrator or with equivalent authorisation.
- By email distribution to individuals who have already expressed an interest in taking part in any clinical trial at the Oxford Vaccine Centre, the Guys and St Thomas NIHR CRF or the Southampton NIHR WTCRF.
- On stalls or stands at exhibitions or fairs.
- Via presentations (e.g. presentations at lectures or invited seminars).
- Direct mail-out: This will involve obtaining names and addresses of adults via the most recent Electoral Roll. The contact details of individuals who have indicated that they do not wish to receive postal mail-shots would be removed prior to the investigators being given this information. The company providing this service is registered under the Data Protection Act 1998. Investigators would not be given dates of birth or ages of individuals but the list supplied would only contain names of those aged between 18 - 45 years (as per the inclusion criteria).
- Oxford Vaccine Centre databases: We may contact individuals registered on the Oxford Vaccine Centre database who have previously expressed an interest in receiving information about future studies for which they may be eligible.
- Southampton NIHR WTCRF Database of Healthy Volunteers: We may contact individuals from this database who have previously expressed an interest in receiving information about future studies for which they may be eligible.

Recruitment to Groups 7 and 8

Volunteers enrolled in Group 5/6 will be asked at one of their follow up visits whether they would be interested in a re-challenge. Those who are will be re-consented, and attend a re-screening visit after their C+90 follow-up appointment.

Recruitment to Group 9 for primary challenge

Volunteers originally screened, eligible, but not enrolled into groups 5 or 6 (e.g., those unable to commit to the schedule or those volunteers who were “back-ups” but were not enrolled), but who agree to remain on the volunteer email distribution list (database), will be contacted to see if they would like to be screened as new controls for a re-challenge study (Groups 9).If this does not result in sufficient volunteers being re-recruited into Group 9, we will formally re-advertise the study as detailed above.

Invitation to visit 1-2.5 years post-final vaccination (Groups 1-5 and 7)

Participants who have received at least three doses of RH5.1/AS01 will be contacted to ask if they would be interested in attending for a further visit which will take place 1-2.5 years after their last dose of vaccination. If interested, a visit will be scheduled and new consent obtained to proceed with the visit and blood tests.

8.2 Informed consent

All volunteers will sign and date the informed consent form before any study specific procedures are performed. The information sheet will be made available to the volunteer at least 24 hours prior to the screening visit. At the screening visit, the volunteer will be fully informed of all aspects of the trial, the potential risks and their obligations. The following general principles will be emphasised:

- Participation in the study is entirely voluntary
- Refusal to participate involves no penalty or loss of medical benefits
- The volunteer may withdraw from the study at any time
- The volunteer is free to ask questions at any time to allow him or her to understand the purpose of the study and the procedures involved
- The study involves research of an investigational vaccine
- There is no direct benefit from participating
- The volunteer's GP will be contacted to corroborate their medical history
- Samples are given as a gift to the University of Oxford and volunteers will not gain any personal direct benefit from this, even if used in the future for commercial purposes (e.g. monoclonal antibody production).
- The volunteer's blood samples taken as part of the study will be stored indefinitely either as part of an ongoing ethically approved study or in a human tissue authority (HTA) licensed biobank.
- Samples may be sent outside of the UK and Europe to laboratories in collaboration with the University of Oxford. These will be anonymised.

The aims of the study and all tests to be carried out will be explained. The volunteer will be given the opportunity to ask about details of the trial, and will then have time to consider whether or not to participate. If they do decide to participate, they will sign and date two copies of the consent form, one for them to take away and keep, and one to be stored in the case report form (CRF) – this is a paper or electronic document used to collect data relating to a particular volunteer. These forms will also be signed and dated by the Investigator.

Volunteers for Groups 5-9 will be asked to complete a questionnaire testing their understanding of the trial as part of the consent process. This helps to ensure that individuals understand the trial sufficiently to give informed consent. Provided the volunteer answers all questions in the questionnaire correctly, they will be asked to sign and date two copies of the consent form, as detailed above. Volunteers who fail to answer all questions correctly on their first attempt will be allowed to re-take the same questionnaire following further discussion with the investigator. Provided they subsequently answer all questions in the questionnaire correctly they may then complete the consent form and be screened for the trial.

Volunteers in Groups 5 and 6 will be re-consented to take part in Groups 7 and 8, respectively, if they choose to participate. At a time-point after the C+90 visit for the preceding challenge, volunteers in Groups

8 and 9 who wish to take place in a further challenge (either tertiary or secondary, respectively) will be re-consented at a re-screening visit for this phase of the study.

For volunteers in Groups 1-5 and 7 who wish to attend for a visit 1-2.5 years after their final vaccination, informed consent to proceed with the visit procedures will be obtained at the start of the visit.

8.3 Inclusion and exclusion criteria

This study will be conducted in healthy adults, who meet the following inclusion and exclusion criteria:

Inclusion Criteria

The volunteer must satisfy all the following criteria to be eligible for the study:

- Healthy adults aged 18 to 45 years.
- Able and willing (in the Investigator's opinion) to comply with all study requirements.
- Willing to allow the Investigators to discuss the volunteer's medical history with their General Practitioner (GP).
- For females only, willingness to practice continuous effective contraception (see below) during the study and a negative pregnancy test on the day(s) of screening and vaccination, and on the day prior to blood-stage CHMI, and prior to the start of antimalarial treatment for Groups 5-9 volunteers.
- Agreement to refrain from blood donation during the course of the study.
- Provide written informed consent.

Additional Inclusion Criteria for Groups 5 - 9

- Agreement to permanently refrain from blood donation, as per current UK Blood Transfusion and Tissue Transplantation Services guidelines [97].
- Reachable (24 hours a day) by mobile phone during the period between CHMI and completion of antimalarial treatment.
- Willingness to take a curative anti-malaria regimen following CHMI.
- Answer all questions on the informed consent questionnaire correctly.
- For Groups 7-9: completion of primary challenge, curative anti-malarials and follow-up (up until at least the C+28 visit)

Exclusion Criteria

The volunteer may not enter the study if any of the following apply:

- Participation in another research study involving receipt of an investigational product in the 30 days preceding enrolment, or planned use during the study period.
- Prior receipt of an investigational vaccine likely to impact on interpretation of the trial data, as assessed by the investigator. For Group 7 volunteers undergoing re-challenge, this exclusion criterion does not extend to the RH5.1/AS01 vaccine previously received.
- Any medical condition that in the judgment of the investigator would make intramuscular (IM) injection unsafe.
- 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 during the period starting six months prior to the first vaccine dose. For corticosteroids, this will mean prednisone 20 mg/day (for adult subjects), or equivalent. Inhaled and topical steroids are allowed.

- Administration of long-acting immune-modifying drugs at any time during the study period (e.g. infliximab).
- Chronic use of antibiotics with antimalarial effects (e.g. tetracyclines for dermatologic patients, sulfa for recurrent urinary tract infections, etc.).
- History of malaria chemoprophylaxis within 60 days prior to vaccination
- History of allergic disease or reactions likely to be exacerbated by any component of the vaccine.
- Any history of anaphylaxis in relation to vaccination.
- Pregnancy, lactation or willingness/intention to become pregnant during the study.
- History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ).
- History of serious psychiatric condition likely to affect participation in the study.
- 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 in the 5 years preceding enrolment.
- Seropositive for hepatitis B surface antigen (HBsAg).
- Seropositive for hepatitis C virus (antibodies to HCV) at screening (***unless*** has taken part in a prior hepatitis C vaccine study with confirmed negative HCV antibodies prior to participation in that study, and negative HCV RNA PCR at screening for this study).
- History of clinical malaria (any species (Not applicable to prior challenge for Groups 7,8 and 9).
- 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 significant disease, disorder or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data.
- Inability of the study team to contact the volunteer's GP to confirm medical history and safety to participate.

Additional exclusion criteria for Groups 5 - 9

- Use of systemic antibiotics with known antimalarial activity within 30 days of CHMI (e.g. trimethoprim-sulfamethoxazole, doxycycline, tetracycline, clindamycin, erythromycin, fluoroquinolones and azithromycin).
- History of sickle cell anaemia, sickle cell trait, thalassaemia or thalassaemia trait or any haematological condition that could affect susceptibility to malaria infection.
- Laboratory evidence of G6PD deficiency at screening
- Laboratory evidence of haemoglobinopathy at screening
- Use of medications known to cause prolongation of the QT interval ***and*** existing contraindication to the use of Malarone.

- Use of medications known to have a potentially clinically significant interaction with Riamet ***and*** Malarone.
- Contraindications to the use of **both** Riamet ***and*** Malarone.
- Any clinical condition known to prolong the QT interval.
- Family history of congenital QT prolongation or sudden death.
- Positive family history in **both** 1st and 2nd degree relatives < 50 years old for cardiac disease.
- History of cardiac arrhythmia, including clinically relevant bradycardia.
- Volunteer unable to be closely followed for social, geographic or psychological reasons.

Effective contraception for female volunteers

Female volunteers are required to use an effective form of contraception during the course of the study. As this is a Phase I, first-in-human, study there is no information about the effect of these vaccines on a foetus.

Acceptable forms of contraception for female volunteers include:

- Established use of oral, injected on implanted hormonal methods of contraception.
- Placement of an intrauterine device (IUD) or intrauterine system (IUS).
- Barrier methods of contraception (condom or occlusive cap with spermicide).
- Male sterilisation, if the vasectomised partner is the sole partner for the subject.
- True abstinence, when this is in line with the preferred and usual lifestyle of the subject (periodic abstinence and withdrawal are not acceptable methods of contraception).

NB: Women in Groups 5-9 using hormonal contraceptives during CHMI and treated with the antimalarial Riamet will be advised to also use a barrier method of contraception whilst on Riamet treatment, and until the start of the next menstruation after treatment.

Prevention of 'Over Volunteering'

Volunteers will be excluded from the study if they are concurrently involved in another trial. In order to check this, volunteers will be asked to provide their National Insurance or Passport number (if they are not entitled to a NI number) and will be registered on a national database of participants in clinical trials (*www.tops.org.uk*).

Re-vaccination exclusion criteria

The following adverse events associated with vaccine immunisation constitute absolute contraindications to further administration of vaccine. If any of these events occur during the study, the subject must be withdrawn and followed until resolution of the event, as with any adverse event:

- Anaphylactic reaction following administration of vaccine
- Pregnancy

The following adverse events constitute contraindications to administration of vaccine at that point in time; if any one of these adverse events occurs at the time scheduled for vaccination, the subject may be vaccinated at a later date, or withdrawn at the discretion of the Investigator. The subject must be followed until resolution of the event as with any adverse event:

- Acute disease at the time of vaccination. (Acute disease is defined as the presence of a moderate or severe illness with or without fever). All vaccines can be administered to persons with a minor illness such as diarrhoea, mild upper respiratory infection with or without low-grade febrile illness, i.e. temperature of ≤37.5°C/99.5°F.

- Temperature of >37.5°C (99.5°F) at the time of vaccination.

8.4 **Withdrawal of Volunteers**

In accordance with the principles of the current revision of the Declaration of Helsinki and any other applicable regulations, a volunteer has the right to withdraw from the study at any time and for any reason, and is not obliged to give his or her reasons for doing so. The Investigator may withdraw the volunteer at any time in the interests of the volunteer's health and well-being. In addition the volunteer may withdraw/be withdrawn for any of the following reasons:

- Administrative decision by the Investigator.
- Ineligibility (either arising during the study or retrospectively, having been overlooked at screening).
- Significant protocol deviation.
- Volunteer non-compliance with study requirements.
- An AE, which requires discontinuation of the study involvement or results in inability to continue to comply with study procedures.

The reason for withdrawal will be recorded in the CRF. If withdrawal is due to an AE, appropriate follow-up visits or medical care will be arranged, with the agreement of the volunteer, until the AE has resolved, stabilised or a non-trial related causality has been assigned. Any volunteer who is withdrawn from the study may be replaced, if that is possible within the specified time frame. The Local Safety Monitor (LSM) may recommend withdrawal of volunteers.

Any volunteer who fails to attend for two or more follow-up visits during the study will be deemed to have withdrawn from the study. If a volunteer withdraws/is withdrawn from the study after CHMI but before reaching the criterion for malaria diagnosis, an appropriate, curative course of anti-malarial therapy must be completed. The importance of this will be emphasised to volunteers at screening. If a volunteer refuses to take anti-malarial therapy after malaria diagnosis, a rapid assessment of mental state and capacity will be undertaken, with the involvement of NHS psychiatric and infectious diseases services. If necessary the volunteer may be detained under section 4 of the UK Mental Health Act until this assessment can be carried out.

If a volunteer withdraws from the study, blood samples collected before their withdrawal from the trial will be used/stored unless the volunteer specifically requests otherwise. Data from volunteers withdrawn from the study after they have had a qPCR result of ≥500 parasites/mL will be included in the analysis of results relating to the study's objectives.

In all cases of subject withdrawal, excepting those of complete consent withdrawal, long-term safety data collection will continue as appropriate if subjects have received one or more vaccine doses or undergone CHMI.

8.5 **Pregnancy**

Should a volunteer become pregnant during the trial, she will be followed up as other volunteers and in addition will be followed until pregnancy outcome, with the volunteer's permission. We will not routinely perform venepuncture on such volunteers. The management of any volunteers found to be pregnant at any time after the blood-stage challenge up to the point of diagnosis with malaria by thick blood smear/ PCR will be discussed with the on-call infectious diseases consultant at the Oxford University Hospitals' NHS Trust.

8.6 Safety Stopping/ Holding Rules

Safety holding rules have been developed considering the fact that this is a first-in-human dose escalation study. Safety reviews will take place prior to each dose escalation. In order for a safety review to take place at least 6/12 volunteers in each group must have received the first two vaccinations.

'Solicited adverse events' are those listed as foreseeable adverse reactions in section 12.3 of the protocol, occurring within the first 7 days after vaccination (day of vaccination and six subsequent days). 'Unsolicited adverse events' are adverse events other than the foreseeable ARs occurring within the first 7 days, or any AEs occurring after the first 7 days after vaccination.

Group holding rules (applies to Groups 1 – 5 and Group 7)

The following holding rules will apply to study Groups 1 – 5 and Group 7 (i.e. vaccinees). If a holding rule is activated, then further vaccinations in any Group will not occur until an internal safety review has been conducted and it is deemed appropriate to restart dosing. The regulatory authority must be informed and a request to restart dosing with pertinent data must be submitted as a request for a substantial amendment. The internal safety review will consider:

- The relationship of the AE or SAE to the vaccine.
- The relationship of the AE or SAE to the vaccine dose, or other possible causes of the event.
- If appropriate, additional screening or laboratory testing for other volunteers to identify those who may develop similar symptoms, and alterations to the current Participant Information Sheet (PIS) are discussed.
- New, relevant safety information from ongoing research programmes on the various components of the vaccine (i.e. the AS01 adjuvant).

The local ethics committee and Adjuvant System manufacturers (GSK) will also be notified if a holding rule is activated or released.

All vaccinated volunteers will be followed for safety until resolution or stabilisation (if determined to be chronic sequelae) of their AEs.

The group holding rules are as follows:

- **Solicited local adverse events:**
    - If more than 25% of doses of a vaccine are followed by the same Grade 3 solicited local adverse event beginning within 2 days after vaccination (day of vaccination and one subsequent day) and persisting at Grade 3 for > 72 hrs.
- **Solicited systemic adverse events:**
    - If more than 25% of doses of a vaccine are followed by the same Grade 3 solicited systemic adverse event beginning within 2 days after vaccination (day of vaccination and one subsequent day) and persisting at Grade 3 for > 48 hrs.
- **Unsolicited adverse events:**
    - If more than 25% of volunteers develop the same Grade 3 unsolicited adverse event (including the same laboratory adverse event) that is considered possibly, probably or definitely related to vaccination and persists at Grade 3 for > 48 hrs.
- **A serious adverse event considered possibly, probably or definitely related to vaccination occurs**

Individual stopping rules

In addition to the above stated group holding rules, stopping rules for individual volunteers will apply (i.e., indications to withdraw individuals from further vaccinations).

- **Local reactions:** Injection site ulceration, abscess or necrosis.

- **Laboratory AEs:**
    - The volunteer develops a Grade 3 laboratory adverse event considered possibly, probably or definitely related within 7 days after vaccination and persisting continuously at Grade 3 for >72hrs.

- **Solicited systemic adverse events:**

    - The volunteer develops a Grade 3 systemic solicited adverse event considered possibly, probably or definitely related within 2 days after vaccination (day of vaccination and one subsequent day) and persisting continuously at Grade 3 for >72hrs.

- **Unsolicited adverse events:**

    - The volunteer has a Grade 3 adverse event, considered possibly, probably or definitely related to vaccination, persisting continuously at Grade 3 for >72hrs.

    - The volunteer has a serious adverse event considered possibly, probably or definitely related to vaccination.

    - The volunteer has an acute allergic reaction or anaphylactic shock following the administration of the vaccine investigational product.

If a volunteer has an acute illness (moderate or severe illness with or without fever) or a fever (oral temperature greater than 37.5°C) at the scheduled time of administration of investigational product, the volunteer will not receive the vaccine at that time. The vaccine may be administered to that volunteer at a later date within the time window specified in the protocol (see Tables 3 - 5) or they may be withdrawn from the study at the discretion of the Investigator.

All vaccinated volunteers will be followed for safety until the end of their planned participation in the study or until resolution or stabilisation (if determined to be chronic sequelae) of their AEs, providing they consent to this.

In addition to these pre-defined criteria, the study can be put on hold upon advice of the Local Safety Monitor, Chief Investigator, Study Sponsor, regulatory authority, Research Ethics Committee (REC) or Local Safety Committee (LSC), for any single event or combination of multiple events which, in their professional opinion, jeopardise the safety of the volunteers or the reliability of the data.

9 STUDY OVERVIEW

9.1 Study Groups

This is an open-label, multi-centre Phase I/IIa dose escalation blood-stage malaria CHMI trial to assess the safety, immunogenicity and efficacy of the candidate malaria vaccine RH5.1/AS01. All volunteers recruited will be healthy, malaria naïve adults aged between 18 and 45 years.

Volunteers will be recruited and vaccinated at the CCVTM, Oxford; Guys and St Thomas' NIHR CRF, London; and the NIHR WTCRF, Southampton for the Phase Ia part of the trial. Volunteers will be recruited and vaccinated at the CCVTM, Oxford, and at Guy's and St Thomas' NIHR CRF, London, for the Phase IIa stage. Volunteers in Group 9 will only be recruited in Oxford. Vaccine efficacy will be assessed using blood-stage CHMI by injection of parasitised red blood cells, as described in section 10.2.

There will be 9 study groups across two phases of the trial, with a total of 84 volunteers (see Table 2).

Phase Ia	**Group**	**Group size**	**Day 0**	**Day 28**	**Day 56**	**Day 182**			
	1	6 – 12	2µg RH5.1/ 0.5mL AS01	2µg RH5.1/ 0.5mL AS01	2µg RH5.1/ 0.5mL AS01				
	2	6 – 12	10µg RH5.1/ 0.5mL AS01	10µg RH5.1/ 0.5mL AS01	10µg RH5.1/ 0.5mL AS01				
	3	12	50µg RH5.1/ 0.5mL AS01	50µg RH5.1/ 0.5mL AS01		10µg RH5.1/ 0.5mL AS01			
	4	12	50µg RH5.1/ 0.5mL AS01	50µg RH5.1/ 0.5mL AS01	50µg RH5.1/ 0.5mL AS01				
Phase IIa	**Group**	**Group size**	**Day 0**	**Day 28**	**Day 56**	**2 weeks post-3rd vaccination**	**~4 months post 3rd vaccination**	**1-2 weeks post 4th vaccination**	
	5	15 (+2)	10µg RH5.1/ 0.5mL AS01	10µg RH5.1/ 0.5mL AS01	10µg RH5.1/ 0.5mL AS01	CHMI			
	6 (controls)	15 (+2)				CHMI			
	7 Subset of Group 5 (vaccinees)	5-15	10µg RH5.1/ 0.5mL AS01	10µg RH5.1/ 0.5mL AS01	10µg RH5.1/ 0.5mL AS01	CHMI	10µg RH5.1/ 0.5mL AS01	CHMI	
	8 Subset of Group 6 (controls)	5-15				CHMI		CHMI	
	9 (controls)	5-6 (+2)						CHMI	

Table 2: Study groups. Given group sizes indicate planned number of recruits to each group.

The study is designed to assess a 'standard' protein-in-adjuvant vaccination regimen of 3 doses given four weeks apart, with dose escalation to assess the best dose in healthy adults (acceptable reactogenicity with good immunogenicity profiles) in Groups 1, 2 and 4. The efficacy of this regimen will also be assessed using the best dose (ascertained from Groups 1, 2 and 4). The other group to receive the vaccine (Group 3) will assess the safety and immunogenicity of giving the 'standard' first two doses of the vaccine followed by a delayed fractional dose. This has been shown to improve efficacy with the RTS,S vaccine given with a similar adjuvant to that being used in this study. This effect has not been widely studied with other candidate vaccines so this study aims to evaluate whether improvements in immunogenicity and/ or *in vitro* efficacy are seen with this regimen.

Vaccination of groups will be sequential from Group 1 to Group 3. Group 3 and 4 can be recruited simultaneously. Volunteers will be able to choose which group they are allocated to (providing there is still space within the group they wish to be in). The vaccination dose for Group 5 was decided following the analysis of safety and exploratory immunology assays from Groups 1, 2 and 4. Given the safety and tolerability are comparable across all dose groups, with no clinical concerns, the immunogenicity (as measured by ELISA) was used to decide the optimum dose for Group 5. The highest median ELISA response at day 70 for the volunteers in Groups 1, 2 and 4, (after at least six volunteers in Group 4 had received all three vaccinations), was in Group 2. Therefore, the 10 μg dose, administrated at Days 0, 28 and 56, was taken forward to the Phase IIa. Two 'back-up' volunteers will be recruited to Groups 5 and 6 in case of withdrawal of volunteers prior to CHMI. The back-up volunteers in Group 5 will be enrolled and vaccinated with the rest of the group but will only undergo CHMI if another vaccinee withdraws from the study. The back up control volunteers (Group 6) will be available at short notice to take part in the trial but will only undergo CHMI if a Group 6 volunteer withdraws prior to CHMI.

The total number of volunteers recruited to Groups 1 and 2 was decided based on the immunogenicity of the vaccines at the 2 μg and 10 μg doses. As both the doses are immunogenic the groups are being recruited to a total of 12 volunteers. Immunogenicity has been assessed according to the results obtained from the VAC057 trial using the viral vectored RH5 vaccines (ChAd63/MVA RH5). If the mean response after 3 vaccinations in Group 1 and Group 2 was not within the 95% CI of the mean response achieved in boosted volunteers in VAC057 the recruitment for these groups was to be held at 6 volunteers per group. This would allow sufficient numbers of volunteers to assess the safety of these doses prior to dose escalation. However, the mean response has exceeded that in boosted VAC057 volunteers, so 12 are being recruited into each group.

Groups 7-9 constitute the re-challenge phase of the study, where we plan to enrol volunteers from Groups 5 and 6 to take part in a second homologous *P. falciparum* challenge. Alongside these we will recruit a fresh group of controls to receive a primary challenge. To summarise (and detailed in both Table 2 and Figure 5) we will have 3 new groups:

Group 7 = (vaccinees from group 5) – these will receive a fourth and final vaccination with RH5.1/AS01 followed 7-14 days later by a repeat homologous malaria challenge.

Group 8 = (malaria-exposed controls from Group 6) – these will be re-challenged in parallel with Group 7.

Group 9 = (malaria-naïve controls) – these will receive a primary malaria challenge in parallel with Groups 7 and 8.

While we will aim to recruit as many volunteers as possible from Groups 5 and 6 into Groups 7 and 8 (respectively), it is important to note that small numbers are routinely studied and reported in numerous other CHMI trials (undertaken in Oxford and elsewhere under similar circumstances). We intend to proceed if ≥4 volunteers from both groups agree to (and are eligible to) participate.

It is important to emphasise that there will be no commitment to undergoing repeat CHMI when volunteers are screened for initial entry into the study.

First volunteers

At each dose of RH5.1 (i.e. 2 µg, 10 µg and 50 µg) the first volunteer to receive each dose will be vaccinated alone and then reviewed on the day following vaccination. Providing there are no safety concerns at the Day 1 review, or recorded in the diary over the next 24 hours, another two volunteers may be immunised 2 days after the first volunteer, at least one hour apart. These volunteers will also be reviewed on the day following vaccination and providing there are no safety concerns 2 days after vaccination the rest of the group can be vaccinated. An independent safety review will be carried out after six volunteers have received two of the three vaccinations in Group 1 before vaccinations are started in Group 2, and similarly between Group 2 and 3/4. Enrolment for Groups 5 and 6 can commence once at least six Group 4

volunteers have received all three vaccinations and completed at least 7 days of safety follow-up with no safety concerns (no holding/ stopping rules met). The vaccination dose for Group 5 (10 μg) is now confirmed.

Duration of study

Volunteers will be considered to be enrolled in the trial on receipt of the first vaccination.

- Groups 1, 2 and 4

  Groups 1, 2 and 4 will attend for three vaccinations on days 0, 28 and 56. The follow up visits are as described in section 11.3, with a last visit in the initial follow-up period approximately 6 months after the final vaccination. Participants will then be invited for a final visit 1-2.5 years following their final vaccination.

- Group 3

  Group 3 volunteers will attend for three vaccinations on days 0, 28 and 182. The follow up visits are as described in section 11.3, with a last visit in the initial follow-up period approximately 6 months after the final vaccination. Participants will then be invited for a final visit 1-2.5 years following their final vaccination.

- Group 5

  Group 5 volunteers will attend for three vaccinations on days 0, 28 and 56. They will undergo blood-stage CHMI 2 weeks after the final vaccination and will be followed up until approximately 6 months after the final vaccination. We will also recruit 2 'back-up' volunteers for Group 5. These will be vaccinated alongside the rest of the group but will only undergo malaria challenge if other volunteers from the group drop out of, or are withdrawn from, the study. Participants will then be invited for a final visit 1-2.5 years following their final vaccination.

- Group 6 (controls)

  Group 6 volunteers will be infectivity controls, so will not receive any vaccinations. Control volunteers will be considered as enrolled in the study once they have attended the C-1 visit (day prior to challenge). In addition to the 15 volunteers to be enrolled in Group 6, back-up volunteers for Group 6 who have been screened and are eligible to participate in the study will be identified. These volunteers will be available to be enrolled in the study at short notice should a planned volunteer withdraw consent or become ineligible immediately prior to CHMI. Back-up volunteers may be enrolled in the study at Day C-1 and asked to attend clinic on day of challenge in case another control volunteer is found to be ineligible at that point. If back-up volunteers are not required on day of challenge they will be withdrawn from the study after this point.

- Group 7

  Group 5 volunteers will be invited to attend for a fourth vaccination with RH5.1/AS01 in the second part of the Phase IIa study, approximately 4 months post their primary malaria challenge. They will then undergo a second homologous blood-stage CHMI 7-14 days later and will be followed up until approximately 6 months after the final vaccination in the initial follow-up period. They will be considered re-enrolled into the study once they have attended their second C-1 visit (day before challenge), and will be referred to as a new Group 7. Participants will then be invited for a final visit 1-2.5 years following their final vaccination.

- Group 8 (controls)

  Group 6 volunteers will be invited to be infectivity controls for a second time (in the second part of the Phase IIa study), so again will not receive any vaccinations. They will undergo a second blood-stage

CHMI in parallel with Groups 7 and 9. They will be considered re-enrolled into the study once they have attended their second C-1 visit, and will be referred to as a new Group 8.

All Group 5 and 6 volunteers who wish to take part in the re-challenge phase will be re-screened (to ensure ongoing eligibility) prior to enrolment into Groups 7 and 8 respectively.

- Group 9 (controls)

Group 9 volunteers will be infectivity controls in the second part of the Phase IIa, so will not receive any vaccinations. Control volunteers will be considered as enrolled in the study once they have attended the C-1 visit. They will undergo a blood-stage CHMI in parallel with the second CHMI administered to Groups 7 and 8. In addition to the 5-6 volunteers to be enrolled in Group 9, 2 back-up volunteers for Group 9 who have been screened and are eligible to participate in the study will be identified. These volunteers will be available to be enrolled in the study at short notice should a planned volunteer withdraw consent or become ineligible immediately prior to CHMI. Back-up volunteers may be enrolled in the study at Day C-1 and asked to attend clinic on day of challenge in case another control volunteer is found to be ineligible at that point. If back-up volunteers are not required on day of challenge they will be withdrawn from the study after this point.

Definition of Start and End of Trial

The start of the trial is defined as the date of the first vaccination of the first volunteer. The end of the trial is the date of the last visit of the last volunteer (LVLV).

9.2 Potential Risks for volunteers

Risks to the volunteers in this trial are associated with phlebotomy, vaccination, malaria infection and receipt of a blood product. These risks are outlined below.

Female participants will be cautioned of the unknown risk of the RH5.1/AS01 vaccine to the foetus and will be advised to use adequate contraceptive methods for the duration of the study.

Phlebotomy

The total amount of blood collected over the study period will be a maximum of approximately 1523mL (in Group 6 volunteers who proceed to a further challenge in Group 8). This amount is drawn over a period of ~6 months and is not expected to compromise the majority of healthy volunteers, however, volunteers will be monitored for anaemia.

Risks occasionally associated with venepuncture include pain and bruising at the site of venepuncture, light-headedness, and syncope (rarely).

Vaccination with RH5.1/AS01

As with any vaccine, Guillain-Barré syndrome or immune-mediated reactions that can lead to organ damage including serious allergic reactions may occur but this should be extremely rare. Serious allergic reactions including anaphylaxis could also occur and for this reason volunteers will be vaccinated in a clinical area where Advanced Life Support trained physicians, equipment and drugs are immediately available for the management of any serious adverse reactions.

The most frequent adverse reactions observed in previous clinical trials using protein antigens with the AS01 adjuvant system include pain, swelling, erythema, and tenderness at the site of injection, and systemic symptoms such as low-grade fever and short-term flu-like symptoms: fatigue, myalgia, headache, malaise.

Febrile seizures assessed as related to RTS,S/AS01 vaccination have been reported in children, but not in adults.

Some individuals vaccinated with adjuvant components identical to those that will be used in this study have reported autoimmune diseases. A causal association between the adjuvant components and occurrence of auto-immune diseases (AID) has however not been established, as these can occur in people who get other vaccines, or no vaccines at all. A meta-analysis of a GSK Vaccines adjuvanted vaccine showed no increased risk of AID associated to the adjuvant [98].

Risk of Infection with Blood Borne Organisms

Experimentally induced blood-stage malaria infection (IBSM) for malaria challenge involves the administration of parasitised red blood cells, originally frozen down from a single donor in Australia. The blood was collected, processed and stored as described in section 5.3. The blood has been stored and monitored for over 15 years. The process basically constitutes a very tiny blood transfusion and therefore the risk of transmission of a blood borne infection cannot be completely ruled out. Measures have been taken to minimise the risks and these are outlined below.

The donor was screened for a range of blood borne infections prior to the inoculum being collected, and has remained well since the first use of the inoculum in 1997 [71]. The donor tested negative for HIV, Hepatitis A, B and C, Syphilis, HTLV1 and Ross River virus. The donor was seropositive for Epstein Barr virus (EBV) and Cytomegalovirus (CMV), indicating previous infection. The blood was initially frozen for a year whilst the donor was monitored and retested. Over 240 volunteers have received the inoculum since 1997 and there have been no serious adverse events, and no cases of blood borne infection associated with this. There were two individuals who were infected with *P. falciparum* malaria with the purpose of preparing the inoculum but all challenge inocula have since come from one of these donors as the other donor had a much lower parasitaemia when blood was collected [71].

More recently, an ampoule of the parasite seed stock from the donor was thawed and cultured within the Queensland Institute of Medical Research (QIMR) laboratory. Culture supernatant tested negative for Mycoplasma by PCR. The blood was also tested for CMV and EBV by PCR using methodologies not available at the time of the initial cryopreservation, and tested negative. Although the donor remains antibody positive for EBV and CMV, it is apparent that the risk of transmission of both CMV and EBV has been greatly reduced by leukodepletion of the donor blood (both EBV and CMV cause latent infection of leukocytes) and washing of the blood with clinical grade saline prior to use. Initially only CMV and EBV seropositive volunteers were enrolled due to a concern about the risk of transmission of these viruses, but the risk has since been deemed extremely low. The requirement for CMV and EBV seronegativity was therefore waived by QIMR and in the most recent blood-stage CHMI trials carried out in both Nijmegen [84] and Oxford (VAC054), serostatus data were collected but not used as inclusion/exclusion criteria. None of the seronegative volunteers in VAC054 or the Nijmegen trial seroconverted during follow-up after CHMI. This study will therefore not exclude volunteers based on EBV and/or CMV seronegativity, and therefore EBV and CMV serostatus will not be assessed.

The risk of transmission of variant Creutzfeldt Jacob Disease (vCJD) from the inoculum appears remote. The two donors are Australian residents, where no cases of either Bovine Spongiform Encephalopathy (BSE) in cattle or vCJD in humans have been reported to date.

As well as the measures outlined above, the risk of infection is further reduced by the very small size of the inoculum. The volume to be transfused for this study is many thousand times smaller than the amount received in typical blood transfusion. Serum from volunteers will be collected before and after challenge for safety serum storage.

Risk of reaction to the blood sample

The donor of the blood used in the inoculum is Blood Group O and Rhesus (Rh) negative. People with Blood Group O negative blood are considered ‘universal donors’, as recipients of their blood are unlikely to develop red cell alloantibodies when given much larger volumes of blood than is envisaged here. The

volume of blood to be used in this study is extremely small. Around 10,000 - 120,000 red cells (around 5% of which contain parasites) from the donor will be injected into each volunteer. One mL of blood contains about 5 billion red cells, demonstrating the very small amount which is to be used in this study. The risk of a transfusion reaction or development of antibodies to donor red cells, which may make blood transfusion more difficult in the future, cannot be completely excluded but is considered extremely unlikely both because of the small volume of blood used and the fact the blood has been leukodepleted. Nevertheless, the volunteers will be monitored for this possibility in the period immediately after the administration of the malaria parasite dose. Volunteers will be reviewed 15 minutes (+/- 5 minutes) and 1 hour (+/- 10 minutes) after receiving the inoculum before they are able to leave the CCVTM. The serum collected for storage (as described above) could be used to test for antibodies at a later date if deemed necessary.

Risks relating to Malaria infection

All non-vaccinated control volunteers and potentially all vaccinees are likely to develop symptomatic malaria infection following CHMI. Symptoms and signs will include feverishness, fever, tachycardia, hypotension, chills, rigors, sweats, headache, anorexia, nausea, vomiting, diarrhoea, myalgia, arthralgia, low back pain, thrombocytopenia and lymphopenia. Unmonitored and untreated *P. falciparum* infection can be fatal and, for this reason, volunteers will be followed up closely post-challenge and only enrolled in the study if they are deemed reliable and capable of complying with the intensive follow-up schedule. If necessary, volunteers may be admitted for in-patient care.

The clone of malaria (3D7) is known to be sensitive to antimalarials including chloroquine, Riamet®, Malarone® and Fansidar and volunteers will be followed up twice daily following challenge and provided with antimalarials as soon as they have:

- One morphologically normal parasite detectable on thick blood film with symptoms consistent with malaria infection, OR
- One morphologically normal parasite detectable on thick blood film and qPCR ≥500 parasites/mL, OR
- Symptoms consistent with malaria infection and qPCR ≥500 parasites/mL.

There is no risk of developing recurrent or relapsing episodes of malaria from these infections as this does not occur with *P. falciparum* malaria.

Groups 7 and 8 will be receiving a second homologous malaria infection. There is no precedent in the modern CHMI era for re-challenge of volunteers who were not sterilely protected and reached thick smear positive on the primary infection. The primary blood-stage infection that these volunteers receive could induce some immunity to a homologous secondary or tertiary challenge (the old neurosyphilis *P. vivax* malaria data [50] showed a reduction in fever episodes and a log reduction the maximum mean parasite count after homologous *P. vivax* re-challenge) but the effect in blood-stage *P. falciparum* is assessed for the first time in this study. In the absence of previous similar data, volunteers will be counselled to expect to experience a similar degree of symptoms as with the primary infection.

Medications Dispensed to Volunteers in the Course of the Trial

(a) Treatment of Plasmodium falciparum Infection

Malaria infection will be treated with oral Riamet (artemether-lumefantrine) unless there is a contraindication to this medication. If there is a contraindication to Riamet, another appropriate antimalarial will be used, either oral Malarone or chloroquine. See the Summary of Product Characteristics (SmPC) for side effects of and contraindications to chloroquine and Malarone.

Riamet is generally well tolerated, but may cause some side effects. Common side effects include headache, dizziness, abdominal pain and loss of appetite, sleeping problems, palpitations, nausea, vomiting, diarrhoea, pruritus, skin rash, cough, muscle or joint pain and fatigue. Volunteers will be

counselled that certain side effects, for example dizziness, may impact on the performance of skilled tasks such as driving.

As Riamet may increase the QT interval, Riamet will not be administered to volunteers at risk for QT prolongation. This includes those with prolonged QT on baseline ECG, a history of long QT syndrome, a family history of congenital QT prolongation or sudden death, cardiac arrhythmias, severe heart disease, and hypokalaemia or hypomagnesaemia. Volunteers will be advised to avoid grapefruit juice whilst taking Riamet. Riamet is also contraindicated in volunteers using concomitant medications affecting the QT interval, and will not be given to these volunteers. Concomitant use of Riamet may decrease effectiveness of hormonal contraceptives. Women using hormonal contraceptives will be advised to also use a barrier method of contraception whilst on Riamet treatment, and until the start of the next menstruation after treatment.

(b) Treatment of Symptoms Associated with Malaria

Volunteers will be dispensed cyclizine and paracetamol for the treatment of symptoms associated with malaria unless there are contraindications to these medications. Cyclizine is generally well tolerated however there is a risk of an allergic reaction. Other side effects include skin rashes or itching, drowsiness, headache, dry mouth nose or throat, blurred vision, palpitations, difficulty urinating, constipation, anxiety, insomnia or hallucinations. Rare side effects include hypotonia, seizures, dizziness, hypertension, paraesthesia, jaundice, hepatitis, confusion or dyskinesias. Paracetamol is generally well tolerated however it can cause an allergic reaction or rarely pancytopaenia.

9.3 **Known Potential Benefits**

Volunteers will not benefit directly from participation in this study. However, it is hoped that the information gained from this study will contribute to the development of a safe and effective malaria vaccine regimen. Volunteers will also receive information about their general health status.

10 EXPERIMENTALLY INDUCED BLOOD-STAGE MALARIA INFECTION INOCULUM

For details on the preparation and previous use of this inoculum please see section 5.3

10.1 **Storage conditions**

Between 1994 and 2003 the cryopreserved samples to be used in this trial were stored in dedicated liquid nitrogen cylinders in a secure facility at QIMR. The liquid nitrogen containers were kept locked and accessible only to approved staff. In 2003 the samples were transferred to Biotec Distribution Ltd., Bridgend, UK and then to Thermo Fisher Bishop's Stortford, Hertfordshire, UK, in 2007 where they have been stored on behalf of Oxford University in temperature-monitored liquid nitrogen.

10.2 **Administration of the inoculum**

Preparation

Thawing and washing of the inoculum will be done with commercial solutions for human use and with disposable syringes and needles according to standard operating procedures used in previous studies at QIMR and Oxford [73,81]. Work will be carried out in the bio safety category III or derogated category III laboratory at the Jenner Institute, Old Road Campus Research Building (ORCRB). Sample manipulations will be performed within a safety cabinet that has been especially fumigated, sterilised and dedicated for this purpose.

After thawing of the blood inoculum and preparation of the syringes, the cold chain (2-8°C degrees) will be maintained at all times until the inoculum has been administered to the volunteer.

Administration

The inoculation will take place in Oxford at the CCVTM. The inoculum will be administered by intravenous injection into an indwelling intravenous cannula. Approximately 1,000 infected red blood cells will be injected in a total volume of 5 mL of normal saline followed by a saline flush. Subjects will be observed for one hour before discharge. The order in which vaccinated and unvaccinated volunteers receive the inoculum will be interspersed in case of time effects on viability of the parasites. If the inoculum is given on more than one day an equal number of control volunteers will receive the inoculum on each day. All volunteers will receive the inoculum within 4 hours of removal from frozen storage.

11 TREATMENT OF TRIAL VOLUNTEERS

11.1 Trial Sites

Volunteers will be recruited and undergo screening visits, vaccination and clinic visits post-vaccination at their local trial site; either at the CCVTM, Oxford, the Guys and St Thomas' NIHR CRF, London or NIHR WTCRF, Southampton for the Phase Ia stage of the trial. Volunteers recruited to the Phase IIa stage of the trial will be recruited in Oxford and at Guy's and St Thomas' NIHR CRF in London (except for Group 9volunteers, who will only be recruited from Oxford). Those recruited in London will undergo screening, vaccination and post-vaccination visits there (pre CHMI). They will then come to Oxford for CHMI and subsequently all post-CHMI follow-up will occur at the CCVTM in Oxford. From the day before challenge until completion of a curative course of anti-malarial therapy, all volunteers must be resident within a 15 mile radius of the CCVTM, Oxford. If necessary, accommodation can be provided for volunteers living beyond this distance.

11.2 Study procedures

Procedures will be performed on the visit time points indicated in the schedule of attendances. Additional procedures or laboratory tests may be performed, at the discretion of the Investigators, e.g. urine microscopy in the event of positive urinalysis.

Observations

Pulse, blood pressure and temperature will be measured at the time points indicated in the schedule of attendances. Height and weight will be measured at on the day prior to challenge.

Blood Tests

Blood will be drawn for the following laboratory tests and processed:

1. At the Oxford University Hospitals NHS Foundation Trust Laboratories, Guys and St Thomas' Hospital or University Hospital Southampton, using NHS standard procedures:
    - **Haematology;** Full Blood Count, Haemoglobinopathy screen, G6PD levels.
    - **Biochemistry;** Sodium, Potassium, Urea, Creatinine, Albumin, Liver Function Tests for all groups. Magnesium will only be measured at screening for volunteers in Groups 5 and 6 (i.e. those undergoing CHMI). For female volunteers only, urine will be tested for beta-human chorionic gonadotrophin (HCG) at screening, immediately prior to each vaccination, the day before challenge, and just prior to starting anti-malarial treatment.
    - **Diagnostic serology;** HBsAg, HCV antibodies, HIV antibodies (specific consent will be gained prior to testing blood for these blood-borne viruses).
    - **Immunology;** Human Leukocyte Antigen (HLA) typing.
2. At University of Oxford research laboratories:
    - **Diagnostic Tests:** Blood films for malaria parasites, PCR for *Plasmodium falciparum* DNA.
    - **Immunology:** Serological analysis of antibody responses (such as ELISA, functional GIA); B cell assays, *ex-vivo* ELISpot assays for interferon gamma and flow cytometry assays will be performed. Other immunological assays including cytokine analysis, DNA and RNA analysis of genetic polymorphisms potentially relevant to vaccine immunogenicity and gene expression studies (both host and parasite) amongst others may be performed at the discretion of the Investigators. Samples may also be used for monoclonal antibody production and B cell repertoire analysis. All initial investigations will be outlined in the VAC063 laboratory plan.

3. **Urinalysis;** Urine will be tested for blood, protein and glucose at screening. For female volunteers only, urine will be tested for beta-human chorionic gonadotrophin (hCG) at screening, immediately prior to each vaccination, the day before challenge, and just prior to starting anti-malarial treatment.
4. **Electrocardiogram**; An electrocardiogram will be performed at screening for volunteers in Groups 5 6 and 9. We will not routinely perform another ECG for volunteers who re-enrol into Groups 7 and 8 if their previous ECG was normal at their first screening visit (unless they were to have experienced new chest pain, palpitations or faints since they were last seen).
5. Samples will be sent to collaborating laboratories for other immunological assays as required. These will include laboratories within and outside the UK (including outside of Europe) for immunomonitoring and harmonisation of key immunological assays. This would involve the transfer of serum, plasma or blood cells to these laboratories, but these would remain anonymised. Informed consent for this will be gained from volunteers.

Immunological assays will be conducted according to the procedures established in the test laboratories. With the volunteers' informed consent, any leftover cells and serum/plasma will be frozen indefinitely for future exploratory immunological analysis of malaria-specific or vaccine-related responses. This may include human DNA and RNA analysis to search for correlates of vaccine immunogenicity and efficacy.

Vaccinations

Before each vaccination, the on-going eligibility of the volunteer will be reviewed. The vaccine will be administered as described above in section 6.4. The injection site will be covered with a sterile dressing and the volunteer will stay in the local trial site for observation for 1 hour, in case of immediate adverse events. Observations will be taken 30 minutes after vaccination (+/- 5 minutes) and the sterile dressing removed and injection site inspected. Observations will also be taken at 60 minutes after vaccination (+/- 10 minutes). An oral thermometer and tape measure will be given to each volunteer, with instructions on use, along with the emergency 24 hour telephone number to contact the on-call study physician if needed. Volunteers will be asked to complete a diary card which may be in paper or electronic form.

11.3 Study visits

The study visits and procedures will be undertaken by one of the clinical trials team. The procedures to be included in each visit are documented in the schedule of attendances. Each visit is assigned a time point and a window period, within which the visit will be conducted. These can be found in the schedule of attendances.

Screening Visit

All potential volunteers will have a screening visit, which may take place up to 90 days prior to enrolment. Volunteers from Groups 5 and 6 who wish to re-enrol into Groups 7 and 8 will be re-screened before re-enrolment to ensure ongoing eligibility. Informed consent will be taken before screening, as described in section 8.2. If consent is obtained, the screening procedures indicated in the schedule of attendances will be undertaken. To avoid unnecessary additional venepuncture, if the appropriate blood test results for screening are available for the same volunteer from a screening visit for another Jenner Institute Clinical Trials group vaccine study, these results may be used for assessing eligibility (provided the results date is within the 3 months preceding enrolment in VAC063).

Abnormal clinical findings from the urine or blood tests at screening and throughout the study will be assessed using the table in Appendix A and the site specific laboratory adverse events tables. If a test is deemed clinically significant it may be repeated to ensure it is not a single occurrence. If an abnormal finding is deemed to be clinically significant, the volunteer will be informed and appropriate medical care

arranged with the permission of the volunteer. Decisions to exclude the volunteer from the enrolling in the trial or to withdraw a volunteer from the trial will be at the discretion of the Investigator.

An electrocardiogram will be done at screening or at the day 0 (D0) visit for vaccinees or day before CHMI (C-1) visit for controls (in cases where ECG was not able to be done at screening).

Day of Vaccination – Groups 1 - 5 (Days 0, 28 and 56/182)

Any new medical issues or symptoms that have arisen will be assessed. Physical observations, urinary β HCG test in female volunteers and venepuncture for immunology and safety bloods will be undertaken according to Tables 3 - 6. The inclusion and exclusion criteria for the study will be reviewed. Vaccines will be administered according to the SOP for mixing and administration for RH5.1/AS01. Diary cards (paper or electronic) will be issued to the volunteer, with instructions for how these should be completed.

Reviews Post Vaccination – Groups 1, 2 and 4 (Days 1, 7, 14, 29, 35, 42, 57, 63, 70, 84, 140, 240 and 600)

Diary cards will be reviewed for details of solicited and unsolicited AEs for 28 days after each vaccination, and any new or undocumented medical issues or symptoms that have arisen will be assessed. Physical observations and venepuncture for immunology and safety bloods will be undertaken according to Table 3.

At day 600, new consent will be obtained prior to visit procedures, including consent to contact the GP. Any new or undocumented medical issues or symptoms that have arisen will be assessed. Due to length of time since last visit, at this visit only, this will include participation in any other trial and any travel history to malaria endemic zones. The GP will be contacted to inform them of the attendance at this additional visit.

Reviews Post Vaccination – Group 3 (Days 1, 7, 14, 29, 35, 42, 56, 183, 189, 196, 210, 266, 366 and 730)

Diary cards will be reviewed for details of solicited and unsolicited AEs for 28 days after each vaccination, and any new or undocumented medical issues or symptoms that have arisen will be assessed. Physical observations and venepuncture for immunology and safety bloods will be undertaken according to Table 4.

At day 730, new consent will be obtained prior to visit procedures, including consent to contact the GP. Any new or undocumented medical issues or symptoms that have arisen will be assessed. Due to length of time since last visit, at this visit only, this will include participation in any other trial and any travel history to malaria endemic zones since the last visit. The GP will be contacted to inform them of the attendance at this additional visit.

Reviews Post Vaccination – Group 5 (Follow-up schedule as per Groups 1, 2 and 4, until C-1 visit)

Diary cards will be reviewed for details of solicited and unsolicited AEs for 28 days after each vaccination, and any new or undocumented medical issues or symptoms that have arisen will be assessed. Physical observations and venepuncture for immunology and safety bloods will be undertaken according to Table 5. CHMI will take place 2 weeks after the final vaccination, and follow-up after CHMI will be undertaken as per Table 5.

At day 530, new consent will be obtained prior to visit procedures. Any new or undocumented medical issues or symptoms that have arisen will be assessed. Due to length of time since last visit, at this visit only, this will include participation in any other trial and any travel history to malaria endemic zones since the last visit. The GP will be contacted to inform them of the attendance at this additional visit.

Reviews Post Vaccination – Group 7

This relates to the final and fourth vaccination at four months and will involve a follow-on Day 1 and Day 7 visit, with a C-1 visit and CHMI 7-14 days after the final vaccination, as for Group 5. Physical observations and venepuncture for immunology and safety bloods will be undertaken according to Table 7.

At day 540, new consent will be obtained prior to visit procedures. Any new or undocumented medical issues or symptoms that have arisen will be assessed. Due to length of time since last visit, at this visit only,

this will include participation in any other trial and any travel history to malaria endemic zones since the last visit. The GP will be contacted to inform them of the attendance at this additional visit.

Day before CHMI (C-1) – Groups 5-9

Any new medical issues or symptoms that have arisen will be assessed. Physical observations, urine β HCG test in female volunteers and venepuncture for immunology, PCR and safety bloods will be undertaken according to Tables 5 and 7. Results of safety bloods taken at this visit must be available and reviewed prior to CHMI.

	S	RH5.1/ AS01 (1)				RH5.1/ AS01 (2)				RH5.1/ AS01 (3)							
Attendance number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Timeline (days)		0	1	7	14	28	29	35	42	56	57	63	70	84	140	240	696
Window (days)	(-90)		0	±2	±2	±5	±1	±2	±2	±5	±1	±2	±2	±5	±14	±14	±275
Inclusion / Exclusion criteria	X	X				X				X							
Informed consent	X																X
Medical History	X	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	X**
Physical Examination	X	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(X)
Urinalysis	X																
β-HCG urine (♀)	X	X				X				X							
Review contraindications	X	X				X				X							
Vaccination		X				X				X							
Physical Observations^	X	X	X	X	X	X	X	X	X	X	X	X	X	X	(X)	(X)	X
AEs reviewed*		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Diary card commenced		X				X				X							
Diary card completed						X				X				X			
HLA typing (mL)		4															
HBV,HCV,HIV (mL)	5																
Haematology (mL)	2	2		2	2	2		2	2	2		2	2	2			
Biochemistry (mL)	3	3		3	3	3		3	3	3		3	3	3			
Immunology		73	13	10	70	73	13	10	70	73	13	10	70	70	70	70	70
Blood volume per visit (mL)	10	82	13	15	75	78	13	15	75	78	13	15	75	75	70	70	70
Cumulative blood volume (mL)	**10**	**92**	**105**	**120**	**195**	**273**	**286**	**301**	**376**	**454**	**467**	**482**	**557**	**632**	**702**	**772**	**842**[$]

Table 3: Schedule of attendances for Groups 1, 2 and 4. **S** = screening visit, **(x)** = If considered necessary, emphasising any acute complaints.

^ Physical observations include blood pressure, pulse and temperature, height and weight; however height and weight will only be measured at screening.

**All AEs will be recorded for 28 days after each vaccination (in an electronic diary). After this, only SAEs or AEs of special interest will be recorded.*

[**] *Medical history at this visit will include travel history to malaria endemic zones and any history of participation in other trials since last visit*

[$] *Approximate cumulative blood volume if blood taken as per schedule, and excluding any repeat safety blood tests that may be necessary.*

	S	RH5.1/ AS01 (1)				RH5.1/ AS01 (2)					RH5.1/AS01 (3)							
Attendance number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Timeline (days)		0	1	7	14	28	29	35	42	56	182	183	189	196	210	266	366	822
Window (days)	(-90)		0	±2	±2	±5	±1	±2	±2	±5	±14	0	±2	±2	±5	±14	±14	±275
Inclusion / Exclusion criteria	X	X				X					X							
Informed consent	X																	X
Medical History	X	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	X**
Physical Examination	X	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(X)
Urinalysis	X																	
β-HCG urine (♀)	X	X				X					X							
Review contraindications	X	X				X					X							
Vaccination		X				X					X							
Physical Observations^	X	X	X	X	X	X	X	X	X	X	X	X	X	X	(X)	(X)	(X)	X
AEs reviewed*		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Diary card commenced		X				X					X							
Diary card completed						X				X					X			
HLA typing (mL)		4																
HBV,HCV,HIV (mL)	5																	
Haematology (mL)	2	2		2	2	2		2	2	2	2		2	2	2			
Biochemistry (mL)	3	3		3	3	3		3	3	3	3		3	3	3			
Immunology		73	13	10	70	73	13	10	70	70	73	13	10	70	70	70	70	70
Blood volume per visit (mL)	10	81	13	15	75	78	13	15	75	75	78	13	15	75	75	70	70	70
Cumulative blood volume (mL)	**10**	**92**	**105**	**120**	**195**	**273**	**286**	**301**	**376**	**451**	**529**	**542**	**557**	**632**	**707**	**777**	**847**	**917$**

Table 4: Schedule of attendances for Group 3. **S** = screening visit, **(x)** = If considered necessary, emphasising any acute complaints.

^ Physical observations include blood pressure, pulse and temperature.

**All AEs will be recorded for 28 days after each vaccination (in an electronic diary). After this, only SAEs or AEs of special interest will be recorded.*

*** Medical history at this visit will include travel history to malaria endemic zones and any history of participation in other trials since last visit $ Approximate cumulative blood volume if blood taken as per schedule, and excluding any repeat safety blood tests that may be necessary.*

	S	RH5.1/ AS01 (1)				RH5.1/ AS01 (2)				RH5.1/ AS01 (3)			C-1	C	C+1	C+2-12	[b] (Day of diagnosis)	C+13-23	C+28	C+90	C+170	C+625
Attendance number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16-37		38-48	49	50	51	52
Timeline (days)		0	1	7	14	28	29	35	42	56	57	63	69	70	71	72-82 (AM+PM)		83-93	98	160	240	696
Window (days)	(-90)		±1	±2	±2		±1	±2	±2		±1	±2		-3-+10	0	0		0	±3	±7	±14	±275
Inclusion / Exclusion criteria	X	X				X				X				X								
Informed Consent (including Questionnaire)	X																					X
Medical History	X	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	X[c]
Physical Examination	X	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(X)
Urinalysis	X																					
Electrocardiogram	X	(X)																				
β-HCG urine (♀)	X	X				X				X			X				X					
Review contraindications	X	X				X				X			X	X								
Vaccination		X				X																
Physical Observations^	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
AEs reviewed*		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	(x)	X
Diary card provided		X				X				X				X								
Diary card collected				X				X				X							X			
Medic Alert Card Given to Volunteers														X								
Treatment for Malaria																	X	(X)				
HLA typing (mL)		4																				
HBV,HCV,HIV (mL)	5																					

	S	RH5.1/ AS01 (1)				RH5.1/ AS01 (2)				RH5.1/ AS01 (3)			C-1	C	C+1	C+2-12	[b] (Day of diagnosis)	C+13-23	C+28	C+90	C+170	C+625
Serum for storage													5							5		
Haematology (mL)	2	2		2	2	2		2	2	2		2	2			2[£]	2		2	2		
Biochemistry ** (mL)	3	3		3	3	3		3	3	3		3	3			3[£]	3		3	3		3
Immunology		70	10	10	70	70	10	50	70	70	10	50	70			10 per visit x 9 visits [a]	70	10 per visit x 8 visits [a]	70	70		70
Blood Film / PCR													3		3	3 x 22	2	3 x 11				
Blood volume per visit (mL)	10	79	10	15	75	75	10	55	75	75	10	55	83	0	3	161	77	113	75	80		70
Cumulative blood volume (mL)	**10**	**89**	**99**	**114**	**189**	**264**	**27 4**	**329**	**404**	**479**	**489**	**544**	**627**	**627**	**630**	**791**	**868**	**981**	**1056**	**1136**		**1206[$]**

Table 5: Schedule of attendances for Group 5. ***S*** *= screening visit,* ***(x)*** *= If considered necessary, emphasising any acute complaints.*

^ Physical observations include blood pressure, pulse and temperature.

**All AEs will be recorded for 28 days after each vaccination (in an electronic diary). After this, only SAEs or AEs of special interest will be recorded.*

^ Physical observations includes blood pressure, pulse and temperature, height and weight, however height and weight will only be measured at screening and C-1.

*** Biochemistry will include Sodium, Potassium, Urea, Creatinine, Albumin, Liver Function Tests & Magnesium , however Magnesium will only be measured at screening.*

[£] Biochemistry and haematology bloods will be checked on day 6 post CHMI

[$] Cumulative blood volume if blood taken as per schedule, and excluding any repeat safety blood tests that may be necessary.

[a] Immunology blood to be taken at AM visits on days 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20 (but only until day of diagnosis).

[b] This bleed to be also carried out at C+21, prior to treatment, if a volunteer is not diagnosed.

[c] Medical history at this visit will include travel history to malaria endemic zones and any history of participation in other trials since last visit

The vaccine schedule for Group 5 will be the same as Groups 1, 2 and 4, using the 10 µg dose of RH5.1. (Windows refer to time since last visit, but windows between vaccinations must be a minimum of 21 days and a maximum of 35 days for the first and second vaccinations. The window between final vaccination and challenge must be a minimum of 11 days and maximum of 24 days).

	S	C-1	C	C+1	C+2-12 (AM+PM)	[b] (Day of diagnosis)	C+13-23	C+28	C+90
Attendance number	1	2	3	4	5-26		27-37	38	39
Timeline (days)		-1	0	1	2-12 (AM+PM)		13-23	28	90
Window (days)	(-90)	-1	0	0	0		0	±3	±7
Inclusion / Exclusion criteria	X	X	X						
Informed Consent (including Questionnaire)	X								
Medical History	X	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
Physical Examination	X	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)
Urinalysis	X								
Electrocardiogram	X								
β-HCG urine (♀)	X	X				X			
Review contraindications	X	X	X						
Physical Observations^	X	X	X	X	X	X	(X)	X	X
AEs reviewed		X	X	X	X	X	(X)	X	X
Diary card provided			X						
Diary card collected								X	
Medic Alert Card Given to Volunteers			X						
Treatment for Malaria						X	(X)		
HLA typing (mL)		4							
HBV,HCV,HIV (mL)	5								
Serum for storage		5							5
Haematology* (mL)	5	2			2[£]	2		2	2
Biochemistry (mL)**	3	3			3[£]	3		3	3
Immunology		95			25 per visit x 9 visits [a]	110		95	95
Blood Film / PCR		3[c]		3	3 x 22	2	3 x 11		
Blood volume per visit(s) (mL)	13	112	0	3	296	117	33	100	105
Cumulative blood volume (mL)	**13**	**125**	**125**	**128**	**424**	**541**	**574**	**674**	**779[$]**

Table 6: Schedule of attendances for Groups 6, 8 and 9 (infectivity controls) in first and second challenges (primary challenges for Groups 6 and 9 and secondary challenge for Group 8).

S = screening visit **(x)** = If considered necessary, emphasising any acute complaints.

*^ Physical observations includes blood pressure, pulse and temperature, height and weight, however height and weight will only be measured at screening and C-1. *Haematology will include Full Blood Count, G6PD levels and Haemoglobinopathy screen.** Biochemistry will include Sodium, Potassium, Urea, Creatinine, Albumin, Liver Function Tests & Magnesium, however Magnesium will only be measured at screening.*

[£] Biochemistry and haematology bloods will be checked on day 6 post malaria challenge

[$] Cumulative blood volume if blood taken as per schedule, and excluding any repeat safety blood tests that may be necessary.

[a] Immunology blood to be taken at AM visits on days 3, 5, 6, 7, 8, 9, 10, 11, and 12 (but only until day of diagnosis).

[b] This bleed to be also carried out at C+21, prior to treatment, if a volunteer is not diagnosed

[c] Blood film (1mL) and PCR (2mL) will be measured for Group 6. PCR (2mL) only will be measured for Groups 8 and 9, so the cumulative blood volume for Groups 8 and 9 will be 35mLs less than for Group 6 (744mLs)

To note: for those Group 6 volunteers that subsequently enter into Group 8 and participate in a second CHMI, the total cumulative blood volume taken over their study experience (7 months total) will be 779 mLs PLUS 744 mLs = 1523 mLs.

	RS	RH5.1/ AS01 (4)			C-1	C	C+1	C+2-12 (AM+PM)	[b] (Day ofdiagnosis)	C+13-23	C+28	C+90	C+640
Attendance number	1	2	3	4	5	6	7	8-29		30-40	41	42	43
Timeline (days)		0	1	7	-1	0	1	2-12 (AM+PM)		13-23	28	90	640
Window (days)	(-90)				-1	0	0	0		0	±3	±7	±275
Inclusion / Exclusion criteria	X	X			X	X							
Informed Consent (including Questionnaire)	X												X
Medical History	X	(x)			(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	X[c]
Physical Examination	X	(x)			(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(X)
Urinalysis	X												
Electrocardiogram	X												
β-HCG urine (♀)	X	X			X				X				
Review contraindications	X	X			X	X							
Physical Observations^	X	X			X	X	X	X	X	(X)	X	X	X
AEs reviewed^^		X			X	X	X	X	X	(X)	X	X	X
Vaccination		X											
Diary card provided		X				X							
Diary card collected											X		
Medic Alert Card Given to Volunteers						X							
Treatment for Malaria									X	(X)			
HLA typing (mL)					4								
HBV,HCV,HIV (mL)	5												
Serum for storage					5							5	
Haematology* (mL)	5			2	2			2[£]	2		2	2	
Biochemistry (mL)**	3			3	3			3[£]	3		3	3	
Immunology		70	10	50	75			10 per visit	70	10 per	70	70	70

	RS	RH5.1/ AS01 (4)			C-1	C	C+1	C+2-12 (AM+PM)	[b] (Day ofdiagnosis)	C+13-23	C+28	C+90	C+640
								x 9 visits [a]		visit x 8 visits			
PCR					2		2	2 x 22	2	2 x 11			
Blood volume per visit(s) (mL)	13	70	10	55	91	0	3	139	77	102	75	80	70
Cumulative blood volume (mL)	**13**	**83**	**93**	**148**	**239**	**239**	**244**	**383**	**460**	**562**	**637**	**717**	**787**

Table 7: Schedule of attendances for Group 7.

RS = Re-screening visit **(x)** = If considered necessary, emphasising any acute complaints.

^ Physical observations includes blood pressure, pulse and temperature, height and weight, however height and weight will only be measured at screening and C-1.

^^All AEs will be recorded for 28 days after each vaccination (in an electronic diary). After this, only SAEs or AEs of special interest will be recorded.

**Haematology will include Full Blood Count, G6PD levels and Haemoglobinopathy screen*

*** Biochemistry will include Sodium, Potassium, Urea, Creatinine, Albumin, Liver Function Tests & Magnesium , however Magnesium will only be measured at screening.*

[£] Biochemistry and haematology bloods will be checked on day 6 post malaria challenge

[$]Cumulative blood volume if blood taken as per schedule, and excluding any repeat safety blood tests that may be necessary. Note that the cumulative blood volume taken over these volunteers' entire study experience (all Group 5 and Group 7 visits included over a year's total follow-up), is 1136+717 mL = 1853 mL.

[a] Immunology blood to be taken at AM visits on days 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20 (but only until day of diagnosis).

[b] This bleed to be also carried out at C+21, prior to treatment, if a volunteer is not diagnosed

[c] Medical history at this visit will include travel history to malaria endemic zones and any history of participation in other trials since last visit

To note: for those Group 5 volunteers that enter into Group 7, the original C+170 visit (Table 5) will be cancelled and instead the extra visits detailed in Table 7 will be required to incorporate the fourth immunisation and second malaria challenge. This makes the cumulative maximum blood volume for Group 7 (taken over their whole study experience - a year's total follow-up plus one visit 1-2 years after last vaccination) 1136 + 792 mL =1928 mL.

Day 70 – follow up after final vaccination (Group 5), Day 14 follow-up after final vaccination (Group 7) and day of CHMI (day of challenge [C]) – Groups 5, 6, 7, 8 and 9

- Perform interim history and examine the injection site and any body systems felt to be necessary by the Investigator and verify continuing eligibility / contraindications.
- Record vital signs.
- Blood-stage CHMI (challenge) will be delivered as per the methods described in section 10.2.
- Insert cannula in forearm vein and flush cannula with normal saline.
- For each volunteer ensure that the inoculum is injected within 4 hours of the inoculum being defrosted. Flush cannula with normal saline again.
- Observe for 1 hour to evaluate for immediate adverse reactions, then remove cannula.
- Medication diary cards, symptomatic treatment (paracetamol and cyclizine) and thermometers will be given out. If medications are not dispensed during this visit they will be dispensed at one of the visits in the following 5 days.

A Medic-Alert type card will be issued to each volunteer with information including Malarone/Riamet®/chloroquine sensitivity of the challenge malaria strain, study physician contact details and a request that the research team be contacted immediately in the event of illness/accident. Each subject will also be issued with an accurate oral thermometer. If the subject does not have their own mobile telephone they will be issued with one for the duration of the study, and counselled about the importance of keeping it switched on or checking the messages regularly. In addition full contact details for each subject will be documented, including home address, mobile, home and work land-line telephone numbers where available and next-of-kin address and telephone numbers. Mobile telephone numbers will be verified prior to challenge to ensure the volunteers are easily contactable. Volunteers must be resident in Oxford / the surrounding area until they have had 2 consecutive negative thick blood films or two consecutive qPCR results with substantial reduction in genome copies/mL after commencing antimalarial treatment, or have reached Day 28 and commenced antimalarial treatment. Subjects must also provide the Investigators with the name and 24 hour telephone number of a close friend, relative or housemate who will be kept informed of their whereabouts for the duration of the study. Volunteers will be counselled that should they fail to return for treatment having been infected with *P. falciparum* they could become very unwell and potentially die. They will be informed that should they fail to attend a scheduled clinic visit post-challenge, their nominated contact, next of kin and the police may be informed and a search started.

Day C+1 (1 day after Challenge) to C+23 (23 days after Challenge)

Follow-up visits in this intensive post-CHMI phase will take place at the CCVTM in Oxford. All volunteers will be seen by one of the clinical study team at each visit. The Investigators are all physicians with experience in acute medicine and infectious diseases and familiarity assessing patients with malaria. The Oxford based research nurses (RNs) are qualified RNs with previous experience conducting CHMI trials.

Each day until a challenge endpoint is passed (i.e. malaria is diagnosed or day 21 post-challenge is reached) volunteers will be required to attend for clinic visits at the CCVTM. Volunteers will be reviewed in clinic once on the first day post-challenge, then twice daily (approximately 12 hours apart) on days 2 to 12 post-challenge. From day 13 until day 21 post-challenge, volunteers will be reviewed daily. Upon malaria diagnosis or day 21 post-challenge being reached, subjects will be reviewed in clinic approximately 24 and 48 hours after diagnosis and / or starting anti-malarial therapy, as described in more detail later.

At each follow-up visit:

- Physical observations will be performed.

- Venepuncture will be performed as per schedule of attendances (Tables 56&8).
- Volunteers will be questioned as to whether they have;
    - Experienced any of the foreseeable symptoms of malaria.
    - Experienced any other symptoms.
    - Taken any medications including over the counter medications.

Subjects will be encouraged to contact one of the Investigators on the 24 hour emergency mobile telephone number if they develop symptoms of malaria or concerning AEs between the regular clinic reviews. The Investigator will consider an extra clinical review and thick film microscopy/ qPCR if the subject's symptoms are moderate or severe. The severity of symptoms will be assessed using grading criteria summarised below. If a volunteer is unwell and unable to attend the CCVTM for a visit, they will be visited at home by one of the Investigators. Such visits will be conducted according to SOP VC022: Lone Working in the Community.

Malaria Diagnosis

Diagnosis of malaria infection following challenge (in Groups 5 and 6) will be defined as positive thick film microscopy (at least one morphologically normal malaria trophozoite seen in 200 high-power (1000x) fields) by one or more experienced microscopists in a patient with symptoms suggestive of malaria.

Real time qPCR for *P. falciparum* will simultaneously be performed, although all Investigators (except the Principal Laboratory Investigator) will be blinded to the results. If a positive thick film for malaria parasites is seen in an asymptomatic volunteer, the most recent qPCR results for this volunteer will be un-blinded to the Chief Investigator by the Principal Laboratory Investigator and the volunteer treated only if any available qPCR result for that individual has been measured as ≥500 parasites/mL. In this scenario, if all available qPCR results are <500 parasites/mL, treatment will be delayed until either the patient develops a further positive thick film in the presence of symptoms suggestive of malaria infection in the opinion of the Investigator, or the volunteer has a further positive thick film with a qPCR measurement ≥500 parasites/mL (Table 9).

Should a volunteer describe symptoms or display signs which are highly likely to represent malaria infection in the opinion of Investigators (such as fever, rigors or severe symptomatology) despite having a negative thick film and the absence of an alternative cause, clinicians may be un-blinded to the qPCR result. If this is positive (≥500 parasites/mL), the volunteer will be treated for malaria.

The Investigators are able to treat any volunteer for malaria regardless of the thick film microscopy or qPCR result if they are clinically concerned (and have discussed the case with the Chief Investigator), or a volunteer wishes to withdraw from the study.

When a case of malaria is diagnosed, each subject will have a clinical evaluation by one of the Investigators (a physician) with appropriate history and physical examination where deemed to be necessary. If necessary, they can be admitted to the John Warin Infectious Diseases ward at the John Radcliffe Hospital, Oxford for observation and further medical management under the care of the Infectious Diseases Consultant on call.

	THICK FILM MICROSCOPY	
MALARIAL SYMPTOMS	**Positive**	**Negative**
Symptomatic	Positive diagnosis	Positive diagnosis if any available PCR result is ≥ 500 parasites/mL
Asymptomatic	Positive diagnosis if any available PCR result is ≥ 500 parasites/mL (Otherwise delay treatment)	Negative diagnosis

Table 9: Malarial Diagnosis Criteria (Groups 5 and 6)

However, in the re-challenge phase of the study (Groups 7-10) we have decided not to use microscopy as a routine diagnostic tool. This is because the qPCR assay that we have used in numerous Oxford-led CHMI challenge studies is now qualified and highly sensitive, reliably detecting parasitaemias as low as 20 parasites per mL of blood (0.02 parasites per µL), long before clinical symptoms manifest. Indeed, based upon results obtained using dilution series of microscopically-counted cultured parasites, this method has a lower limit of quantification (LLQ, defined as %CV<20%) of around 20 p/mL blood. Counted parasite dilution series results suggest that the lower limit of probable detection (LLD, i.e. a probability of >50% of ≥1 positive result among three replicate qPCR reactions) is in the region of 5 p/mL, while samples at 1 p/mL are consistently negative (24/24 qPCR reactions). Positive results in this assay (even at very low level) are thus essentially 100% specific for genuine parasitaemia, with positive results beneath the LLQ likely to signify parasitemia in the range 2-20 p/mL. The qPCR assay has also performed well in an international External Quality Assurance (EQA) exercise (https://www.ncbi.nlm.nih.gov/pubmed/24838112). By also establishing a revised treatment threshold of 5,000 parasites per mL (5 parasites per µL), any potential complications associated with clinical malaria will be minimised.

It became apparent in the initial challenge (Groups 5 and 6) that microscopy not only added no benefit as a diagnostic tool but also contributed nothing to volunteer safety, as by the time parasites were microscopically detectable all volunteers had already reached the qPCR threshold for treatment (>500 parasites per mL). By applying a revised increased qPCR threshold of 5,000 parasites per mL to the data from the first CHMI, analysis suggests that approximately 25% of volunteers would have had a slightly delayed diagnosis, but the concomitant criterion of compatible symptoms would ensure that this minor delay would not incur any increased risk to volunteer safety.

This change also means that we are strengthening the scientific output of the study by ensuring that volunteers are not diagnosed prematurely by a chance microscopy finding (in blood-stage malaria there is always a possibility that a parasite can be detected at any time, regardless of the absolute parasitaemia). Indeed, our data from the VAC054 blood-stage CHMI study show that thick film microscopy diagnosed volunteers with a median of 16,374 p/mL (range 949 – 164,509 p/mL); n=26 (sse Figure 6 below) [39]. Thus diagnosis is occurring over a range of > 2 logs parasitaemia, with over half the volunteers at < 10,000 p/mL. Premature diagnosis of > 50% of these volunteers by microscopy reduces the qPCR data available for the primary / PMR analysis, thus reducing the quality of the scientific output of the study.

In light of the above, the new proposed criteria for diagnosis and immediate treatment of volunteers in Groups 7-10, are:

Asymptomatic with any available qPCR result ≥ 10,000 parasites/mL

Symptomatic with any available qPCR result ≥ 5,000 parasites/mL

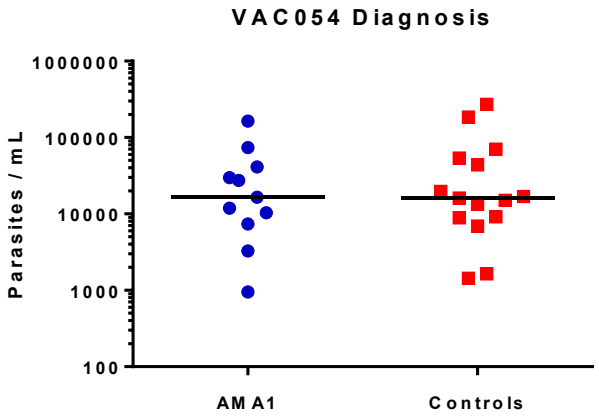

Figure 6: Parasite genome copies per mL at diagnosis by thick film microscopy, as determined by quantitative PCR for volunteers vaccinated with Apical Membrane Antigen 1 vaccine, FMP2.1/AMS01 and control volunteers (VAC054)

By applying these revised diagnostic criteria we are certain that the safety of our volunteers will not be compromised. It is also important to note that other trial centres in the USA have recently adopted a similar approach - NCT020-15091 was a multi-institution, phase 1, open-label, dose-escalation trial with CHMI, designed to assess the safety, immunogenicity, and protective efficacy of PfSPZ Vaccine. In this study PCR was used as the primary diagnostic [58]**.**

Importantly, in the unlikely event of a technical failure with the qPCR machine, we have a working back-up qPCR machine that will be available for use 24/7 if required.

In addition, Investigators, as always, are able to treat any volunteer for malaria regardless of the qPCR result or its availability if they are clinically concerned (and have discussed the case with the Chief Investigator).

Malaria Management

Volunteers will be treated with oral Riamet on diagnosis unless this is contraindicated (see SmPC for Riamet). Riamet is a licensed drug in the UK for treatment of *Plasmodium falciparum* infection. Riamet is a combination drug consisting of 20mg artemether and 120mg lumefantrine per tablet. A treatment course of Riamet consists of 6 doses of 4 tablets. The first 4 tablets will be given when diagnosis is made, followed by additional doses after 8, 24, 36, 48 and 60 hours (window period +/- 1 hour for each dose). If a diagnosis is made late at night (after the evening clinic has been completed) and the volunteer is asymptomatic, they will be informed of the result and given the choice to return to clinic immediately or delay treatment until first thing the following morning.

Prior to starting Riamet, volunteers will be screened for drug interactions and contraindications. This includes checking for pregnancy (urinary β HCG test in female volunteers) and prolonged QT (on pre-challenge ECG). Volunteers will be reminded of the potential side effects and given the patient information sheet for Riamet and a card outlining when their doses of Riamet should be taken. Volunteers will be advised to avoid grapefruit juice. Tablets should be taken together with a fatty meal (a light snack will provided when doses are observed in clinic). At least three doses will be directly observed in clinic.

- Volunteers who remain undiagnosed with malaria at Day 21 will start a treatment course of Riamet at their Day 21 visit..
- If a volunteer is unable to tolerate an oral anti-malarial, they will be admitted for inpatient care and an appropriate parenteral anti-malarial therapy prescribed following discussion with the on call infectious diseases consultant covering the John Warin ward (Infectious Diseases Unit, Oxford University Hospitals' NHS Trust).

If a volunteer withdraws/is withdrawn from the study after administration of CHMI but before reaching the criteria for malaria diagnosis, a complete, appropriate, curative course of anti-malarial therapy must be completed. The importance of this will be emphasised to volunteers at screening.

Malaria Management – Alternative Anti-Malarial Medications

If a volunteer has a contraindication to Riamet or is unable to tolerate Riamet, oral chloroquine or malarone (atovaquone/proguanil) may be prescribed as an alternative treatment for malaria.

Malarone is a licensed drug in the UK for treatment of acute uncomplicated malaria caused by *Plasmodium falciparum*. Malarone is a combination drug consisting of proguanil hydrochloride and atovaquone. A treatment course of Malarone consists of 4 'standard tablets' of Malarone (proguanil hydrochloride 100mg, atovaquone 250mg) once daily, orally for 3 days. All three doses of Malarone will be directly observed in clinic. The infecting parasites are known to be fully sensitive to Malarone. Prior to starting Malarone, volunteers will be screened for drug interactions and contraindications (including a urinary β HCG test in female volunteers). Volunteers will be reminded of the potential side effects of Malarone and given the patient information sheet for Malarone.

Chloroquine (See SmPc for chloroquine) is a licensed drug in the UK for treatment of acute, uncomplicated malaria caused by *Plasmodium falciparum*. The 3D7 clone parasites are known to be fully sensitive to chloroquine. Prior to starting chloroquine, volunteers will be screened for drug interactions and contraindications to chloroquine. A urinary β HCG test will be performed in female volunteers. Volunteers will be reminded of the potential side effects of chloroquine, given the patient information sheet for chloroquine and a card outlining when their doses of chloroquine should be taken. A treatment course of chloroquine consists of tablets containing 155mg of chloroquine base administered as 620mg (4 tablets) orally at time 0, then 310mg (2 tablets) at 8 hours, 310mg (2 tablets) at 24 hours and 310mg (2 tablets) at 48 hours (window period +/- 1 hour for each dose). The treatment administration will be directly observed by one of the Investigators or the research nurse, for the doses at 0, 24 and 48 hours.

Malaria Management – Supportive Medications

Provided there are no contraindications, all volunteers will be provided with a 3 day course of paracetamol (1g orally up to four times a day) and a 3 day course of cyclizine (50mg orally three times a day) (see SmPC for Paracetamol & Cyclizine). Volunteers will be given the patient information sheet for these medications and advised how frequently they can take doses. Volunteers will be issued with a medication diary card on which they will be asked to document all doses of medications taken post-CHMI.

All medications used in trial will be handled and dispensed according to SOP VC021: Handling, Storage and Dispensing of Non-IMP Medication.

Criteria for Hospital Admission

If any of the following criteria are met, admission to the John Warin ward (Infectious Diseases Unit, Oxford University Hospitals' NHS Trust) will be considered:

- Failure of symptoms to improve within 48 hours of starting anti-malarial therapy.
- Unable to tolerate oral antimalarial therapy.
- Dehydration requiring intravenous fluid therapy.
- Signs or symptoms suggestive of pulmonary oedema.
- Signs or symptoms of neurological dysfunction including altered consciousness.
- Signs, symptoms or laboratory evidence of significant renal dysfunction.
- Unanticipated concern about subject's home circumstances.
- Any other significant finding which the Investigator feels warrants inpatient admission.

Ultimately, the decision regarding admission will be taken by the Investigators in conjunction with the Infectious Diseases Consultant on call.

Follow-up Post Diagnosis

Subjects will be reviewed in clinic approximately 24 and 48 hours after the start of anti-malarial therapy for observation of Riamet dosing. At least half of the Riamet doses for each volunteer will therefore be observed by the study team (for volunteers taking chloroquine, the same applies, and for volunteers taking Malarone, all doses will be observed). At the visits 24 and 48 hours after diagnosis, physical observations, assessment of symptoms and venepuncture will also be performed. If blood films taken at 24 and 48 hour post-diagnosis are negative for parasites (Groups 5 and 6) or qPCR over 48 hours shows a substantial reduction in parasitaemia (Groups 7-10) and the patient is asymptomatic or has mild, resolving symptoms, the volunteer will not be seen again in clinic until day 28 post-CHMI (C+28, Groups 5-9, first and second challenge periods or 6 days post treatment initiation(Groups 8-10, tertiary challenge period). If not, the volunteer will continue to be reviewed in clinic daily until they have 2 consecutive negative blood films (Groups 5 and 6) or rapid reduction in parasitaemia on 2 consecutive PCR readings (Groups 7-10) at least 24 hours apart following start of antimalarial treatment, and all symptoms are mild or resolving.

Measures to be taken if a Volunteer Goes Missing Post CHMI

In the unlikely event that a volunteer should (a) fail to attend for a scheduled clinical visit or (b) be un-contactable by telephone after blood-stage challenge and before completion of an appropriate course of anti-malarial therapy, the following stakeholders will be informed:

- All Investigators.
- The volunteer's nominated contact and next of kin.
- The trial Sponsor.
- The local safety committee.
- The Research Ethics Committee.
- The competent authority.
- Relevant NHS Hospital Trust R&D departments.
- The local police department.
- Local Accident and Emergency departments.

All efforts will be made to locate the volunteer by the police. While all parties will aim to preserve the volunteer's confidentiality, if necessary, details of the volunteer's identity and participation in the study may be passed to the national media in order to help locate the missing individual. Volunteers will be informed of this during screening.

Day C+28 (28 days after Challenge)

Physical observations will be performed and AEs assessed. Venepuncture will be performed as per schedule of attendance (Tables 5 & 6). Where applicable, medication diary cards and unused, dispensed medications will be collected from volunteers.

Volunteers will be counselled to contact the study team or their GP if they feel feverish or unwell in the 6 months following the challenge.

Where applicable, medication diary cards and unused, dispensed medications will be collected from volunteers.

Volunteers will be counselled to contact the study team or their GP if they feel feverish or unwell in the 6 months following the challenge.

Day C+90 (90 days after Challenge)

Physical observations will be performed and AEs assessed. Venepuncture will be performed as per schedule of attendance (Tables 5,6 and 8).

Day C+170 (170 days after challenge) – Groups 5 and 7 only

This review may be conducted by telephone or as a clinic visit. Any SAEs occurring since the previous visit (day 90 post CHMI) will be assessed.

12 ASSESSMENT OF SAFETY

Safety will be assessed by the frequency, incidence and nature of adverse events and serious adverse events arising during the study.

12.1 Interim Safety Review

Prior to each dose escalation (i.e. between Groups 1 and 2, and between Groups 2 and 3/4) the local safety monitor will be consulted to provide a review of safety data and adverse events in volunteers before proceeding to the next vaccine dose. Six volunteers should have received the first two doses of the vaccine as per the group schedules, and have been followed up for at least 7 days following the second dose, before the safety review is conducted. Interim safety data may also be made available to manufacturers (in coded format) as specified in the contract with the manufacturer(s).

12.2 Definitions

Adverse Event (AE)

An AE is any untoward medical occurrence in a volunteer, which may occur during or after administration of an Investigational Medicinal Product (IMP) and does not necessarily have a causal relationship with the intervention. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the study intervention, whether or not considered related to the study intervention.

Each participant reported adverse event will be graded by the participant according to the table for grading severity of adverse events (see section 12.10). Severity gradings may be reviewed and discussed with the participants at the clinic visits.

Adverse Reaction (AR)

An AR is any untoward or unintended response to an IMP. This means that a causal relationship between the IMP and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by the reporting medical Investigator as having a reasonable suspected causal relationship to an IMP (i.e. possibly, probably or definitely related to an IMP) will qualify as adverse reactions.

Unexpected Adverse Reaction

An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., IB for an unapproved IMP).

Serious Adverse Event (SAE)

An SAE is an AE that results in any of the following outcomes, whether or not considered related to the study intervention.

- Death.
- Life-threatening event (i.e. the volunteer was, in the view of the Investigator, at immediate risk of death from the event that occurred). This does not include an AE that, if it occurred in a more severe form, might have caused death.
- Persistent or significant disability or incapacity (i.e. substantial disruption of one's ability to carry out normal life functions).
- Hospitalisation or prolongation of hospitalisation, regardless of length of stay, even if it is a precautionary measure for continued observation. Hospitalisation (including inpatient or outpatient hospitalisation for an elective procedure) for a pre-existing condition that has not worsened unexpectedly does not constitute a serious AE.
- An important medical event (that may not cause death, be life threatening, or require hospitalisation) that may, based upon appropriate medical judgment, jeopardise the volunteer and/or require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic

reaction requiring intensive treatment in an emergency room or clinic, blood dyscrasias, or convulsions that do not result in inpatient hospitalisation.

- Congenital anomaly or birth defect.

Serious Adverse Reaction (SAR)

An adverse event (expected or unexpected) that is both serious and, in the opinion of the reporting Investigator or Sponsors, believed to be possibly, probably or definitely due to an IMP or any other study treatments, based on the information provided.

Suspected Unexpected Serious Adverse Reaction (SUSAR)

A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out in the IB or Summary of Product Characteristics (SmPC).

12.3 Foreseeable Adverse Reactions:

The foreseeable ARs following vaccination with RH5.1/AS01 include injection site pain, erythema, warmth, swelling, and pruritus, and generalised myalgia, arthralgia, fatigue, and feverishness, as well as fever, malaise, headache and nausea. These adverse events will be listed as solicited adverse events providing they occur within 7 days of the day of vaccination. AEs other than these, or those AEs occurring outside of the 7 days after vaccination, will be listed as unsolicited adverse events.

12.4 Other Foreseeable Medical Occurrences

The following medical occurrences are foreseeable:

- Clinical *P. falciparum* disease resulting in hospitalisation.
- Clinical *P. falciparum* disease resulting in fever, tachycardia, hypotension, feverishness, chills, rigor, sweats, headache, anorexia, nausea, vomiting, myalgia, arthralgia, low back pain, fatigue, lymphopenia and thrombocytopenia.
- Adverse reactions to Malarone, Riamet, chloroquine, paracetamol and cyclizine, as detailed in the SmPCs for these medications.

12.5 Expected Serious Adverse Events

No serious adverse events are expected in this study.

12.6 Causality Assessment

For every unsolicited AE, an assessment of the relationship of the event to the administration of the vaccine will be undertaken by the CI or the CI-delegated clinician at the coordinating site (Oxford). An intervention-related AE refers to an AE for which there is a probable or definite relationship to administration of a vaccine. An interpretation of the causal relationship of the intervention to the AE in question will be made, based on the type of event; the relationship of the event to the time of vaccine administration; and the known biology of the vaccine therapy (Table 10). Causality assessment will take place during planned safety reviews, interim analyses (e.g. if a holding or stopping rule is activated) and at the final safety analysis, except for SAEs, for which causality should be assigned by the reporting Investigator.

0	**No Relationship**	No temporal relationship to study product ***and*** Alternate aetiology (clinical state, environmental or other interventions); ***and*** Does not follow known pattern of response to study product
1	**Unlikely**	Unlikely temporal relationship to study product ***and*** Alternate aetiology likely (clinical state, environmental or other interventions) ***and*** Does not follow known typical or plausible pattern of response to study product.
2	**Possible**	Reasonable temporal relationship to study product; ***or*** Event not readily produced by clinical state, environmental or other interventions; ***or*** Similar pattern of response to that seen with other vaccines
3	**Probable**	Reasonable temporal relationship to study product; ***and*** Event not readily produced by clinical state, environment, or other interventions ***or*** Known pattern of response seen with other vaccines
4	**Definite**	Reasonable temporal relationship to study product; ***and*** Event not readily produced by clinical state, environment, or other interventions; ***and*** Known pattern of response seen with other vaccines

Table 10: Guidelines for assessing the relationship of vaccine administration to an AE.

12.7 Reporting Procedures for All Adverse Events

All AEs occurring in the 28 days following each vaccination observed by the Investigator or reported by the volunteer, whether or not attributed to study medication, will be recorded. Recording and reporting of all AEs will take place as detailed in SOP VC027. AEs occurring after CHMI in Groups 5-9 will also be recorded- these will be entered directly into the eCRF. All AEs that result in a volunteer's withdrawal from the study will be followed up until a satisfactory resolution occurs, or until a non-study related causality is assigned (if the volunteer consents to this). Serious adverse events (SAEs) will be collected throughout the entire trial period.

Reporting Procedures for Serious AEs (see SOP OVC005 Safety Reporting)

In order to comply with current regulations on serious adverse event reporting to regulatory authorities, the event will be documented accurately and notification deadlines respected. SAEs will be reported on the SAE forms to members of the study team immediately after the Investigators become aware of their occurrence, as described in SOP OVC005. Copies of all reports will be forwarded for review to the Chief Investigator and Principal Investigators (as the Sponsor's representatives) within 24 hours of the Investigator being aware of the suspected SAE. The local safety committee (LSC) will be notified of SAEs which are deemed possibly, probably or definitely related to study interventions; the LSC will be notified immediately (within 24 hours) of the Investigators' being aware of their occurrence. SAEs will not normally be reported immediately to the REC unless there is a clinically important increase in occurrence rate, an unexpected outcome, or a new event that is likely to affect safety of trial volunteers, at the discretion of the Chief Investigator and/or LSC. In addition to the expedited reporting above, the Investigator shall include all SAEs in the annual Development Safety Update Report (DSUR) report.

Reporting Procedures for SUSARS

The Chief Investigator will report all SUSARs to the MHRA and REC within required timelines (15 days for all SUSARs, unless life threatening in which case 7 days, with a final report within a further 8 days (total 15)). The Chief Investigator will also inform all Investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants.

All SUSARs and deaths occurring during the study will be reported to the Sponsor. For all deaths, available autopsy reports and relevant medical reports will be made available for reporting to the relevant authorities.

Development Safety Update Report

A Development Safety Update Report (DSUR) will be submitted by the Sponsor to the competent authority and ethics committee on the anniversary of the first approval date from the regulatory authority for each IMP.

12.8 Volunteers to whom an IMP has not been administered

Untoward medical occurrences in clinical trial participants not having received an IMP (Group 6, 8, 9 and 10 participants) will also be solicited, collected, and recorded in the CRF as for Adverse Events. The severity grading will be undertaken as for AEs. Where these events result in a patient's withdrawal from the study or are present at the end of the study, they will be followed up until a satisfactory resolution or stabilisation occurs, or until a non-study related causality is assigned.

Should an untoward and unintended response to the blood-stage challenge (non-IMP) occur in a Group 6, 8,9 or 10 participant, such unexpected serious reactions would be reported to the Local Safety Committee, ethics committees, CTRG and other interested parties, including other centres worldwide conducting CHMI studies, would be made aware of this. Such serious adverse reactions which are related not to an IMP but to the malaria challenge cannot be reported as SUSARs, but they will be notified to ethics and the MHRA under urgent safety measures (as per CT-1 and CT-3 guidance http://ec.europa.eu/health/files/eudralex/vol-10/2011_c172_01/2011_c172_01_en.pdf; accessed 28 September 2012).

Reporting Procedures for Serious Adverse Events in Group 6, 8, 9 and 10 (volunteers who have not received an IMP)

A serious adverse event (SAE) occurring to an unvaccinated participant should be reported to the REC that gave a favourable opinion of the study where in the opinion of the Chief Investigator the event was 'related' (resulted from administration of any of the research procedures) and 'unexpected' in relation to those procedures. Reports of related and unexpected SAEs should be submitted within 15 working days of the Chief Investigator becoming aware of the event, using the NRES report of serious adverse event form.

12.9 Adverse Events of Special Interest

Adverse events of special interest will be reported as SAEs. These are:

- Severe hypersensitivity reactions (e.g. anaphylaxis)
- Any new, suspected auto-immune disease

12.10 **Assessment of Severity**

The severity of clinical and laboratory adverse events will be assessed according to the scales in Tables –11-13.

Adverse Event	Grade	Intensity
Pain at injection site	1	Pain that is easily tolerated
	2	Pain that interferes with daily activity
	3	Pain that prevents daily activity
Erythema at injection site*	1	>3 - ≤50 mm
	2	>50 - ≤100 mm
	3	>100 mm
Swelling at injection site*	1	>3 - ≤20 mm
	2	>20 - ≤50 mm
	3	>50 mm

erythema or swelling ≤3mm is an expected consequence of skin puncture and will therefore not be considered an adverse event.

Table 11: Severity grading criteria for local adverse events.

	Grade 1 (mild)	Grade 2 (moderate)	Grade 3 (severe)
Fever (oral)	37.6°C - 38.0°C	38.1°C – 39.0°C	>39.0°C
Tachycardia (bpm)*	101 - 115	116 – 130	>130
Bradycardia (bpm)**	50 – 54	40 – 49	<40
Systolic hypertension (mmHg)	141 - 159	160 – 179	≥180
Diastolic hypertension (mmHg)	91 - 99	100 – 109	≥110
Systolic hypotension (mmHg)***	85 - 89	80 – 84	<80

Taken after ≥10 minutes at rest

****When resting heart rate is between 60 – 100 beats per minute. Use clinical judgement when characterising bradycardia among some healthy subject populations, for example, conditioned athletes.***

*****Only if symptomatic (e.g. dizzy/ light-headed)***

Table 12: Severity grading criteria for physical observations

GRADE 0	None; Symptom not experienced
GRADE 1	Mild; Short-lived or mild symptoms; medication may be required. No limitation to usual activity
GRADE 2	Moderate: Mild to moderate limitation in activity. Medication may be required
GRADE 3	Severe: Considerable limitation in activity. Medication or medical attention required

Table 13: Severity grading criteria for local and systemic AEs.

12.11 **Procedures to be followed in the event of abnormal findings**

Eligibility for enrolment in the trial in terms of laboratory findings will be assessed as detailed in Appendix A. Abnormal clinical findings from medical history, examination or blood tests will be assessed as to their clinical significance throughout the trial. Laboratory adverse events will be assessed using the site-specific tables in the Institutional site file (ISF)/ trial master file (TMF). If a test is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence. If a test remains clinically significant, the volunteer will be informed and appropriate medical care arranged as appropriate and with the permission of the volunteer. Decisions to exclude the volunteer from enrolling in the trial or to withdraw a volunteer from the trial will be at the discretion of the Investigator.

12.12 **Local Safety Committee**

A Local Safety Committee (LSC) will be appointed to provide real-time safety oversight. The LSC will review SAEs deemed possibly, probably or definitely related to study interventions. The LSC will be notified within 24 hours of the Investigators' being aware of their occurrence. The LSC has the power to place the study on hold if deemed necessary following a study intervention-related SAE. At the time of writing the LSC will be chaired by Dr Brian Angus, a Clinical Tutor in Medicine, Honorary Consultant Physician and Director, Centre for Tropical Medicine at the University of Oxford. There will be a minimum of two other appropriately qualified committee members. All correspondence between Investigator and LSC will be conveyed by the Investigator to the trial Sponsor.

The chair of the LSC may be contacted for advice and independent review by the Investigator or trial Sponsor in the following situations:

- Following any SAE deemed to be possibly, probably, or definitely related to a study intervention.
- Any other situation where the Investigator or trial Sponsor feels independent advice or review is important.

The study can be put on hold upon advice of the Local Safety Monitor, Chief Investigator, Study Sponsor, REC or Local Safety Committee, for any single event or combination of multiple events which, in their professional opinion, jeopardise the safety of the subjects or the reliability of the data. If the study is placed on hold it may only be restarted following discussion with and approval from the LSC, the REC, the trial Sponsor and Chief Investigator. If the clinical trial is halted and is unable to resume, a formal letter will be sent to the regulatory authorities by the Sponsor explaining the reasons for cessation of the study.

Safety Profile Review

The safety profile will be assessed on an on-going basis by the Investigators. The LSM will perform independent external safety reviews prior to dose escalations. The Chief investigator, Principal Investigator, and relevant Investigators (as per the trial delegation log) will also review safety issues and SAEs as they arise.

13 STATISTICS

13.1 Sample Size Selection for Groups 1 - 4

This is a descriptive Phase I trial that will balance the safety of volunteers with the aims to assess the vaccine's safety profile and immunogenicity after selected doses of the vaccines. Groups 1 and 2 will have between 6 and 12 volunteers recruited to each, based on the immunogenicity of the vaccines at the 2 μg and 10 μg doses. Immunogenicity will be assessed according to the results obtained from the VAC057 trial using the viral vectored RH5 vaccines (ChAd63/MVA RH5). If the mean response after 3 vaccinations in Group 1 and Group 2 is not within or greater than the 95% CI of the mean response achieved in boosted volunteers in VAC057 the recruitment for these groups will be held at 6 volunteers per group. This will allow sufficient numbers of volunteers to assess the safety of these doses prior to dose escalation. If the 2 μg and 10 μg doses are sufficiently immunogenic 12 volunteers will be enrolled into each of these groups.

Groups 3 and 4 will have 12 volunteers enrolled into each group, which will be sufficient for assessing safety at this early phase and should allow for comparison in immunogenicity between the full dose (50 μg) and low dose (2 μg and 10 μg) groups.

13.2 Description of Power Calculations for Groups 5 and 6

Any statistically significant change in PMR in a vaccine group would be considered as success, although it remains unclear what level of change in PMR would be likely to be significant in terms of clinical outcome. The calculations below suggest that the proposed trial design would provide good power to detect vaccine-induced reductions in PMR of >33%, and excellent power to detect reductions of 50%.

Statistical power calculations are heavily dependent upon the assumptions made regarding the distribution of data in controls, and the effect of the intervention upon both the location and dispersion of data in the intervention group.

For the current study the trial design mirrored that of a previous study undertaken in Oxford, VAC054 [39,99]. When the VAC054 study was designed, data on the distribution of PMRs in controls was available from previously conducted blood-stage challenge trials conducted in Oxford [73,81] and in Nijmegen (RUNMC) [84]. The historical mean PMR in Oxford had been 12-fold per 48 hours, and at RUNMC has been 10-fold.

We wished to assess the statistical power of studies with a variety of group sizes up to a maximum total number of volunteers of 30, based upon clinical trial site capacity, as follows:

- 10 controls (N1) *vs* 10 vaccinees (N2)
- 10 controls (N1) *vs* 20 vaccinees (N2)
- 15 controls (N1) *vs* 15 vaccinees (N2)

We wished to consider the power of such studies to detect vaccine efficacy of either a 33% or 50% mean reduction in PMR relative to controls, on the basis that preliminary analyses had suggested that a 50% PMR reduction was likely to be fairly readily detectable regardless of other variables, whereas a 33% PMR reduction may be challenging to detect in some situations.

We felt that the SD of PMR in the control group and the SD of the vaccine effect upon PMR were sufficiently uncertain that we should consider a number of scenarios with various values of these parameters, as follows:

1. Three possible values for SD of the PMR in the control group: 2, 3 and 4-fold per 48 hours, based upon the extremes of observed data from Oxford and RUNMC.
2. Three possible values for SD of the vaccine effect upon PMR– currently unknown. We used estimates of 2, 3 and 4-fold per 48 hours.

Thus a total of 9 possible scenarios (combinations of PMR SD in the control group and vaccine effect PMR) were considered for each combination of mean vaccine efficacy (33 v 50%), group size, and underlying PMR in controls.

Power calculations were performed using a two-sample two-sided t-test power analysis by Dr Nicola Williams at the Centre for Statistics in Medicine in Oxford in consultation with the group at the Jenner Institute.

Conclusions:

All three options for group sizes provide sufficient power to detect a 50% reduction in PMR – irrespective of whether analysis is based on historical data from Oxford or RUNMC.

Greater consideration needs to be given to the group sizes required to observe a more modest reduction in PMR, as depicted in Table 14.

	Number of scenarios under which power ≥80% to detect a 33% reduction in mean PMR *		
Group Size	**Oxford data (mean control PMR = 12)**	**RUNMC data (mean control PMR = 10)**	**Total**
10 vs 10	4/9	1/9	5/18
10 vs 20	6/9	4/9	10/18
15 vs 15	8/9	4/9	12/18

Table 14: 9 situations of underlying SD in the controls and vaccinees were assessed for each combination of group size, underlying PMR in control groups, and vaccine effect; data shown here relate to the lower level of vaccine efficacy considered, i.e. 33% PMR reduction.

Small differences in the dispersion of PMR between the two groups had a major impact on power of studies with smaller numbers of volunteers in the control groups, such that it was not possible to be confident that trials with 10 controls would be adequately powered to detect a 33% vaccine effect upon PMR.

A study design with 15 controls versus 15 vaccinees consistently did however provide adequate power to detect a 33% vaccine effect upon PMR under most circumstances, including those with relatively high dispersion of PMR in the controls and/or high dispersion of vaccine efficacy.

A key comparison is the power of a 10 *vs* 10 trial as compared to a 15 *vs* 15 trial to detect a 33% reduction in PMR. The former gives ≥80% power under 5/18 tested situations; the latter gives ≥80% power under 12/18 scenarios (as shown in Table 14).

A study design with 15 controls *vs* 15 vaccinees was therefore chosen for VAC054.

Since obtaining the VAC054 data[39] , these power calculations have been repeated, using the mean PMR (10.31) in the 15 unvaccinated infectivity controls, with the SD of 2.36, and using three possible values for SD of the vaccine effect upon PMR – currently unknown for RH5.1/AS01. We used estimates of 2, 3 and 4-fold per 48 hours and a group size of 15 vaccinees vs 15 controls:

Comparison of scenarios comparing SDs of 2, 3 and 4 for sample size of 15 vaccinees to 15 controls with SD 2.36 and mean PMR 10.31		
Vaccinated Group SD	**Power to detect a 33% reduction in mean PMR**	**Power to detect a 50% reduction in mean PMR**
2	99%	100%
3	93.7%	99.9%
4	81.8%	99%

Table 15: Power to detect a 33% or 50% change in PMR in vaccinated volunteers assuming a SD or 2, 3 or 4.

13.3 **Sample Size Selection for Groups 7, 8, 9 and 10**

We will invite all of Group 5 and 6 back to participate in a re-challenge (Groups 7 and 8), so that we can continue to have maximum power to detect any significant difference between the 2 groups. However, all malaria re-challenge studies rely on volunteer re-participation – as such, for the second challenge, we intend to proceed if ≥4 volunteers in both groups consent to the re-challenge. Small numbers are routinely studied and reported in numerous other CHMI trials (undertaken in Oxford and elsewhere under similar circumstances). In addition we shall recruit a minimum of 5 new controls into Group 9, to act as infectivity controls for repeat CHMI of the other 2 groups. 5-6 infectivity controls are commonly recruited for CHMI studies and this would provide a control group of similar sample size to the expected number of re-challenges.

14 QUALITY CONTROL AND ASSURANCE PROCEDURES

Investigator procedures

Approved site-specific standard operating procedures (SOPs) will be used at all clinical and laboratory sites.

Monitoring

Monitoring will be performed according to ICH Good Clinical Practice (GCP) by CTRG. Following written SOPs, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements. The Investigator sites will provide direct access to all trial related source data/documents and reports for the purpose of monitoring and auditing by the Sponsor and inspection by local and regulatory authorities.

Modification to protocol

No substantial amendments to this protocol will be made without consultation with, and agreement of, the Sponsor. Any substantial amendments to the trial that appear necessary during the course of the trial must be discussed by the Investigator and Sponsor concurrently. If agreement is reached concerning the need for an amendment, it will be produced in writing by the Chief Investigator and will be made a formal part of the protocol following ethical and regulatory approval.

The Investigator is responsible for ensuring that changes to an approved trial, during the period for which regulatory and REC approval has already been given, are not initiated without regulatory and REC's review and approval except to eliminate apparent immediate hazards to the subject.

Protocol deviation

Any deviations from the protocol will be documented in a protocol deviation form and filed in the trial master file.

Audit & inspection

The QA manager will perform internal audits to check that the trial is being conducted and data recorded, analysed and accurately reported according to the protocol, Sponsor's SOPs and in compliance with ICH GCP. The audits will also include laboratory activities according to an agreed audit schedule. The internal audits will supplement the external monitoring process and will review processes not covered by the external monitor.

The Sponsor, trial sites, REC, and authorised individuals from Leidos may carry out audit to ensure compliance with the protocol, GCP and appropriate regulations. GCP inspections may also be undertaken by the regulatory authority to ensure compliance with protocol and national regulations. The Sponsor will assist in any inspections.

Serious Breaches

The Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the MHRA within 7 days of the Sponsor becoming aware of the breach.

A serious breach is defined as "A breach of GCP or the trial protocol which is likely to effect to a significant degree –

(a) the safety or physical or mental integrity of the subjects of the trial; or

(b) the scientific value of the trial".

In the event that a serious breach is suspected the Sponsor will be informed within one working day.

Trial Progress

The progress of the trial will be overseen by the Chief Investigator.

Publication Policy

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge that the study was funded through financial support to Oxford University by the Infectious Disease Division, Bureau for Global Health, United States Agency for International Development (USAID). Other contributors, including GSK Vaccines, will be acknowledged in accordance with the

International Committee of Medical Journal Editors (ICMJE) guidelines. Other funding support for this trial comes from the National Institutes of Health Research (NIHR) through the Oxford Biomedical Research Centre (BRC).

15 ETHICS

15.1 Declaration of Helsinki

The Investigator will ensure that this study is conducted according to the principles of the current revision of the Declaration of Helsinki 2008.

15.2 ICH Guidelines for Good Clinical Practice

The Investigators will ensure that this study is conducted in full conformity with the ICH Good Clinical Practice (GCP), the requirements of the Medicines for Human Use (Clinical Trial) Regulations 2004, and local regulatory requirements.

15.3 Informed Consent

Written, informed consent will be obtained, as described in section 8.2.

15.4 Research Ethics Committee (REC)

A copy of the protocol, proposed informed consent form, other written volunteer information and the proposed advertising material will be submitted to the REC for written approval. The Investigator will submit and, where necessary, obtain approval from the REC for all subsequent amendments to the protocol and associated trial documents. A non-substantial amendment does not require UK ethics committee approval. The Investigator will notify deviations from the protocol or SAEs occurring at the site to the sponsor and will notify the REC of these if necessary in accordance with procedures.

15.5 Volunteer Confidentiality

All data will be anonymised; volunteer data will be identified by a unique study number in the CRF and database. Any blood samples taken in Oxford will be labelled with the volunteer's unique study number and barcode only. Samples taken at other trial sites will be labelled as per local procedures. Separate confidential files containing identifiable information will be stored in secured locations. Only the Sponsor representative, Investigators, the clinical monitor, authorised individuals from Leidos and USAID, the REC and the regulatory authorities will have access to the records. Photographs taken of vaccination sites (if required, with the volunteer's written, informed consent) will not include the volunteer's face and will be identified by the volunteer's trial-specific identification number only. Once developed, photographs will be stored as confidential records, as above. This material may be shown to other professional staff, used for educational purposes, or included in a scientific publication.

16 DATA HANDLING AND RECORD KEEPING

16.1 Data Handling

The Chief Investigator will be the data manager with responsibility for delegating the receiving, entering, cleaning, querying, analysing and storing all data that accrues from the study. The Investigators will enter the data into the volunteers' CRFs, which will be in a paper and/or electronic format (using an OpenClinica™ database stored on a secure server). Electronic data will be stored on secure servers which are outsourced by OpenClinica™. OpenClinica™ meets FDA part 11B standards. This includes safety data, laboratory data (clinical) and outcome data. Safety data will also be collected through an electronic diary, which is stored on a secure server.

16.2 Record Keeping

The Investigators will maintain and retain appropriate medical and research records and essential documents for this trial in compliance with ICH E6 GCP and regulatory and institutional requirements for the protection of confidentiality of volunteers. The Chief Investigator, co-Investigators and clinical research nurses will have access to records. The Investigators will permit authorised representatives of the Sponsor, REC, regulatory agencies, authorised individuals from Leidos and USAID, and the monitors to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress.

16.3 Source Data and Case Report Forms (CRFs)

All protocol-required information will be collected in CRFs designed by the Investigator. All source documents will be filed in the CRF. Source documents are original documents, data, and records from which the volunteer's CRF data are obtained. For this study, these will include, but are not limited to, volunteer consent form, blood results, GP response letters, laboratory records, diaries, and correspondence. In the majority of cases, CRF entries will be considered source data as the CRF is the site of the original recording (i.e. there is no other written or electronic record of data). In this study this will include, but is not limited to medical history, medication records, vital signs, physical examination records, urine assessments, blood results, adverse event data and details of vaccinations. All source data and volunteer CRFs will be stored securely.

16.4 Data Protection

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorised third party, without prior written approval of the Sponsor.

17 FINANCING AND INSURANCE

17.1 **Financing**

The study will be funded through financial support to Oxford University from the Infectious Disease Division, Bureau for Global Health, US Agency for International Development (USAID) and the National Institutes of Health Research through the Oxford Biomedical Research Centre.

17.2 **Insurance**

The University of Oxford has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London).

18 COMPENSATION

Volunteers will be compensated for their time and for the inconvenience caused by procedures. Approximate total amounts are shown in Table 15.

Compensation will be calculated according to the following:

- Screening visits will be paid at £25 flat rate.
- Travel expenses (enrolled volunteers):
    - £10 per visit. Where travel expenses are greater than £10 per visit because the volunteer lives outside the city of the trial site, the volunteer will be given further reimbursement to meet the cost of travel necessary for study visits.
- Inconvenience of blood tests (enrolled volunteers):
    - £10 per blood donation
- Time required for visit (enrolled volunteers):
    - £20 per hour
- Illness Compensation (Groups 5-10 during CHMI): £20 per hour
- Back-up volunteers (section 9) who are not enrolled in the study will be compensated £200. This is in addition to compensation for visits they may have attended.
- Extra visits (e.g. where a volunteer is brought back to clinic for a repeat blood test):
    - £20 per visit as a flat rate.

Group No.	Time in Trial (approximately)	Maximum No. of Clinic Visits	Maximum Volume of Blood Taken (mL)	Maximum Compensation Amount
1	8 months	17	842	£755
2	8 months	17	842	£755
3	12 months	18	987	£795
4	8 months	17	842	£795
5	8 - 12 months	53	1206	£2715-£2755
6	3 months	39	779	£1765
7	6 months	42	787	£1930
8	3 months	39	744	£1765
9	3 months	39	744	£1765

Table 15: Approximate compensation amounts for volunteers (including addition of late final bleed time-point for Groups 1-5 and 7). Note the approximate time in trial for the vaccinated groups is not inclusive of the lag until the late final bleed; this will vary for each volunteer depending on when they received their final vaccination.

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20 APPENDIX A: LABORATORY VALUES FOR EXCLUSION

Laboratory parameters for inclusion/exclusion in the trial will be considered on an individual basis, with investigator discretion for interpretation of results and the need for repeated tests. In general, volunteers will be excluded if a result at screening constitutes what would qualify as a grade 1 (or higher) laboratory adverse event, according to the site-specific laboratory adverse event tables (filed in the ISF/TMF). Urinalysis at screening will be assessed as per the table below:

ABNORMAL URINE ANALYSIS (using MULTISTIX)	
Protein*	2+ or Protein creatinine ratio of ≥50mg/mmol
Blood[£]	≥1+ on two dipstick tests
Glucose	1+

**In the event of the dipstick testing positive for protein with ≥1+ protein urine should be sent for a protein creatinine ratio.*

[£] In the event of urine dipstick testing positive for ≥1+ blood with, or without, protein in volunteers a repeat dipstick test will be carried out to confirm haematuria. In female volunteers, a menstrual history will be taken to elicit whether the subject is currently menstruating and if they are, urine dipstick will be repeated after 1 - 2 weeks. If blood and/or proteinuria persist in any volunteer, they will be excluded from the trial, and the appropriate follow-up arranged.