

ASSESSMENT OF NOVEL PRE-ERYTHROCYTIC MALARIA VACCINES IN HUMANS

By:

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

The University of Oxford
Kellogg College

Submission Date: October 2017

Word count: ~40,000

This thesis is based on my own independent work, except where acknowledged.

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October 2017

Acknowledgements

I owe enormous gratitude and thanks to all the people mentioned, for making this thesis possible. The clinical trials presented in this thesis are a culmination of huge efforts by a large team of people in Oxford, and at collaborating sites in Southampton and London. I would particularly like to mention a few people here.

First and foremost, I would like to thank my supervisors, Professor Adrian Hill and Professor Katie Ewer for providing me with this invaluable opportunity to undertake this DPhil project. I would especially like to extend my gratitude for support provided during the difficult times over the last four years.

We are blessed with an excellent team of staff at the Centre for Vaccinology and Tropical Medicine, especially the nurses who undertake most of the follow-up visits during the trials and are always willing to go the extra mile during extremely busy periods. In particular, I would like to thank Ian Poulton for being very supportive during these periods. Oliver Griffiths played a fundamental role in ensuring that VAC065 was a smoothly run malaria challenge trial.

I have had the good fortune to have worked with an excellent bunch of clinicians who have always managed to maintain a light-hearted ambience in the office during stressful periods. I would particularly like to thank Dr Daniel Silman who has undertaken most of the clinical trial activity over the last six months to allow me to focus on writing my thesis.

I would particularly like to thank Georgina Bowyer and Jonathan Powlson in the laboratory at the Jenner Institute for being patient with me and teaching me to undertake the immunological assays detailed in this thesis.

Finally, I owe enormous gratitude to my girlfriend, Julia who has been exceptional in providing moral support, believing in me and providing counsel during the final stages, while going through the endeavours of writing up her own PhD. Last but not least, I would not have submitted this thesis if it wasn't for the unconditional love and support from my brother and parents through difficult times over the last four years.

Abstract

Falciparum malaria remains one of the leading infectious causes of morbidity and mortality worldwide. Though the search for an effective vaccine has seen unprecedented advances in recent years and one of the leading vaccine candidates, RTS,S/AS01 is going to enter pilot deployments in Africa in 2018, there has been no vaccine that has demonstrated durable high level efficacy. This thesis describes four Phase I and Phase II clinical trials assessing novel pre-erythrocytic malaria vaccines in healthy adult UK volunteers.

R21 has been developed at the Jenner Institute, University of Oxford. This is an improved RTS,S construct, an antigen derived from the pre-erythrocytic circumsporozoite protein, which is an abundant coat protein involved in sporozoite development and hepatocyte invasion. R21 comprises recombinant particles expressing the central repeat and the C-terminus of the circumsporozoite protein (CSP) fused to the Hepatitis B surface antigen (HBsAg), but without the excess of unfused HBsAg protein found in RTS,S. Both RTS,S and R21 predominantly function by inducing antibodies against the circumsporozoite protein. In addition to humoral immunity, it is widely recognised that T-cell mechanisms are likely to play an important role in malaria immunity and heterologous prime-boost regimes using viral-vectored vaccines have been shown to induce potent T-cell responses. ChAd63-MVA ME-TRAP is a regime that has been developed in the Jenner Institute and the first of the clinical trials described in Chapter 3 assesses the safety and immunogenicity of adjuvanting this regime with a novel saponin-based adjuvant,

Matrix-M™. This trial showed that this regime was safe, well tolerated and did not result in any immunological interference.

R21 had never been administered to humans prior to the work described in this thesis. In Chapter 4, I describe two Phase I clinical trials assessing the safety and immunogenicity of R21 given at varying doses administered with two different adjuvants, Matrix-M (MM) and $AS01_B$. Both these trials showed that R21 was safe and well tolerated when administered with either adjuvant and elicited comparable humoral immune responses to RTS,S/$AS01_B$ even at one-fifth of the dose (10μg). This formed the basis to test the efficacy of R21/MM, which had a favourable reactogenicity profile, using controlled human malaria infection in malaria-naïve UK volunteers. High level efficacy (> 80%) is reported in Chapter 5 with a three-dose schedule of 10/10/10μg R21/MM given 4 weeks apart. This provides the first evidence of proof-of-concept that this vaccine regime should be tested in malaria-endemic populations.

Publications resulting from this work

Navin Venkatraman, Nicholas Anagnostou, Carly Bliss, Georgina Bowyer, Danny Wright, Karin Lövgren-Bengtsson, Rachel Roberts, Ian Poulton, Alison Lawrie, Katie Ewer, Adrian V. S. Hill. Safety and immunogenicity of heterologous prime-boost immunization with viral-vectored malaria vaccines adjuvanted with Matrix-M™. *Vaccine. 2017 Sep 20. pii: S0264-410X(17)31248-3. doi: 10.1016/j.vaccine.2017.09.028.*

Navin Venkatraman, Alfred B. Tiono, Georgina Bowyer, Saranya Sridhar, Daniel Silman, Katharine Collins, Philip Angell-Manning, Jonathan Powlson, Pedro Folegatti, Carly Bliss, Greg Glenn, Megan Baker, Ian Poulton, Sarah Moyle, Eleanor Berrie, Nicola Green, Ekta Mukhopadhyay, Alison Lawrie, Rachel Roberts, David J.M. Lewis, Katie Ewer, Adrian V. S. Hill. Phase I assessment of the first-in-human administration of a novel malaria vaccine candidate, R21 in UK and Burkinabe volunteers. Manuscript in preparation.

Navin Venkatraman, Georgina Bowyer, Nick J. Edwards, Oliver Griffiths, Jonathan Powlson, Daniel Silman, Richard Morter, Pedro M. Folegatti, Angela Minassian, Ian Poulton, Katharine Collins, Florian Brod, Philip Angell-Manning, Eleanor Berrie, Nathan Brendish, Greg Glenn, Louis Fries, Jake Baum, Andrew M. Blagborough, Rachel Roberts, Alison M. Lawrie, David J.M. Lewis, Saul N. Faust, Sarah Gilbert, Katie J. Ewer, Adrian V. S. Hill. High Level Efficacy in Humans of a Next-Generation *P. falciparum* Anti-Sporozoite Vaccine: R21 in Matrix-M™ adjuvant. Manuscript in preparation.

Navin Venkatraman, Georgina Bowyer, Katharine Collins, Philip Angell-Manning, Jonathan Powlson, Carly Bliss, Nathan Brendish, Oliver Griffiths, Ian Poulton, Sarah Moyle, Eleanor Berrie, Nicola Green, Ekta Mukhopadhyay, Marc Lievens, Danielle Morelle, Alison Lawrie, Rachel Roberts, Saul Faust, Katie Ewer, Adrian V. S. Hill. Safety, immunogenicity and durability of a novel malaria vaccine candidate, R21 adjuvanted with $AS01_B$. Manuscript in preparation.

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Abbreviations

aa	Amino acid
Ab	Antibodies
AdHu5	Human adenovirus 5
AE	Adverse event
ALP	Alkaline phosphatase
AMA1	Apical membrane antigen 1
AR	Adverse reaction
AS01	Adjuvant System 1 comprising the immunoenhancers QS-21 and MPL with proprietary liposomes.
CBF	Clinical Bio-Manufacturing Facility
CCVTM	Centre for clinical vaccinology and tropical medicine
CEF	Chick embryo fibroblasts
ChAd63	Chimpanzee adenovirus serotype 63
CHMI	Controlled human malaria infection
CI	Chief investigator
CRF	Clinical research facility
CSP	Circumsporozoite protein
CTL	Cytotoxic T lymphocyte
CTRG	Clinical trials research governance
DAMP	Damage-associated molecular patterns
DMSO	Dimethyl sulfoxide
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immunosorbent assay

ELISpot	Enzyme linked immunospot assay
EPI	Expanded program of immunisation
FDA	Food and drug Administration
FP9	Fowlpox 9
GMSC	Genetically modified organisms safety committee
GCP	Good clinical practice
GMO	Genetically modified organisms
GMP	Good manufacturing practice
GSK	GlaxoSmithKline
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HEK293	Human embryonic kidney cells 293
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
ICH	International conference on harmonisation
ICS	Intracellular cytokine staining
IFN-γ	Interferon-gamma
IM	Intramuscular
IMP	Investigational medicinal product
IQR	Interquartile range
LLPC	Long-lived plasma cell
LPS	Lipopolysaccharide
LSC	Local safety committee
LSM	Local safety monitor

ME	Multiple epitope
MedDRA	Medical dictionary for regulatory activities
MHRA	Medicines and healthcare products regulatory agency
MM	Matrix-M
MPL	Monophosphoryl lipid A
MSP	Merozoite surface protein
MVA	Modified vaccinia virus Ankara
μg	Microgram
NANP	The peptide asparagine-alanine-asparagine-proline
NaSCN	Sodium thiocyanate
NHP	Non-human primate
NHS	National health service
NIHR	National institute for health research
NRES	National research ethics service
PAMP	Pathogen-associated molecular patterns
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
Pf	*Plasmodium falciparum*
*Pf*SPZ	Attenuated, aseptic, purified, cryopreserved sporozoites
PHA-L	Phytohaemmagglutinin-L
PIS	Participant information sheet
PRR	Pattern recognition receptors
QP	Qualified person

QS	*Quillaja saponaria*
R21	Protein particle malaria vaccine candidate consisting of a portion of the CSP antigen of the malaria parasite *P. falciparum* strain NF54, fused to the amino terminal end of the Hepatitis B virus S protein
RBC	Red blood cell
RH5	Reticulocyte binding protein homologue 5
RTS,S	A hybrid polypeptide consisting of a portion of the CSP antigen of the malaria parasite *P. falciparum* strain NF54, fused to the amino terminal end of the Hepatitis B virus S protein
SAE	Serious adverse event
SAR	Serious adverse reaction
SEB	Staphylococcal enterotoxin B
SFC	Spot forming cells
SMFA	Standard membrane feeding assay
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction
TBV	Transmission blocking vaccine
TBA	Transmission-blocking activity
T_{fh}	Follicular helper T cell
TMF	Trial master file
TLR	Toll-like receptors
TNF-α	Tumour necrosis factor-alpha
TOPS	The over-volunteering prevention system

TRAP	Thrombospondin-related adhesion protein
TRM	Tissue-resident memory
UK	United Kingdom
UOXF	University of Oxford
US	United States
VE	Vaccine efficacy
VLP	Virus-like particle
WBC	White blood cell
WHO	World health organisation
WTCRF	Wellcome trust clinical research facility

CHAPTER ONE:

Introduction

1.1 Background

The concept of vaccination, discovered by Edward Jenner, in the late 18th century is one of the greatest feats of modern medicine. There are a number of diseases whose course in history has been significantly changed by the development of an effective vaccine. However, there remain a number of infectious diseases for which the development of an efficacious vaccine has remained elusive for many years, including falciparum malaria.

1.2 Falciparum malaria

1.2.1 Lifecycle of *Plasmodium falciparum* [1]

Plasmodium species are apicomplexan protozoan parasites transmitted by the female *Anopheles* mosquito. The lifecycle of *Plasmodium falciparum (Pf)* is complex with stages in both human and mosquito hosts (Figure 1-1). The female mosquito injects malaria sporozoites into the human host during her blood meal. These sporozoites briefly circulate in the blood before they travel to the liver and invade hepatocytes. Here, the sporozoites multiply within hepatocytes as schizonts and mature into merozoites for approximately 7 days, which is the incubation period during which the parasites are not detectable in the bloodstream and patients are asymptomatic. The hepatocytes then rupture, releasing a large number of merozoites into the bloodstream. The blood stage parasites are responsible for the clinical manifestations of the disease. Subsequently, these merozoites invade red blood cells where they multiply. After 48 hours, the erythrocytes rupture, releasing progeny merozoites that in turn invade new erythrocytes. A few of these merozoites

differentiate into sexual erythrocytic stages – gametocytes. These are ingested by a mosquito during her blood meal. The male (microgametocyte) and female (macrogametocyte) unite in the mosquito midgut to create a zygote. The zygotes invade the mosquito midgut wall where they mature into oocysts. The oocysts grow, rupture and release sporozoites which migrate to the mosquito's salivary glands and are injected into the human when the mosquito feeds.

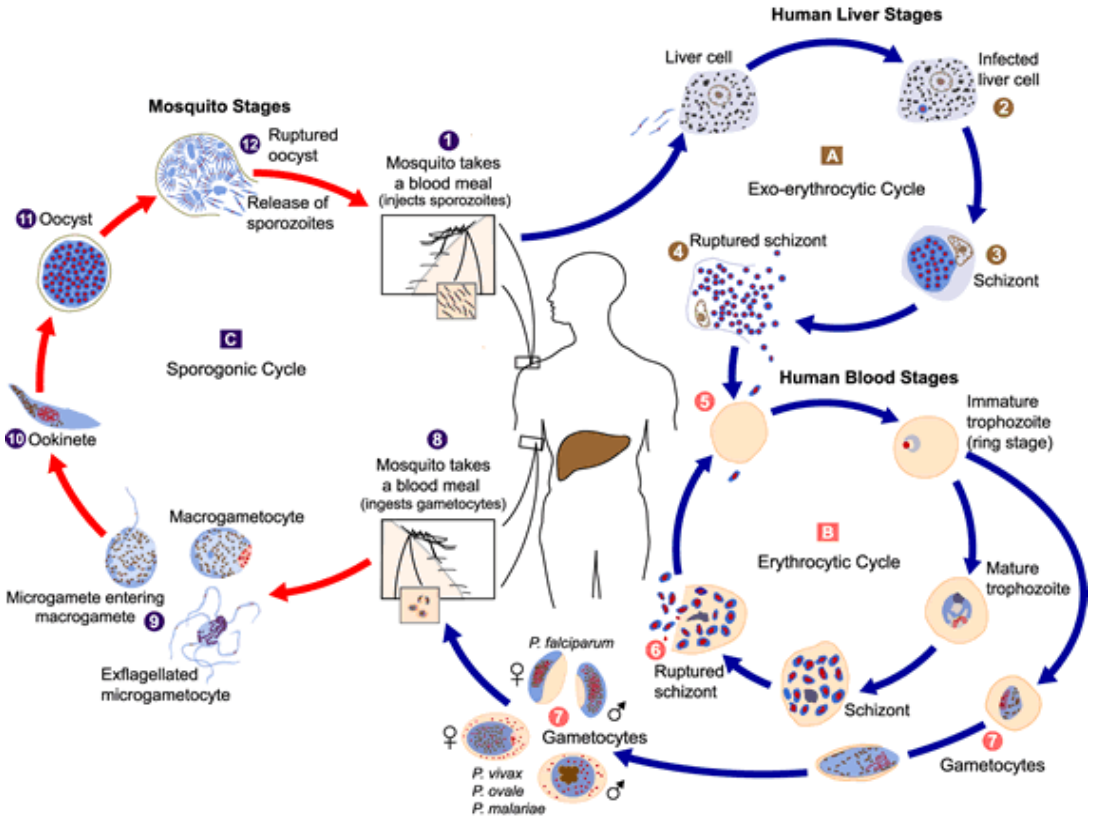


Figure 1-1. Lifecycle of Plasmodium falciparum [1]

1.2.2 Clinical manifestations of *Plasmodium falciparum* malaria

The clinical manifestations of *Pf* malaria occur during the blood stage of infection. This usually happens 7-14 days after the acquisition of infection from the bite of an infected female *Anopheles* mosquito. The nature of the clinical presentation is significantly dependant on the pattern and intensity of local transmission, which can

confer varying levels of protective immunity. In areas of stable transmission throughout the year, partial immunity to clinical disease and a reduced risk of developing severe disease are acquired in early childhood [2]. This accounts for the predominance of severe disease and mortality occurring in the under-five's in sub-Saharan Africa.

The presenting symptoms of malaria are highly variable, non-specific and similar to a systemic viral illness. Fever is a common presentation, however may be absent in some cases. Other features include headache, malaise, fatigue, abdominal pain, myalgia, arthralgia and chills. Clinical examination is often unremarkable; however, the liver and spleen may be palpable. Prompt diagnosis and treatment with effective antimalarials usually results in a rapid and full recovery. Untreated malaria often results in progressive increases in parasite burden and development of the manifestations of severe disease. High parasite counts are indisputably a risk factor for mortality, but the relation between parasitaemia and outcomes is dependent on the levels of local transmission. In low-transmission areas, mortality rises with parasite counts > 2%, whereas in areas of higher transmission much higher parasite densities may be well tolerated. Parasitaemia > 10% is associated with a high mortality risk in any epidemiological context [3]. In high-transmission areas, the risk is greatest among young children and visitors (of any age) from non-endemic areas. In other areas, severe malaria is more evenly distributed across all age groups. Severe malaria can involve multiple organs and the clinical manifestations are shown in Table 1-1. The most important complications of *Pf* malaria are cerebral malaria, respiratory

distress due to acidosis, hypoglycaemia, acute kidney injury, jaundice and pulmonary oedema [3].

1.2.3 Treatment of *Plasmodium falciparum* malaria

Artemisinin-based therapies are the recommended first-line treatment for *Pf* malaria. Quinine with an additional drug, such as doxycycline or Atovaquone-Proguanil are alternatives to artemisinin-combination therapy in uncomplicated falciparum malaria [4]. All cases of severe falciparum malaria should be admitted to a hospital and treatment commenced promptly with intravenous artesunate, which has been shown to significantly reduce mortality in comparison to quinine [5, 6].

Clinical manifestations of severe malaria
Clinical features • Impaired consciousness (including unrousable coma); • Prostration, i.e. generalized weakness so that the patient is unable to sit, stand or walk without assistance • Multiple convulsions: more than two episodes within 24h • Deep breathing and respiratory distress (acidotic breathing) • Acute pulmonary oedema and acute respiratory distress syndrome • Circulatory collapse or shock, systolic blood pressure < 80mm Hg in adults and < 50mm Hg in children • Acute kidney injury • Clinical jaundice plus evidence of other vital organ dysfunction • Abnormal bleeding

Laboratory and other findings
• Hypoglycaemia (< 2.2mmol/l or < 40mg/dl) • Metabolic acidosis (plasma bicarbonate < 15mmol/l) • Severe normocytic anaemia (haemoglobin < 5g/dl, packed cell volume < 15% in children; <7g/dl, packed cell volume < 20% in adults) • Haemoglobinuria • Hyperlactataemia (lactate > 5mmol/l) • Renal impairment (serum creatinine > 265μmol/l) • Pulmonary oedema (radiological)

Table 1-1. Clinical manifestations of severe malaria [3].

1.3 Burden and progress towards elimination

There are five *Plasmodium* species known to cause human malaria - *P. falciparum, P. vivax, P. ovale*, *P. malariae* and *P. knowlesi.* 3.2 billion people (half the world's population) live in areas at risk of malaria transmission in 91 countries and territories [7]. There were an estimated 212 million cases with around 429,000 deaths in 2015 and there is a huge socioeconomic burden associated with this parasitic disease [7, 8]. *Pf* is the predominant species and falciparum malaria remains one of the leading infectious causes of morbidity and mortality worldwide, mainly affecting children and pregnant women in sub-Saharan Africa [7].

The advent of artemisinin-combination therapy, increased uptake of insecticide-treated nets, indoor residual spraying and substantial increase in global impetus and funding for malaria elimination has resulted in significant recent reductions in mortality in many places [9]. However, emergence and spread of resistance to

artemisinins [10-12] and resistance to insecticides [13] may hinder progress being made towards the ultimate goal of eradication. In this regard, the development of a vaccine would be an invaluable complementary tool in the fight against malaria. *Pf* is a complex pathogen, which is highly immunoevasive with significant antigenic diversity, and therefore the development of an efficacious vaccine has remained elusive for many years. The Malaria Vaccine Technology Roadmap was updated in 2013 and calls for the development of a vaccine with at least 75% durable efficacy against clinical malaria suitable for administration to appropriate at-risk groups, by 2030 [14].

1.4 Biological plausibility for the development of a malaria vaccine

The feasibility of a vaccine against malaria is supported by two main observations. Firstly, there is clear evidence of acquisition of natural immunity against clinical malaria in populations living in malaria endemic regions [15, 16]. It is well recognized that adults living in high-transmission settings, such as sub-Sahel Africa, develop natural immunity to clinical disease despite the presence of a parasitaemia, which would be of clinical significance in a malaria-naïve population [17, 18]. It is also known that persistence of this natural immunity requires frequent exposure and immunity wanes once an individual does not reside in a high-transmission setting [17]. This suggests that malaria vaccine development could mimic the mechanisms of the natural immune system to reduce morbidity and mortality but multiple booster doses may be required. Unfortunately, the exact mechanisms of naturally acquired immunity, which is likely to involve a complex interplay between the innate and adaptive immune response directed at the blood stage of the life cycle, are not fully

understood [19, 20]. Possible mechanisms of humoral immunity that could be harnessed by an effective vaccine include antibodies that block sporozoite invasion of hepatocytes, or inhibit invasion of erythrocyte by merozoites, or inhibit cytoadherence and sequestration in the capillary bed that are the hallmarks of severe disease, or prevent development of the sexual stages. In addition, T-cell mediated mechanisms that kill infected hepatocytes or induce macrophages to phagocytose erythrocytic parasites could also be playing a role [19]. It is also worth considering that a pre-erythrocytic vaccine that provides sterile protection against blood stage malaria in infants would result in a lack of exposure to malaria and development of natural immunity, which could result in a 'rebound phenomenon' once the initial efficacy of the vaccine has waned as observed with the most advanced malaria vaccine candidate, RTS,S/AS01 [21].

Secondly, it has long been known that immunization with irradiated *P. berghei* sporozoites can achieve protection in murine models [22]. This was replicated in humans in the 1970s by Clyde et al. [23, 24] and Rieckman et al. [25, 26]; however, this efficacy was short-lived and required hundreds of infected mosquito bites over a prolonged period. The use of the whole sporozoite vaccine approach has been re-visited more recently [27] and further details of their clinical development are given in Section 1.8.

1.5 Progress towards a licensed malaria vaccine

Despite attempts for more than 50 years to develop a highly efficacious, durable malaria vaccine, there is still not a licensed vaccine. There are numerous approaches

to the development of a malaria vaccine targeted at the different stages of its complex life cycle. It is also widely accepted that a highly efficacious malaria vaccine is likely to be a multi-stage combination vaccine targeting distinct antigens expressed at the different stage of its life cycle. The most advanced vaccine candidates to date are all targeted at the pre-erythrocytic stage and there have been significant advances over the last decade. There are two key proteins that are expressed during the sporozoite stage that have been extensively studied and are the targets for the most advanced pre-erythrocytic vaccines – the circumsporozoite protein (CSP) and thrombospondin-related adhesion protein (TRAP).

1.6 Pre-erythrocytic subunit vaccines

The pre-erythrocytic stage of *Pf* infection presents an attractive target as the number of sporozoites injected by the mosquito during the blood meal represents a significantly lower parasite load in comparison to the number of merozoites that emerge on hepatic schizont rupture. However, the sporozoites that reach the dermal circulation are only circulating for a few hours before they reach the liver and invade hepatocytes. Therefore, there is only a brief window to destroy circulating sporozoites prior to hepatocyte invasion, for a pre-erythrocytic vaccine like RTS,S/AS01 that predominantly works by inducing antibodies to sporozoites. The liver stage of infection provides a longer window of opportunity for cell-mediated immunity to recognize and destroy infected hepatocytes. Pre-erythrocytic vaccines do not delay the initial emergence of parasites in to the blood, or the rate of parasite multiplication in the blood, but rather reduce the size of this initial inoculum [28]. A delay to patent blood stage infection in persons receiving these vaccines reflects a

reduced liver-to-blood inoculum. Ideally, a highly efficacious vaccine would achieve complete sterile protection before the development of blood stage malaria.

1.6.1 Circumsporozoite protein

The leading vaccine candidate antigen is the circumsporozoite protein (CSP), which was first discovered in the early 1980s, and is an abundant sporozoite surface coat protein [29]. The CSP is highly conserved among *Plasmodium* species. It comprises a central four amino acid (aa) NANP (asparagine-alanine-asparagine-proline) repeat region, that is diverse among *Plasmodium* species, but conserved within *P. falciparum*. This is flanked by two conserved domains: region I, a 5-aa sequence at the N-terminus and a COOH-terminal cell-adhesion domain termed the type I thrombospondin repeat (TSR) [30-32]. There are a number of reasons that support the selection of the CSP as a potential target for a malaria vaccine candidate.

In the human host, this protein plays a pivotal role in sporozoite maturation and motility during its journey from the mosquito midgut to the mammalian liver and in the invasion of hepatocytes [31, 33-35]. In addition, the CSP is crucial in the formation of sporozoites from oocysts in the mosquito midgut [36] and may also be involved in sporozoite binding to the mosquito salivary gland [37]. In vitro, antibodies directed against B cell epitopes derived from this protein can inhibit the infectivity of sporozoites to liver cells [38]. In murine models, passive transfer of antibodies to the immunodominant B cell repeat epitope of the CSP as well as active immunisation with constructs containing this epitope, confer protection against sporozoite challenge [39, 40]. Furthermore, it has been shown that adoptive transfer of $CD8^+$

cytotoxic T-cell (CTL) or $CD4^+$ T-cell clones specific for epitopes on the CSP can provide protection against a sporozoite challenge in mice [41, 42].

The CSP therefore became the focus of malaria vaccine development and provided significant hope towards the development of an efficacious subunit malaria vaccine. Though numerous vaccine candidates with whole CSP and the immunodominant NANP repeat region utilizing a variety of vaccine delivery platforms were initially tested in humans, there was limited humoral immunogenicity [43, 44] and no evidence of significant protection in the human malaria sporozoite challenge model [44-50] or in the field [51-53]. The development of the first licensed recombinant vaccine (for Hepatitis B) produced in the yeast *Saccharomyces cerevisiae* and recognition of the utility of Hepatitis B surface antigen as a carrier protein for *Pf* antigens from the CSP was a significant breakthrough [54, 55].

This provided the basis for Smith Kline Beecham Biologicals, now GlaxoSmithKline (GSK) to develop a novel malaria vaccine candidate, R16HBsAg. It comprised 16 CSP repeats (15 NANP and 1 NVDP) fused to a 42 aa sequence of the pre-S2 region of the Hepatitis B surface antigen (HBsAg), followed by 226 aa from the HBsAg itself. The first Phase I trial of this vaccine showed it to be safe and immunogenic in humans at much lower doses than previous CSP-based vaccines [56]. The desire to include the C-terminal flanking region of the CSP, which also includes important T- cell epitopes led to the development of the RTS,S particle adsorbed to alum.

RTS comprised of 19 NANP repeats and the entire C-terminal flanking region of the CSP fused to 226 aa from the HBsAg. It was co-expressed in the yeast *Saccharomyces cerevisiae* and formed RTS,S particles at a ratio of RTS:S of 1:4. RTS,S adjuvanted with

alum or adjuvanted with alum and 3-deacylated monophosphoryl lipid A (MPL) was well tolerated in humans and induced humoral responses, but minimal cellular responses. There was no protective efficacy seen in the RTS,S/alum group and only 25% (2/8) of volunteers were protected in the RTS,S/alum/MPL group [57]. In parallel, GSK were also developing a number of novel, more potent adjuvant systems (AS**) to improve immunogenicity and efficacy of a number of vaccines. Further details of vaccine adjuvants including the AS** adjuvants are given in Section 1.11. In addition to RTS,S, the CSP was also tested in humans using other vaccine delivery platforms including DNA vaccines [58-60] and viral-vectored vaccines [61-63] including heterologous prime-boost regimes [64-71]. However, none of these regimes showed significant sterile protection against sporozoite challenge [64, 66, 69-71].

1.6.2 Development of RTS,S/AS** vaccines

RTS,S was initially assessed in malaria-naïve adults to establish proof-of-concept in combination with three different novel adjuvants – alum and MPL (designated SBAS4), an oil-in-water emulsion (SBAS3) and this emulsion plus MPL and QS21, which is a saponin-based immunostimulant (SBAS2). High level efficacy (86%) was only observed in the group receiving RTS,S with SBAS2 (later renamed as AS02) [72]. However, the antibody responses and efficacy waned by 6 months after the initial malaria sporozoite challenge [73]. Subsequently, studies demonstrated safety, humoral responses to the CSP and modest protection against natural infection in semi-immune adult Gambian men [74, 75]. However, this efficacy was again short-lived. In parallel, comparable safety and immunogenicity was also demonstrated in

Kenyan adults living in hyper-endemic settings [76]. These promising results provided the impetus for the first field trials in African children. Concurrent studies in The Gambia and Mozambique in two different age groups showed the vaccine was safe, immunogenic and well tolerated [77-79].

These results paved the way for the first Phase IIb efficacy study of RTS,S/AS02 in African children aged 1–4 years in two centres in Mozambique [80]. In the per-protocol population, vaccine efficacy of 29·9% for the first clinical episode of malaria was observed. Vaccine efficacy for severe malaria was 57.7%. Contrary to previous studies in malaria naïve [73] and endemic populations [75], there was also some suggestion that this low-level efficacy could be maintained over 45 months [81, 82]. Antibody responses to the CSP were also still ~30-fold higher than pre-vaccination levels at 45 months [82]. The first trials in the target population of African infants began in 2005 where a protective efficacy of ~65% was seen in two Phase IIb trials where three doses, given 4 weeks apart were administered either staggered or in co-ordination with the Expanded Programme of Immunisation (EPI) [83, 84].

During this time, GSK were also developing a novel adjuvant, AS01 in an attempt to improve on AS02. ASO1 was also a combination of QS21 and MPL; however, it was prepared in a liposomal-based formulation. Initial pre-clinical data showed that ASO1 elicited comparable humoral and superior cellular immunogenicity to AS02 [85]. Subsequently, RTS,S/AS01 demonstrated superior efficacy to RTS,S/AS02 in malaria-naïve adults, which was associated with improved humoral and cellular immunogenicity [86]. Head-to-head assessment of the adjuvants in an efficacy trial in Kenyan adults showed comparable efficacy of ~30%; however, antibody responses

to the CSP were significantly improved with RTS,S/AS01 [87]. This improved immunogenicity was again replicated in African children aged 5-17 months [88, 89] and an efficacy against clinical malaria of ~50% was observed [90]. Durability of efficacy up to 19 months was also demonstrated in this population [91, 92].

These promising results resulted in RTS,S/AS01 being chosen as the preferred formulation in the pivotal Phase III efficacy study - a large double-blinded, randomized, controlled multicentre trial, which included 15,460 infants and young children in seven sub-Saharan African countries (Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique, and Tanzania) with diverse malaria transmission settings. In children aged 5-17 months at first immunization, the estimated overall efficacy was a 55.8% reduction in the number of all malaria episodes during the first 12 months of follow-up, with 47.3% efficacy against severe, life-threatening malaria [93]. The vaccine did not perform as well in children vaccinated with the first dose when aged 6-14 weeks of age, in co-administration with other EPI vaccines. Estimated overall efficacy in this age group over 12 months of follow-up was 33% for all malaria episodes, and 37% for severe, life-threatening malaria [94].

In July 2014, efficacy was reported for both age groups over 18 months of follow-up. In the 5-17 month age group, the incidence of clinical malaria in the per-protocol population was 0.69/person-year in the RTS,S/AS01 group and 1.17/person-year in the control group, resulting in an overall VE of 46%. This waned over time, but persisted throughout the 18-month period. Efficacy against severe malaria was 36% and reductions in both malaria hospitalizations (41%) and all-cause hospitalizations

(19%) were noted over 18 months [95]. In the 6-14 week age group, an overall efficacy of 27% was observed in the incidence of all episodes of clinical malaria [95].

The final results of this Phase 3 trial were published in April 2015 [96]. 8922 children aged 5-17 months were included in the modified intention-to-treat analysis and followed up for a median of 48 months (IQR 39-50). 9585 episodes of clinical malaria met the primary case definition in the control group who received a comparator vaccine at months 0, 1, 2 and 20. In comparison, there were 6616 episodes of clinical malaria in those that received RTS,S/AS01 at months 0, 1, 2 and 20, giving an overall vaccine efficacy of 36.3%. However, achieving this efficacy required four immunisations at time points not currently in the standard schedule of infant immunisations and therefore may be logistically difficult to deploy. In those that received RTS,S/AS01 at months 0, 1, 2 and a dose of comparator vaccine at month 20, overall vaccine efficacy was 28%. Vaccine efficacy against severe malaria was 32% in those that received RTS,S/AS01 at months 0, 1, 2 and 20, but only 1% in those administered the vaccine at 0, 1 and 2 months [96].

6537 young infants aged 6-12 weeks were included in the modified intention-to-treat analysis and followed up for a median of 38 months (IQR 34-41). These infants were administered the RTS,S/AS01 vaccine with other infant vaccinations at months 0, 1 and 2, and some received a fourth booster dose at a non-standard time point of 20 months. Overall vaccine efficacy against episodes of clinical malaria in those that received RTS,S/AS01 at months 0, 1, 2 and 20 was 26% and 18% in those receiving immunisations at 0, 1 and 2 months. Efficacy against severe malaria was 17% with

four doses and 10% with three doses. There was no vaccine efficacy against malaria mortality but power was low.

There are a number of reasons that could potentially explain the reduced efficacy in 6-12 week old infants. It is well recognised that there is an age-dependant stepwise increase in antibody responses, including affinity maturation and antibody persistence, during the early stages of life [97]. It is also known that maternal antibodies can inhibit vaccine responses to a number of diseases; these antibodies would have waned by 6-9 months, which could have resulted in improved responses in the older age group [98]. It is also possible that co-administration with other EPI vaccines or the excess of HBsAg present in the formulation interferes with the induction of a potent immune response to the CSP.

Though these results show significant low level short-term efficacy against most endpoints, there remains an urgent need to improve efficacy to achieve goals set down by the Malaria Vaccine Technology Roadmap. Furthermore, over 7 years of follow-up, though a three-dose schedule with RTS,S/AS01 was initially protective against clinical malaria in the 5-17 month olds, this result was offset by rebound in later years in areas with higher than average exposure to malaria parasites [21].

1.6.3 Thrombospondin-related adhesion protein

The second most important sporozoite protein, which was discovered in 1988, is the thrombospondin-related adhesion protein (TRAP) [99-101]. TRAP is a type I transmembrane protein that consists of an A domain, a TSR and a repeat region of variable length and sequence depending on the *Plasmodium* species [102]. It is found in the micronemes and the sporozoite surface [100] and plays a pivotal role in the gliding motility of sporozoites and hepatocyte invasion in the human host [103, 104]. In addition, TRAP is critical for the sporozoite invasion of mosquito salivary glands [104]. Murine studies using the rodent homologue, called sporozoite surface protein 2 (SSP2) in *P yoelii*, showed that vaccination induced protection against sporozoite challenge that was dependent on T-cell mediated elimination of infected hepatocytes [105, 106]. It was also shown that 100% protection could be achieved by adoptive transfer of a $CD8^+$ CTL clone recognizing SSP2. This high level efficacy was still observed even when administered three hours after sporozoite inoculation at a time when sporozoites had entered hepatocytes, reiterating that it is likely to be due to the elimination of infected hepatocytes [107].

The increasing recognition of the superior ability of viral-vectored vaccines to induce cellular immunogenicity during the 1990s was a significant milestone in the development of vaccines against complex pathogens like malaria, tuberculosis and HIV, where this arm of the adaptive immune system is likely to be playing a pivotal role. This was coupled with the demonstration that sequential immunization with two different viral vectors encoding the same antigen could induce a strong cellular response against murine malaria [108]. It was also shown that this 'heterologous

prime-boost approach' had a synergistic effect, even when replication-incompetent viral vectors were used and induced very potent cellular responses [108].

The successful worldwide eradication of smallpox using vaccination with vaccinia virus highlighted it as a potential vaccine vector. Modified vaccinia virus Ankara (MVA), a replication-defective form, was developed due to concerns regarding the potential pathogenicity of vaccination with replication-competent virus. It is derived from over 500 serial passages in chick embryo fibroblasts and has six major genomic deletions compared to the parental Ankara genome that render it unable to replicate in mammalian cells. Murine studies using the *P. berghei* model showed that sequential immunisation with a DNA prime followed by a MVA boost expressing pre-erythrocytic antigens conferred high level protection against sporozoite challenge [109]. The order of immunisation was proven to be important as a MVA-DNA prime-boost regime did not result in any protection [109]. Studies in chimpanzees showed that the DNA-MVA prime-boost regime expressing *Pf*TRAP given intramuscularly 6 weeks later induced antibodies and antigen-specific CTLs [110].

ME-TRAP is a recombinant 2398 base-pair insert encoding a single polypeptide of 789 amino acids, which is a multiple epitope (ME) string fused to the entire sequence of the T9/96 strain of *Pf*TRAP. The ME string comprises fourteen *Pf* pre-erythrocytic $CD8^+$ T cell epitopes, three $CD4^+$ T cell epitopes and two B cell epitopes [111]. The first studies in humans using the ME-TRAP insert tested various combinations of DNA and MVA vaccines administered intramuscularly and intradermally, respectively. Though the overall safety profile of the vaccines was acceptable, a 1-2mm blister, which resolved spontaneously developed following 15% of vaccinations with

intradermal MVA ME-TRAP [112]. Assessment of immunogenicity showed that three sequential DNA priming vaccinations, followed by two MVA boosting vaccinations encoding TRAP elicited a significant cellular immune response that conferred partial protection as demonstrated by a delay in patency. However, none of the volunteers were sterilely protected by this regime and it required five immunisations [113]. Only one out of eight volunteers were sterilely protected in a further sporozoite challenge study in malaria-naïve volunteers assessing a shorter regime of two DNA vaccinations followed by a MVA boost [114]. Though the DNA-MVA ME-TRAP vaccine regime was shown to be safe and elicited superior cellular immune responses in populations with previous malaria exposure [115], no significant efficacy was seen in a randomised controlled field trial [116].

Attempts to improve on cellular immune responses led to the development of recombinant avipox viruses as vaccine vectors in prime-boost regimes with MVA. Superior immunogenicity and efficacy was shown in murine models compared to DNA/MVA immunisation, particularly when an attenuated fowl pox virus (called FP9) was used as a prime, rather than a boost [117]. Prime-boost vaccination with FP9-MVA ME-TRAP was shown to be safe, immunogenic, and partially protective in malaria-naïve adults [118, 119] and adults and children in endemic populations [120, 121]. However, there was no evidence of protective efficacy against clinical malaria in a field trial using this regime [122, 123].

Adenoviruses are attractive vaccine vectors as their genome is well characterised and relatively easy to render replication-defective by gene deletions, they possess a stable virion so that inserts of foreign genes are usually not deleted and they can

infect large numbers of cells without any evidence of insertional mutagenesis. Simian adenoviruses were developed as vaccine vectors to circumvent pre-existing immunity to human adenoviruses. They do not cause pathological illness in humans and it has been shown that there is a low seroprevalence of neutralising antibodies to chimpanzee-origin adenoviruses (< 5%) in US and African populations [124, 125]. Chimpanzee adenovirus serotype 63 (ChAd63) was developed as a replication-deficient vaccine vector by deletion of the essential E1 region that is required for propagation. Pre-clinical studies in murine models showed that simian adenoviruses induce superior cellular immune responses and efficacy in comparison to human adenoviruses [126] and boosting with a MVA vaccination resulted in improved durability of protection [127]. ChAd63 ME-TRAP was shown to be safe and immunogenic in humans [128]. The ChAd63-MVA ME-TRAP malaria vaccine strategy has demonstrated durable partial efficacy in a Phase IIa sporozoite challenge trial in the UK [129], and partial efficacy was again evident in two controlled human malaria infections (CHMI) studies with *Pf*-infected sporozoites [66], [Rampling, Payne et al. in preparation]. A Phase IIb randomised controlled single-blind trial undertaken in Kenyan male adults showed that vaccination with ChAd63-MVA ME-TRAP reduced the risk of malaria infection by 67% [130]. However, a similar trial in Senegalese adults did not show any vaccine efficacy of this regime, which was thought to be due to lack of power. [131] More recently, this vaccination regime was tested in the target population – African infants. This study in 5-17 month old children in Burkina Faso demonstrated acceptable reactogenicity but substantially reduced T-cell immunogenicity compared to UK adults and Gambian infants and no significant

protection over two years of follow-up [Tiono et al. manuscript in preparation]; [132, 133].

1.7 Combination pre-erythrocytic vaccine strategies

There is a general consensus that a highly efficacious malaria vaccine would have to be a multicomponent vaccine that induced both humoral and cellular responses targeting different stages of its complex life cycle. Ideally, an efficacious pre-erythrocytic vaccine would induce high level sterile efficacy, with no evidence of blood stage parasitaemia in vaccinated subjects. In addition to the sterile efficacy of 20-50% observed individually with the aforementioned pre-erythrocytic vaccine approaches, a significant number of additional vaccinated volunteers have a delay in patency to infection compared to control subjects. This is very likely to represent a reduction in the hepatic parasite burden and therefore the number of parasites emerging from the liver [28]. Combining different vaccines that function by different mechanisms in the same regime could have synergistic effects in comparison to either vaccine given alone. Mathematical modelling demonstrated that combining RTS,S, which predominantly acts by inducing antibodies to the CSP and viral-vectored vaccines that are associated with protection that correlates with T-cell mediated killing of infected hepatocytes could have a synergistic effect [134], which is in keeping with findings from pre-clinical studies [135].

The first clinical trial to assess whether a combination regime of RTS,S/AS01 and ChAd63-MVA ME-TRAP would result in improved efficacy was conducted in Oxford. It was shown that this combination was safe and did not interfere with humoral

responses to the CSP. High level sterile efficacy of 82.4% was observed in the group that received a three dose standard schedule of RTS,S/AS01 in combination with ChAd63 ME-TRAP and MVA ME-TRAP given at week 2 and week 10, respectively. However, this was a small sporozoite challenge study and there was no statistically significant improvement to the efficacy of RTS,S/AS01 alone in this study [136], in part due to the higher than average level of efficacy induced by RTS,S alone, compared with previous CHMI studies.

1.8 Whole sporozoite vaccines

The very first attempts to develop a pre-erythrocytic malaria vaccine in humans were based on the delivery of radiation-attenuated sporozoites by mosquito bites [22-26]. During the 1990s, extensive investigation of immune responses and efficacy in humans showed that high level efficacy [137] that was durable [138] could be achieved if volunteers were immunized with > 1000 immunising bites with irradiated sporozoites. However, it was considered to be logistically difficult to use this method to immunize large numbers of people in endemic regions. Initial attempts to circumvent this issue began with a vaccine of metabolically active, non-replicating, purified, aseptic, cryopreserved, whole parasites. Though this was shown to be safe and well tolerated when administered either intradermally or subcutaneously it elicited suboptimal cellular and humoral responses and demonstrated minimal protective efficacy against sporozoite challenge [139]. In parallel, NHP studies showed that intravenous administration elicited potent and durable cellular responses, peripherally and within the liver [139].

This provided the impetus for the first clinical trial to assess the intravenous administration of attenuated, aseptic, purified, cryopreserved sporozoites (*Pf*SPZ) in humans [140]. 100% efficacy (6/6 volunteers were sterilely protected) was observed at 3 weeks after the immunisation schedule in volunteers who received 5 doses of 1.35×10^5 *Pf*SPZ [140]. However, this high level efficacy was not replicated when the fifth dose was increased to 4.5×10^5 *Pf*SPZ, where a corrected vaccine efficacy of 62% (8/12 volunteers protected) was reported [141]. A number of further CHMI studies testing increasing doses in order to reduce the number of vaccinations and to assess durability were initiated. Cellular and humoral responses declined significantly over time and a vaccine efficacy of 55% was observed at 59 weeks after an immunisation schedule of 4 doses of 2.7×10^5 *Pf*SPZ given at 0, 4, 8 and 20 weeks [141]. A further attempt to reduce the number of doses resulted in testing of a three dose schedule of 4.5×10^5 *Pf*SPZ given at 0, 8 and 16 weeks. 13/15 subjects did not develop parasitaemia at CHMI 3 weeks after the immunisations, resulting in high level protective efficacy of 86.7%. However, this efficacy waned to ~55% at re-challenge, 6 months after the vaccinations [142]. In addition, the ability of these vaccines to provide protection against a heterologous challenge strain has been assessed as most preceding CHMI studies have been undertaken using the *Pf*NF54 strain, which is the same as the strain used for the production of the vaccines. Increasing the dose to 9×10^5 *Pf*SPZ given at 0, 8 and 16 weeks resulted in durable protection of ~50% against a heterologous CHMI strain (*Pf*7G8) at 33 weeks after the final immunisation [143]. The first trial to assess the intravenous *Pf*SpZ vaccine in the field was published recently where a five dose regime $2{\cdot}7 \times 10^5$ *Pf*SPZ given at days 0, 28, 56, 84, and 140 resulted in a 48% reduction in infection in high transmission settings but only 9%

efficacy against clinical malaria [144]. These results are promising; however, the optimal dosing and timing of the vaccine regime still remains to be identified. A five dose regime given over 7 months poses questions about deliverability and there remain concerns about the acceptability and deployability of an intravenous, cryopreserved vaccine in African populations.

An alternative approach to using irradiation attenuated sporozoites is the delivery of infectious sporozoites with antimalarial prophylaxis that prevents onset of clinical disease. This approach was initially explored in the 1970s in murine models and Beaudoin et al. reported induction of sterile protection [145]. However, this approach was not assessed in humans until more recently. The first clinical trial using infectious mosquitoes under chloroquine chemoprophylaxis reported that all 10 volunteers were protected against CHMI performed 8 weeks after three exposures to infectious mosquitoes and 4 weeks after the termination of chemoprophylaxis [146]. Rechallenge of previously protected volunteers at 28 months after the immunisations showed that 4 out of 6 volunteers were sterilely protected [147]. More recently, the use of direct venous inoculation to administer the infectious sporozoites under chloroquine chemoprophylaxis also demonstrated 100% efficacy [148]. These clinical trials provide encouraging proof-of-concept for this method of immunisation; however, the logistically difficult immunisation and chemoprophylaxis regimes, and the risks if chemoprophylaxis is not adequately adhered to, may hinder progression to an appropriately deployable vaccine that can be tested in African populations.

1.9 Blood stage vaccines

The development of natural immunity to malaria that protects against severe disease in adults living in endemic populations is thought to be a cumulative acquisition of protective antibodies against blood stage antigens. However, this immunity is dependent on repeated boosting by regular infection and is therefore present in adults but not in infants and young children who remain at risk of severe and often fatal disease. A blood stage malaria vaccine is an attractive option as it could generate immune responses that mimic natural immunity, or could control parasitaemia by alternative mechanisms while allowing exposure to parasitaemia and persistence of complementary host immune mechanisms. As described previously, one of the major disadvantages of pre-erythrocytic vaccines that induce sterile protection is the risk of a rebound of severe disease once the vaccine efficacy wanes [21]. There are several lines of evidence supporting the feasibility of a blood stage vaccine including induction of protective immunity with defined antigens that correlated with the humoral response, the ability of passive transfer of antibodies to confer protection in animal models [149, 150] and the ability of passively transferred antibodies from immune adults to protect against progression of severe malaria in children in endemic settings [151].

The most advanced blood stage antigen is the apical membrane antigen 1 (AMA1), which is a micronemal protein [152] that is secreted onto the surface of merozoites and AMA1-Abs block invasion of erythrocytes [153]. Based on evidence of partial efficacy in non-human primates (NHP) [154] *Pf*-AMA1 based vaccines have been tested in humans using both protein-in-adjuvant and viral-vectored approaches.

Initial Phase I studies demonstrated that these vaccines were safe and immunogenic in malaria-naïve [155-159] and endemic populations [160-163]. This led to assessment of efficacy in CHMI studies and field trials; however, none of these studies demonstrated any significant efficacy [164-169]. *Pf* merozoite surface protein 1 (MSP1), the major surface coat antigen on merozoites has also been developed as a candidate blood stage malaria vaccine. Though numerous studies demonstrated that both protein-in-adjuvant and viral-vectored formulations were safe and immunogenic [170-174], no candidate vaccines containing any portion of MSP have shown efficacy or reduction in parasite multiplication rates [166, 175].

More recently, *Pf* reticulocyte binding protein homologue 5 (RH5) has been identified as the most promising candidate blood stage antigen. It is thought to be essential for parasite survival as it is refractory to genetic deletion [176, 177] and is under low immune pressure in endemic populations [178]. It binds to its receptor basigin and this interaction mediates an essential interaction required for red blood cell invasion by all tested strains of *P. falciparum* [179]. Antibodies can block erythrocyte invasion to high efficiency, requiring lower antibody concentrations compared to *Pf*AMA1 and *Pf*MSP1 [180, 181], and secondly, there is clear evidence of strain-transcending activity [178, 181-183]. Initial assessment of both viral-vectored and protein-in-adjuvant formulations showed efficacy in *Aotus* monkeys [184]. Clinical trials assessing this antigen using viral-vectored (ClinicalTrials.gov NCT02181088) and protein-in-adjuvant (ClinicalTrials.gov NCT02927145) regimes are currently ongoing.

1.10 Transmission blocking vaccines

This innovative method of vaccination targets parasite antigens in the sexual or mosquito-stage that are likely to be highly conserved due to minimal selective pressure from the human immune system. These vaccines are intended to induce antibodies in vaccinees that are ingested by the mosquito during their blood meal and can potentially block fertilization of the gametes, or development of ookinetes to form oocysts at the basal face of the midgut, or invasion of salivary glands by sporozoites. This mode of vaccination does pose an ethical dilemma as it does not confer direct protection to the vaccinated individual but an effective transmission-blocking vaccine (TBV) will have public health benefits at the community level by reducing onward transmission and protecting close family members. In addition, it could be used as a complementary component to a multi-stage vaccine when used in combination with a pre-erythrocytic or blood stage vaccine that does protect the vaccinated individual. Though a number of potential antigens have been identified, only a few have made it to human clinical trials due to difficulties in production of recombinant vaccines that induce adequate humoral immune responses with acceptable reactogenicity profiles [185]. This mode of vaccination also poses difficulties in providing proof-of-concept in efficacy studies as it cannot be initially tested in malaria challenge studies. In this regard, the standard membrane feeding assay (SMFA) is regarded as the surrogate 'gold standard' to assess transmission-blocking activity (TBA). This assay measures the TBA of induced antibodies by feeding cultured *P. falciparum* gametocytes to *Anopheles* mosquitoes through a membrane feeding apparatus, in the presence of serum, and approximately one week later the mosquitoes are dissected to enumerate oocysts in their midguts [186].

*Pf*s25 is the most advanced TBV candidate and is expressed most abundantly, post-activation on the surface of the developing zygote and ookinete. Pre-clinical studies showed that *Pf*s25 was able to induce antibodies with potent transmission blocking activity. An early Phase I trial assessing a vaccine containing *Pf*s25 formulated with Montanide ISA 51 was halted early due to unexpected reactogenicity, which was thought to be due to the specific antigen-adjuvant combination [187]. A recently published clinical trial showed that *Pf*s25 conjugated to a detoxified protein from *Pseudomonas Aeruginosa* adjuvanted with Alhydrogel was well tolerated and induced antibodies that correlated with transmission reducing activity in the SMFA [188]. On the basis of promising pre-clinical data showing that vaccination with a *Pf*s25-IMX313 fusion protein resulted in significant improvement of functional antibody responses, a Phase Ia study of this fusion protein expressed in the ChAd63 and MVA viral vectors has been initiated (ClinicalTrials.gov NCT02532049). The only other TBV candidate that has entered human clinical trials is *Pf*s230 (ClinicalTrials.gov NCT02334462; NCT02942277), a protein on the surface of freshly emerged, activated gametes.

The level of TBA required to have a meaningful impact in preventing malaria transmission in the field is not known. The SMFA is a biologically complex assay involving feeding of laboratory-reared mosquitoes through a membrane; it is highly variable and difficult to consistently reproduce. Furthermore, to prove field efficacy would require large-scale trials in high transmission settings to detect temporal reductions in new infections.

1.11 Adjuvants

Many licensed vaccines are formulated with adjuvants that boost the immune response. Following the serendipitous discovery that substances that induce local inflammation at the vaccination site can enhance adaptive immune responses to antigens, aluminium salts were first used for their immune-enhancing properties in the 1930s in combination for vaccines against diphtheria, tetanus and pertussis. Since then, aluminium adjuvants were the only adjuvants used in licensed vaccines and there were no significant advances in this field until the 1980s. These advances were prompted by the birth of subunit vaccines that were often inherently poor at stimulating a robust immune response in the absence of a vaccine adjuvant. Today, a range of different adjuvants are in the pipeline and a few of the vaccines that have been licensed in the recent past contain more novel vaccine adjuvants [189].

To date, the exact mechanism of any vaccine adjuvant has not been fully delineated. The prevailing theory regarding the classical emulsion and aluminium salt adjuvants is that the boosting of immune responses is related to a depot effect, which results in persistence of antigen and local inflammation. Other potential mechanisms of action include improved uptake of antigen by antigen presenting cells (APC), modifying the type of immune response (eg. Th1 v Th2 response; CD8 vs CD4), increasing the generation of memory cells, a more rapid initial response and altering the breadth, specificity or affinity of the response [190].

1.11.1 Matrix-M™

Matrix-M (MM) is a novel adjuvant technology patented by Isconova (now Novavax),

which is a 40nm-sized complex containing the adjuvant-active saponin *Quillaja saponaria*, phospholipid and cholesterol. Quillaja saponins are triterpene glycoside substances derived from the tree *Quillaja saponaria*. Saponins are surface-active compounds and in aqueous solution, they are potent adjuvants and are used in commercial veterinary vaccines, e.g., vaccines against foot-and-mouth disease, bovine mastitis, feline leukemia and equine influenza. A HPLC-purified fraction of the same saponin, called QS21, is a component of the AS01 adjuvant, described in Section 1.11.2.

In animal studies, MM has been shown to induce a multifaceted response including antibody production, T cell responses and recruitment of innate immune cells into draining lymph nodes [191, 192]. Mixed with a virosomal H9N2 avian influenza vaccine, MM induced superior antigen-specific humoral and $CD8^+$ T cell responses in comparison to other adjuvants [193]. MM administered with an intramuscular H5N1 virosomal influenza vaccine induced a strong immediate and long-term humoral and cellular immune response and showed a dose-sparing potential [194]. It was also shown that MM adjuvanted H5N1 vaccine conferred protection against lethal viral challenge in mice [195]. This novel saponin-based adjuvant has been safely administered to ~900 healthy human volunteers in combination with vaccines for a number of infectious diseases [196, 197].

1.11.2 AS01 adjuvant system

GlaxoSmithKline began development of the AS adjuvant system in the 1990s based on the combination of classical adjuvants such as aluminium salts, oil-in-water emulsions, liposomes and immunomodulatory molecules. The recognition of toll-like

receptors (TLR) and their key role in initiation of an innate immune response and subsequent adaptive immune response, and the discovery that bacterial lipopolysaccharide (LPS) required the TLR-4 gene to activate the innate immune response led to the development of 3-O-desacyl-4'-monophosphoryl lipid A (MPL) as an immunoenhancer [198]. The MPL molecule consists of a chemically detoxified form of the parent lipopolysaccharide (LPS) from the R595 strain of Gram-negative bacterium *Salmonella minnesota*.

Three formulations that incorporate MPL have been tested in humans – AS01 contains MPL in combination with QS21, formulated with liposomes [199]. QS21 is a natural saponin molecule purified from the bark of the South American tree, *Quillaja saponaria*. AS02 is an oil-in-water emulsion with MPL and QS21 and AS04 is a combination of alum and MPL. Activation of the innate immune system, particularly dendritic cells and induction of cytokines and costimulatory molecules that promote cellular and humoral immune responses were thought to be responsible for the immunoenhancing properties of the MPL [200]. Initial pre-clinical assessment of QS21 showed that it promotes high antibody responses and $CD8^+$ T-cell responses in mice [201, 202] and high antibody responses in humans [203]. AS01 has been tested in combination with candidate vaccines against a number of diseases including malaria, HIV [204], TB [205-208] and varicella zoster [209]. It has been most extensively studied in humans in combination with the leading malaria vaccine candidate RTS,S (See Section 1.6.2).

1.12 Assessment of vaccines in humans

Clinical development of potential vaccine candidates that have been shown to be safe, non-toxic, immunogenic and efficacious in pre-clinical studies begins with the initiation of Phase I studies. These are usually small studies that entail administering lower doses of the vaccine in a staggered fashion with regular review of the safety parameters before escalating to higher doses. These studies also provide the opportunity to assess the adaptive immune responses elicited by the vaccine, which could prove as an indicator of potential efficacy.

Traditionally, most vaccines work by inducing antibodies and assessment of the humoral response induced by the vaccine is most commonly assessed using an enzyme-linked immunosorbent assay (ELISA). This measures the level of antigen-specific antibody present in the serum of vaccinated individuals using antigen-coated plates and a secondary antibody attached to a chromogenic substrate. In addition to measuring quantitative responses, it is also of relevance to assess the quality of the induced antibodies and their functionality. The avidity of the induced antibodies can also be assessed using an ELISA where increasing concentrations of a solution of a chemical compound, such as sodium thiocyanate is used to displace antigen-antibody interactions. One of the key mechanisms of action of RTS,S/AS01 is thought to be due to inhibition of sporozoite invasion of hepatocytes. The functional ability of the antibodies to inhibit sporozoite invasion can be evaluated by co-incubating serum from vaccinated volunteers with freshly dissected sporozoites on hepatocyte-coated plates. T-cell immunity has also been shown to be important in malaria immunity and has led to development of vaccines that are potent inducers of cellular immunity. Evaluation of the ability of vaccines to induce T cells is assessed by the quantification

of interferon-γ secretion using an ELISPOT assay, which is produced by both $CD4^+$ and $CD8^+$ T cells, in response to antigens incorporated in the vaccine. Flow cytometry can be used to differentiate between the proportions of $CD4^+$ and $CD8^+$ cells induced and define the cytokine expression profiles of these cells.

These *in vitro* assays provide the initial indications of whether the vaccine is likely to be efficacious. In contrast to a number of other infectious diseases, there is currently no defined unequivocal correlate of protection that could predict efficacy of a potential malaria vaccine candidate in humans. Initial assessment of potential efficacy can be undertaken in animal models and utilisation of murine malaria has provided significant insights in the investigation of potential vaccine efficacy. Animal models can also facilitate investigation of mechanisms of protection that may be more difficult to elucidate in humans; for example, in the analysis of vaccines that function by inducing liver stage immune responses. However, these models do have limitations and may not accurately reflect human disease. Non-human primates such as monkeys have also been used in the investigation of potential vaccine candidates; however, they do pose ethical concerns and malaria efficacy studies of vaccines in NHPs are only carried out in a few specialised centres worldwide [210].

The gold standard for proving vaccine efficacy in humans would require randomised controlled efficacy trials in the target population. The only malaria vaccine that has completed a large Phase III efficacy trial in African children and infants is RTS,S/AS01. This trial required large numbers of volunteers in varying transmission settings with significant logistic and cost implications. It is therefore important to assess efficacy in smaller, less expensive studies in order to efficiently choose the best potential

candidates to take forward to these larger studies. For a select number of infectious diseases, including malaria, the deliberate infection of human volunteers has provided valuable inroads in proving proof-of-concept to select potential vaccine candidates to be tested in larger efficacy studies [211]. *Pf* malaria is particularly well suited to challenge studies as it has a relatively short asymptomatic period, well-established diagnostic tests and no long term sequelae following appropriate and timely treatment. Furthermore, the more recent introduction of PCR detection has provided a reproducible, sensitive and powerful tool to assess efficacy. These studies require much smaller numbers of participants, can be conducted in a controlled environment for malaria-naïve volunteers, allow specific strains of malaria to be assessed and are not subject to the variations in seasonal malaria transmission. However, they do pose ethical and logistical considerations due to limitations of the number of volunteers that can be infected and the intensive close monitoring required.

Deliberate infection of humans with malaria was initially performed in the early 20th century, primarily as a therapy for patients with neurosyphilis [212]. Subsequent development of *in vitro* continuous culture of *Pf* and generation of mature gametocytes in the 1980s [213, 214] made it possible to produce laboratory-reared infectious mosquitoes. These advances led to the first controlled human malaria infection (CHMI) study by mosquito bite being conducted in the United States (US) where six malaria-naïve volunteers were infected with the bites of infected female *Anopheles* mosquitoes [215]. CHMI is now a well-established tool to assess efficacy of vaccine candidates in malaria-naïve volunteers and has been administered to over

1500 volunteers in centres worldwide. There is now a worldwide consensus on the key ethical considerations and the conduct of mosquito bite CHMI trials [216]. All centres use *Anopheles stephensi* mosquitoes that feed on either the chloroquine-sensitive NF54 strain of *Pf* or the 3D7 clone of NF54. In addition, limited numbers of volunteers have been challenged with the chloroquine-resistant 7G8 strain of *P. falciparum* [142, 217].

More recently, aseptic, cryopreserved *Pf* sporozoites administered intradermally, intramuscularly or intravenously have been shown to be an effective method to assess vaccine efficacy and has been tested in malaria-naïve and endemic populations [218-224]. Though this method doesn't mimic the natural mode of infection, it does have some advantages compared to CHMI delivered by mosquito bite. It does not require a CL3 insectary, which may not be available in endemic settings and there can be increased standardisation across centres as the challenge dose can be more accurately described. In addition to the above methods, which are required to assess pre-erythrocytic vaccines, an intravenous blood stage challenge model has also been developed that has been used in clinical trials to assess blood stage vaccine efficacy against clinical malaria and reductions in parasite multiplication rates [169, 225].

1.13 Hypothesis and aim of work

The development of numerous approaches to a malaria vaccine targeting different stages of its life cycle has been described. There remains an urgent need to improve on current approaches in terms of field efficacy, deployability and durability. R21 is a

novel malaria vaccine candidate that has been developed at the Jenner Institute, University of Oxford. This is an improved RTS,S construct that comprises recombinant particles expressing the central repeat and the C-terminus of the circumsporozoite protein (CSP) fused to HBsAg, but without the excess of unfused HBsAg protein found in RTS,S, which was required to allow it to form a particle [226]. R21 had never been administered to humans prior to the work described in this thesis. It is well recognised that protein particle subunit vaccines require an adjuvant to boost immune responses to levels required for protective efficacy. In addition, it has been described that a highly efficacious malaria vaccine is likely to be a combination of different approaches acting on different stages of the life cycle.

The hypothesis relating to work in this thesis is that R21 administered with novel adjuvants can elicit superior immunological responses and efficacy in comparison to the most advanced malaria vaccine candidate, RTS,S/AS01. The aim of this work was to provide proof-of-concept of a highly efficacious multifaceted pre-erythrocytic falciparum malaria vaccine that would be suitable for testing in malaria-endemic regions. In addition, we wanted to assess whether Matrix-M given in combination with ChAd63-MVA ME-TRAP would result in immunological interference.

Chapter three describes the assessment of the safety and immunogenicity of ChAd63-MVA ME-TRAP administered with the novel saponin based adjuvant, Matrix-M.

Chapter four describes the first-in-human assessment of the safety and immunological assessment of R21 administered alone and with the vaccine adjuvants, Matrix-M and $AS01_B$.

Chapter five describes the assessment of protective efficacy against sporozoite challenge in malaria-naïve volunteers of two different dosing regimens of R21 adjuvanted with Matrix-M and the combination regime of R21/MM given with ChAd63-MVA ME-TRAP.

CHAPTER TWO:

Materials and Methods

2.1 Vaccines

2.1.1 ChAd63 ME-TRAP

ChAd63 ME-TRAP is a replication-deficient viral-vectored vaccine comprising of the simian adenovirus, chimpanzee adenovirus 63 (ChAd63) containing a sequence of genes which express a series of known epitopes from *Plasmodium falciparum* pre-erythrocytic stage antigens fused to TRAP, which is an abundant pre-erythrocytic stage antigen. Replication defective adenovirus is produced by deletion of genes from the E1 locus, which is essential for viral replication. These viruses are propagated easily with good yields in human embryonic kidney cells 293 (HEK 293), which express E1 from human adenovirus 5 (AdHu5) [227]. The ME string is fused in frame to the entire T9/96 strain of *P. falciparum* TRAP [116]. ChAd63 ME-TRAP has been administered to 926 healthy adults, children and infants in clinical studies in the UK and Africa and has an excellent safety record. Further details of the clinical development of this vaccine are detailed in Section 1.6.3.

2.1.2 MVA ME-TRAP

MVA is a highly attenuated strain of vaccinia virus that that was originally derived from the vaccinia strain Ankara by over 570 serial passages in primary chick embryo fibroblasts (CEF cells). MVA has six major genomic deletions compared to the

parental Ankara genome and is severely compromised in its ability to replicate in mammalian cells. The replication defect occurs at a late stage of virion assembly such that viral and recombinant gene expression is unimpaired. Therefore, MVA is an efficient single round expression vector that is incapable of causing disease in mammals. MVA ME-TRAP has been administered safely to ~1500 healthy volunteers including adults in the UK and infants, children and adults in malaria-endemic settings. It been shown to enhance cellular immunity several-fold when given as a booster vaccination following ChAd63 ME-TRAP priming [128, 129, 132, 228, 229]. Further details of the clinical development of this vaccine are detailed in Section 1.6.3.

2.1.3 R21

R21 is a novel pre-erythrocytic malaria vaccine candidate that was developed at the Jenner Institute, University of Oxford. It consists of a virus-like particle (VLP) made from monomers of the HBsAg fused to the NANP repeats and the C-terminus of the CSP and a C-Tag. The sequence of R21c protein contains from N- to C- terminus: the NANP repeat region and the C-terminus of the CSP followed in frame by HBsAg followed by a C-Tag (consisting of the four amino acid residues EPEA; included to allow immunoaffinity chromatography purification of the protein) and then a stop codon. The DNA sequence coding for the CSP-HBsAg fusion protein was synthesised by GeneArt GmbH (Regensburg, Germany) using codons most often used in Pichia to encode the sequence. The insert was ligated into the expression vector pPink-HC (Invitrogen) to make the plasmid pPink-HC-R21. The C–Tag was then added to the R21 sequence from pPink-HC-R21 by PCR. Large white colonies were selected from

the plates and used for R21 expression. A single clone of Pichia Pink strain 1 that had been screened positive for R21 expression was supplied to the Clinical Biomanufacturing Facility (CBF), University of Oxford to express and purify R21 to Good Manufacturing Practice (GMP) standards. R21 had never been previously administered to humans prior to the work described in this thesis. It has been shown to be safe and highly immunogenic in pre-clinical studies [226].

2.2 Adjuvants

2.2.1 Matrix-M™

Matrix-M adjuvant is a novel nanoparticle adjuvant technology patented by Isconova (now Novavax). It is manufactured by mixing defined, partially-purified extracts of the bark of the *Quillaja saponaria* Molina tree, termed Fraction A and Fraction C, with cholesterol and phosphatidylcholine in the presence of a detergent. Matrix-M, which has been used in VAC048, VAC053 and VAC065 refers to a blend of Matrix-A and Matrix-C at a ratio of 85:15. Matrix C is a highly active adjuvant but reactogenic saponin and Matrix A is well tolerated but a weaker adjuvant. They are mixed together to reduce the dose of Matrix C whilst maintaining the adjuvant activity, therefore substantially increasing the safety margin. It has been safely administered to ~900 healthy human volunteers in combination with vaccines for a number of infectious diseases.

2.2.2 AS01

The AS01 family of Adjuvant Systems developed by GlaxoSmithKline (GSK) contains 2 immuno-enhancers, monophosphoryl lipid A (MPL) and QS21, formulated with

liposomes [199]. The MPL molecule consists of a chemically detoxified form of the parent lipopolysaccharide (LPS) from the R595 strain of Gram-negative bacterium *Salmonella minnesota*. QS21 is a natural saponin molecule purified from the bark of the South American tree, *Quillaja saponaria*. There are two formulations in the AS01 family: an adult formulation $AS01_B$ containing 50μg of MPL and 50μg of QS-21 per dose and a paediatric formulation $AS01_E$, which contains half the quantity of each immunoenhancer. ASO1 has been safely tested in adults, children and infants in candidate vaccines against a number of diseases, including malaria. It has been most extensively studied in combination with the leading malaria vaccine candidate RTS,S (See Section 1.6.2).

2.3 Study preparation, participant enrolment and safety analysis

VAC048 had received ethics and regulatory approvals and 13 volunteers had been enrolled in the trial when I took over the conduct of the trial in November 2013. I enrolled 10 further volunteers in order to complete the trial. I prepared the study documents (clinical trial protocols, volunteer information sheets, consent forms, letters to their GP) for VAC053, VAC056 and VAC065. These were reviewed by the Sponsor [Clinical Trials Research Governance, University of Oxford (CTRG)] prior to submission to the National Research Ethics Service (NRES) and the Medicines and Healthcare products Regulatory Agency (MHRA). In addition, I prepared the documents to attain approval from the Research and Development departments at the NHS collaborating sites in Southampton and Imperial College, London. I also obtained approval from the Genetic Modification Safety Committee, Oxford University Hospitals NHS Trust to use the viral vectored vaccines in VAC065 as a Class

I activity under the UK Genetically Modified (Contained Use) Regulations, 2014. The Chief Investigator for all trials was Professor Adrian Hill, but the conduct of the trials was delegated to me as lead clinician. All trials were independently monitored by CTRG to ensure protection of the rights and wellbeing of the volunteers. The studies were conducted according to the principles of the Declaration of Helsinki (2008) and the International Conference on Harmonization Good Clinical Practice guidelines. Study-specific methods, such as study design, approvals and inclusion/exclusion criteria are described in the chapters relating to each trial.

2.3.1 Recruitment of volunteers

Healthy volunteers between the age of 18 and 50 years were recruited to the studies at all three sites (Oxford, London, and Southampton) by various means. Trial-specific and generic posters and adverts that were approved by the ethics committee were posted in public places and in local newspapers. I also ran a stall with my colleagues at the universities' fresher's fairs in Oxford. There are healthy volunteer databases maintained in Oxford, Southampton and London through which some of the volunteers were contacted. We also delivered a mail shot to potential volunteers living locally. Social networking sites (Facebook and Twitter) were also used to increase awareness amongst potential participants. Recruitment of volunteers was coordinated by local study coordinators. We used the departmental website and TrialSpark to provide information about the trials including the volunteer information sheet (See Appendix 1) and they would be given an opportunity to register for the trial. Volunteers who register their interest received a copy of the volunteer information sheet by email and an invitation to arrange a screening visit. There was

always a minimum of 24 hours between the information sheet being sent out and the screening visit.

2.3.2 Screening visit and informed consent

The screening visit took place up to 180 days before participants were enrolled in the study. Participants were considered enrolled in the study once they had a vaccination or on the day of malaria challenge, if they were a control volunteer and had not received any vaccinations. Most of the screening visits were undertaken by me for volunteers recruited in Oxford. Screening visits were carried out by the local study team in Southampton and London. The details of the trial, the potential risks and the schedule of attendances were discussed and any doubts clarified before the participant signed two copies of the consent form. In addition, VAC065 volunteers had to also complete a questionnaire consisting of multiple choice questions regarding the information, risks and the commitments of the CHMI prior to signing the consent form. It was required that the participants answered all questions correctly before proceeding to sign the consent form. Volunteers who failed to answer all questions correctly on their first attempt were allowed to re-take the questionnaire following further discussion. The following principles of informed consent were 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. However, if the volunteer has undergone CHMI and not completed a course of appropriate

antimalarial therapy then the volunteer will need to maintain contact with the investigators for monitoring and treatment.

- 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.
- There is no direct benefit from participating.
- The volunteer's GP will be contacted to corroborate their medical history and confirm that the volunteer is eligible to take part in the study. Volunteers will only be enrolled in the study if written or verbal information regarding the volunteer's medical history is obtained from the GP.
- The volunteer will be registered on the TOPS database (The Over volunteering Prevention System; www.tops.org.uk).

Following informed consent, the volunteers were asked their medical history, underwent a physical examination and investigations including baseline blood tests (full blood count, urea & electrolytes, liver function tests and serology for Hepatitis B, Hepatitis C and HIV), and urinalysis were performed. All female volunteers also had a urinary pregnancy test. In VAC065, an ECG was also undertaken and cholesterol and magnesium levels were also checked with the other biochemistry tests. The volunteers were informed of any abnormal clinical findings and if an abnormal finding was deemed to be clinically significant, appropriate medical care was arranged with the permission of the volunteer. The decision to exclude any volunteer was made by me in discussion with the chief investigator. Volunteer's GPs were contacted and informed about their patient's potential interest, giving information about the trial, and asked to respond with a summary of their medical history and if they had any

clinical objections to the participant being involved in the trial. The inclusion and exclusion criteria for each trial are detailed in each trial chapter.

2.3.3 Study design and procedures

The study design for each trial is detailed in the respective chapters. The schedule of attendances detailing the visit schedule and the procedures and interventions undertaken at each visit can be found in Appendix 2. All data at clinic visits were entered in real time into the OpenClinica database. All data were anonymised. Safety blood tests were carried out in the NHS laboratories and paper copies of the results were signed off by me in Oxford or one of the trial clinicians at the collaborating sites. The blood results were then entered into the OpenClinica database by the study team and these data were exported and processed by the data manager prior to analysis and grading by me.

2.3.4 Assessment of safety

Participants were requested to record solicited adverse events (AEs) on diary cards for 7 days following each vaccination. In VAC048, volunteers were provided with a paper diary card to record their symptoms, which were subsequently entered into the OpenClinica database by the study team. In addition, unsolicited AEs were collected actively during each study visit and recorded in the paper case report form and subsequently entered in the OpenClinica database by the study team. The adverse event data were exported and processed by the data manager prior to analysis by me. An electronic diary card (e-diary) specific for each trial was used in VAC053, VAC056 and VAC065. This was designed in collaboration with Sylwester

Pawluk and was validated by me prior to the clinical trial commencing. Solicited AEs were recorded for 7 days following each vaccination and unsolicited AEs were collected for 28 days following each vaccination. The frequency, nature and incidence of AEs were analysed directly from exports of the data from the e-diary into an Excel spreadsheet. Serious adverse events were collected throughout the course of each study. A Development Safety Update Report for each Investigational Medicinal Product (IMP), written by me was submitted to the regulatory body annually. This would also include an annual update of the Investigator's Brochure with up to date safety information.

2.3.4.1 Definitions

The following definitions for labelling adverse events reported by volunteers are listed in the clinical trial protocols.

Adverse Event (AE): An AE is any untoward medical occurrences in a volunteer, including a dosing error, which may occur during or after administration of an 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.

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 either the reporting medical investigator or the sponsors 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.*, Investigator's Brochure for an unapproved investigational medicinal product) is considered as an unexpected adverse drug reaction.

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 (i.e., results in death from any cause at any time)
- 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 serious 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 hospitalization 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 hospitalization) that may, based upon appropriate medical judgment, jeopardize the volunteer and/or require medical or surgical intervention to prevent one of the outcomes listed above.

- Congenital anomaly or birth defect.

Reporting and causality assignment of SAEs were performed by me within 24 hours of becoming aware of their occurrence.

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 Reactions (SUSARs): A SUSAR is a SAE that is unexpected and thought to be possibly, probably or definitely related to an IMP.

2.3.4.2 Solicited adverse events

Solicited AEs are those that are commonly expected to occur after vaccination and are specifically enquired about in the first 7 days after vaccination. Their frequency, incidence and nature are included in the Reference Safety Information, which is updated annually in the Investigators' Brochure. Local AEs include pain, redness, swelling, itch and warmth. Systemic AEs include fever/feverishness, arthralgia, myalgia, headache, nausea, fatigue and malaise. There were specific criteria for the grading of fever, pain, swelling and erythema (Table 2-1). The remaining solicited AEs were graded by the volunteers based on the limitation in daily activities and whether assistance or medical interventions were required.

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
Fever ^{O}C	1	37.6 – 38.0
	2	>38.0
	3	>39.0

Table 2-1. **Severity grading criterion for injection site pain, erythema and swelling.** **erythema and swelling ≤3mm is an expected consequence of skin puncture and was therefore not considered an adverse event.*

2.3.4.3 Unsolicited adverse events

Any AEs that are not listed as a solicited AE or those that occur 7 days after vaccination are termed unsolicited AEs. For every unsolicited AE, an assessment of the relationship of the AE to the IMP was undertaken by me. Alternative causes of the AE, such as the natural history of pre-existing medical conditions, concomitant therapy, other risk factors and the temporal relationship of the event to vaccination or CHMI were considered and investigated, as deemed appropriate after discussion with the volunteer. The causality assignment was also blindly peer-reviewed by another independent clinician and any discrepancies discussed before the final causality is assigned to the AE. The assignment of a causal relationship for adverse events were conducted according to predefined guidelines (Table 2-2). Unsolicited adverse events deemed definitely, possibly or probably related to vaccination were coded according to the Medical Dictionary for Regulatory Activities (MedDRA) classification and the maximum severity, duration and relationship to IMP are

tabulated in the results section for each clinical trial. Causality assessment was undertaken at the final safety analysis, except for SAEs, where causality assignment was undertaken in real-time and the Chief Investigator was informed within 24 hours of me becoming aware of the event.

2.3.4.4 Laboratory adverse events

Blood samples were taken during the course of the trial on days 0, 7 and 28 after each vaccination to monitor for any changes in haematological and biochemical parameters. These samples were analysed in the local NHS laboratories. VAC053, VAC056 and VAC065 were multi-centre trials and site-specific tables based on the FDA toxicity grading scale [230] were used in these trials (Tables 2-3, 2-4 and 2-5 for Oxford, Southampton and Imperial College (London), respectively). VAC048 was only conducted in Oxford and laboratory AEs were graded according to Table 2-6. If abnormal results or undiagnosed conditions were found during the course of the study, they were discussed with the volunteer and appropriate medical care arranged (GP or hospital specialist). Any newly diagnosed conditions were looked after within the NHS.

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 2-2. Guidelines for assessing the relationship of vaccine administration to an adverse event.

(Ox) Routine Haematology			Lab Range	Grade 0	Grade 1	Grade 2	Grade 3
Haemoglobin	**Male**	g/l	130 - 170	126 - 170	115 - 125	100 - 114	<100
	Female		120 - 150	114 - 150	105 - 113	90 - 104	<90
White Blood Cells	**Elevated**	x109/l	4.00 - 11.00	3.51 - 11.49	11.50 - 15.00	15.01 - 20.00	>20
	Low				2.50 - 3.50	1.50 - 2.49	<1.50
Platelets		x109/l	150 - 400	136 - 400	125 - 135	100 - 124	<100
Neutrophils		x109/l	2.0 - 7.0	1.5 - 7.0	1.00 - 1.49	0.50 - 0.99	<0.50
Lymphocytes		x109/l	1.0 - 4.0	1.0 - 4.0	0.75 - 0.99	0.50 - 0.74	<0.50
Eosinophils		x109/l	0.0 - 0.5	0.0 - 0.64	0.65 - 1.50	1.51 - 5.00	>5.00

Routine Biochemistry			Lab Range	Grade 0	Grade 1	Grade 2	Grade 3
Sodium	**Elevated**	mmol/l	135 - 145	135 - 146	147 - 148	149 - 150	>150
	Low				132 - 134	130 - 131	<130
Potassium	**Elevated**	mmol/l	3.5 - 5.0	3.4 - 5.0	5.1 - 5.2	5.3 - 5.4	>5.4
	Low				3.2 - 3.3	3.0 - 3.1	<3.0
Urea		mmol/l	2.5 - 7.4	2.5 - 8.1	8.2 - 8.9	9.0 - 11.0	>11.0
Creatinine		µmol/l	49 - 104	49 - 113	1.1-1.5*ULN 114 -156	>1.5-3.0*ULN 157 - 312	>3.0*ULN >312
Bilirubin	**Normal LFT**	µmol/l	0 - 21	0 - 26	1.3-1.5*ULN 27 - 31	>1.5-2.0*ULN 32-42	>2.0*ULN >42
Bilirubin	**Abormal LFT**	µmol/l	0 - 21	0 - 22	1.1-1.25*ULN 23 - 26	>1.25-1.5*ULN 27 - 31	>1.5-1.75*ULN >31
When accompanied by any increase in LFT							
ALT		IU/l	10 - 45	10 - 55	1.25 - 2.5*ULN *56 - 112*	>2.5 - 5.0*ULN *113 - 225*	>5.0*ULN *>225*
Alk Phosphatase		IU/l	30 - 130	30 - 142	1.1 - 2.0*ULN *143 - 260*	>2.0 - 3.0*ULN *261 - 390*	>3.0*ULN *>390*
Albumin		g/l	32 - 50	32 - 50	28 - 31	25 - 27	<25

Table 2-3. Severity grading criteria for laboratory abnormalities in VAC053, VAC056 and VAC065 using Oxford University Hospitals NHS Foundation Trust laboratory reference ranges. *Adapted from FDA guidelines [230].*

(S) Routine Haematology			Lab Range	Grade 0	Grade 1	Grade 2	Grade 3
Haemoglobin	**Male**	g/l	130 - 170	126 - 170	115 - 125	100 - 114	<100
	Female		120 - 150	114 - 150	105 - 113	90 - 104	<90
White Blood Cells	**Elevated**	x109/l	4.00 - 11.00	3.51 - 11.49	11.50 - 15.00	15.01 - 20.00	>20
	Low				2.50 - 3.50	1.50 - 2.49	<1.50
Platelets		x109/l	150 - 400	136 - 400	125 - 135	100 - 124	<100
Neutrophils		x109/l	2.0 - 7.5	1.5 - 7.5	1.00 - 1.49	0.50 - 0.99	<0.50
Lymphocytes		x109/l	1.5 - 4.0	1.0 - 4.0	0.75 - 0.99	0.50 - 0.74	<0.50
Eosinophils		x109/l	0.0 - 0.5	0.0 - 0.64	0.65 - 1.50	1.51 - 5.00	>5.00

Routine Biochemistry			Lab Range	Grade 0	Grade 1	Grade 2	Grade 3
Sodium	**Elevated**	mmol/l	133 - 146	133 - 146	147 - 148	149 - 150	>150
	Low				131 - 132	129 - 130	<129
Potassium	**Elevated**	mmol/l	3.5 - 5.3	3.4 - 5.3	5.4 - 5.5	5.6 - 5.7	>5.7
	Low				3.2 - 3.3	3.0 - 3.1	<3.0
Urea		mmol/l	2.5 - 7.8	2.5 - 8.1	8.2 - 8.9	9.0 - 11.0	>11.0
Creatinine		µmol/l	53 - 97	53 - 106	1.1-1.5*ULN 107 - 145	>1.5-3.0*ULN 146 - 291	>3.0*ULN >291
Bilirubin	**Normal LFT**	µmol/l	0 - 20	0 - 25	1.3-1.5*ULN 26 - 30	>1.5-2.0*ULN 31 - 40	>2.0*ULN >40
Bilirubin	**Abnormal LFT**	µmol/l	0 - 20	0 - 21	1.1-1.25*ULN 22 - 25	>1.25-1.5*ULN 26 - 30	>1.5-1.75*ULN >30
When accompanied by any increase in LFT							
ALT/AST	**Male**	IU/l	10 - 40	10 - 49	1.25-2.5*ULN *50 - 100*	>2.5-5.0*ULN *101 - 200*	>5.0*ULN *>200*
	Female		7 - 35	7 - 43	*44 - 87*	*88 - 175*	*>175*
Alk Phosphatase		IU/l	30 - 130	30 - 142	1.1-2.0*ULN *143 - 260*	>2.0-3.0*ULN *261 - 390*	>3.0*ULN *>390*
Albumin		g/l	35 - 50	32 - 50	28 - 31	25 - 27	<25

Table 2-4. Severity grading criteria for laboratory abnormalities in VAC056 and VAC065 using University Hospital Southampton NHS Foundation Trust laboratory reference ranges. *Adapted from FDA guidelines [230].*

(Imp) Routine Haematology			Lab Range	Grade 0	Grade 1	Grade 2	Grade 3
Haemoglobin	**Male**	g/l	130 - 168	126 - 168	115 - 125	100 - 114	<100
	Female		114 - 150	114 - 150	105 - 113	90 - 104	<90
White Blood Cells	**Male**	x109/l	4.2 - 10.6	3.51 - 11.49	11.50 - 15.00	15.01 - 20.00	>20
Elevated	**Female**		4.2 - 11.2				
White Blood Cells	**Male**	x109/l	4.2 - 10.6	3.51 - 11.49	2.50 - 3.50	1.50 - 2.49	<1.50
Low	**Female**		4.2 - 11.2				
Platelets	**Male**	x109/l	130 - 370	121 - 370	110 - 120	95 - 109	<95
	Female		135 - 400	121 - 400			
Neutrophils		x109/l	2.0 - 7.1	1.5 - 7.1	1.00 - 1.49	0.50 - 0.99	<0.50
Lymphocytes		x109/l	1.1 - 3.6	1.0 - 3.6	0.75 - 0.99	0.50 - 0.74	<0.50
Eosinophils		x109/l	0.0 - 0.5	0.0 - 0.64	0.65 - 1.50	1.51 - 5.00	>5.00

Routine Biochemistry			Lab Range	Grade 0	Grade 1	Grade 2	Grade 3
Sodium	**Elevated**	mmol/l	133 - 146	133 - 146	147 - 148	149 - 150	>150
	Low				131 - 132	129 - 130	<129
Potassium	**Elevated**	mmol/l	3.5 - 5.3	3.5 - 5.3	5.4 - 5.5	5.6 - 5.7	>5.7
	Low				3.2 - 3.3	3.0 - 3.1	<3.0
Urea		mmol/l	2.5 - 7.8	2.5 - 8.1	*8.2 - 8.9*	*9.0 - 11.0*	*>11.0*
					1.1-1.5*ULN	>1.5-3.0*ULN	>3.0*ULN
Creatinine	**Male**	µmol/l	60 - 125	60 - 137	138 - 187	188 - 375	>375
	Female		55 - 110	55 - 120	121 - 165	166 - 330	>330
					1.3-1.5*ULN	>1.5-2.0*ULN	>2.0*ULN
Bilirubin	**Normal LFT**	µmol/l	0 - 21	0 - 26	27 - 31	32-42	>42
					1.1-1.25*ULN	>1.25-1.5*ULN	>1.5-1.75*ULN
Bilirubin	**Abnormal LFT**	µmol/l	0 - 21	0 - 22	23-26	27-32	>32
When accompanied by any increase in LFT							
ALT					1.25-2.5*ULN	>2.5-5.0*ULN	>5.0*ULN
Alanine aminotransferase		IU/l	0 - 40	0 - 49	50 - 100	101 - 200	>200
					1.1-2.0*ULN	>2.0-3.0*ULN	>3.0*ULN
Alk Phosphatase		IU/l	30 - 130	30 - 142	143 - 260	261 - 390	>390
Albumin		g/l	35 - 50	32 - 50	28 - 31	25 - 27	<25

Table 2-5. Severity grading criteria for laboratory abnormalities in VAC053 and VAC065 using Imperial College Healthcare NHS Trust laboratory reference ranges. *Adapted from FDA guidelines [230].*

Laboratory Test	Grade 1	Grade 2	Grade 3
Hgb (female) – decrease from testing laboratory LLN in gm/dl	1.0 - <1.5	≥1.5 & <2.0	≥2.0
Hgb (male) – decrease from testing laboratory LLN in gm/dl	≥1.5 & <2.0	≥2.0 & <2.5	≥2.5
Absolute neutrophil count (ANC, cells/mm^3)	1000-1499	500-999	<500
Leukopenia (WBC, cells/mm^3)	<3500 - ≥2500	<2500 - ≥1500	<1500
Platelets (cells/mm^3)	125,000 – 135,000	100,000 – 124,000	20,000-99,000
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN
ALT	1.25 – 2.5 x ULN	>2.6 – 5.0 x ULN	>5.0 x ULN
Creatinine	1.1 – 1.5 x ULN	>1.6 – 3.0 x ULN	>3.0 x ULN

Table 2-6. Severity grading criteria for laboratory adverse events in VAC048.

2.3.4.5 Group holding rules

The safety holding rules described below were developed for use in VAC053, VAC056 and VAC065 considering the fact that these trials were the first time that R21 was being administered to humans.

- **Solicited local adverse events:**

More than 25% of doses of the vaccine at a given time point (e.g. Day 0, Day 28) in a study group 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 >48 hrs

- **Solicited systemic adverse events:**

More than 25% of doses of the vaccine at a given time point (e.g. Day 0, Day 28) in a study group 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 >48hrs

- **Unsolicited adverse events:**

More than 25% of subjects at a given timepoint (e.g. Day 0, Day 28) in a study group develop the same Grade 3 unsolicited adverse event (including the same laboratory adverse event) that persists at Grade 3 for > 48hrs

- **A serious adverse event considered possibly, probably or definitely related to vaccination occurs**
- **Death or any life-threatening SAE considered possibly, probably or definitely related to vaccination occurs**
- **Necrosis at the injection site, within the 7-day (days 0-6) post-vaccination period**

Activation of a holding rule would mean that further vaccinations in any Group could not occur until an internal safety review by the Local Safety Committee, study sponsor and the chief investigator had been conducted and it was deemed appropriate to restart dosing.

2.3.4.6 Individual stopping rules

In addition to the above group holding rules, stopping rules for individual subjects were also stipulated. Participants who fulfilled at least one of the following criteria were withdrawn from further vaccinations in the study:

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

The subject 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

- **Systemic solicited adverse events:**

The subject 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 subject has any Grade 3 adverse event considered possibly, probably or definitely related to vaccination, persisting continuously at Grade 3 for >72hrs

- **The subject has a serious adverse event considered possibly, probably or definitely related to vaccination.**
- **The subject has an acute allergic reaction or anaphylactic shock following the administration of vaccine investigational product.**

2.3.4.7 Local safety committee

A Local Safety Committee (LSC) was appointed to provide real-time safety oversight. This was chaired by Prof Brian Angus, a Clinical Tutor in Medicine, Honorary Consultant Physician and Director, Centre for Tropical Medicine at the University of Oxford. There were also two other appropriately qualified committee members. In addition to real-time safety oversight of the clinical trials undertaken by me, the Local Safety Monitor (LSM) performed independent external safety reviews prior to dose escalations. The LSC had the power to place the study on hold if deemed necessary following a study intervention-related SAE. In addition, I contacted the LSM for advice on an ongoing basis if independent advice and review were deemed important.

2.3.5 Compensation for trial participants

Volunteers were compensated for their time and for the inconvenience caused by procedures as below.

- Screening visit	£25 per visit
- Travel expenses	£10 per visit
- Inconvenience of blood tests	£10 per blood donation
- Time required for visit:	£20 per hour
- Illness Compensation (volunteers undergoing CHMI)	£480

Backup control volunteers who were not enrolled in the study were compensated £200.

2.4 Immunological assays

In all trials detailed in this thesis, blood samples for immunology were collected on day 0, prior to vaccination and at numerous time points as detailed in the schedule of attendances (See Appendix 2). Blood was collected in lithium heparinised or serum tubes and transported to the Jenner Institute laboratories for processing. Most of the immunological assays presented in this thesis were undertaken by the team of scientists at the Jenner Institute, under the supervision of Prof Katie Ewer. In addition, I have also performed some of the ELISAs, IFN-γ ELISpots and functional assays of induced antibodies detailed in this thesis. All protocols and record keeping were performed in compliance with Good Clinical Practice and all assays were performed as per well-established Jenner Standard Operating Procedures (SOP). The methods for these assays have been previously published and I will give a brief description of the methods for these assays in this chapter [136].

Reagent	Company	Catalogue no.
4-Nitrophenyl phosphate	Sigma	N2765
5 x Diethanolamine buffer	Pierce	34064
BCIP/NBT plus	Euorpa Bioproducts	NBTH-1000
Blocker Casein in PBS	Perbio	37528
Brefeldin A	eBioscience	00-4506-51
Carbonate-bicarbonate buffer	Sigma	C3041-100CAP
Casyton counting buffer	Sedna Scientific	43003
CD107a- PE Cy5 (eBioH4A3)	eBioscience	15-1079-42
CD14-eFluor 450 (TuK4)	eBioscience	48-0149-42
CD19-eFluor 450 (SJ25-C1)	eBioscience	48-0199-42
CD28 purified	eBioscience	16-0289-85
CD3-Alexa Fluor 700 (UCHT1)	eBioscience	56-0038-82
CD49d purified	eBioscience	16-0499-85
CD4-APC (RPA-T4)	eBioscience	17-0049-42
CD8-APC-AF780 (RPAT8)	eBioscience	47-0088-42
Dimethyl sulfoxide (DMSO)	Sigma	D2650
Dulbecco's PBS	Sigma	D8537
Goat anti-human IgG-Alkaline Phosphatase	Sigma	A3187
Heat-inactivated foetal calf serum	Biosera	S1810
IFN-γ Antibody-Catcher (1-DIK)	Mabtech	3420-2A
IFN-γ Antibody-Detector (7-1B6-Biotin)	Mabtech	3420-2A
IFN-γ-FITC (4S.B3)	eBioscience	11-7319-82
IL-2-PE (MQ1-17H12)	eBioscience	12-7029-82
L-Glutamine	Gibco	25030-24
Lymphoprep	Axis Shield	1114545
Monensin	eBioscience	00-4505-51
Penicillin/Streptomycin	Gibco BRL/Invitrogen	15140-122
Phosphate Buffered Saline (PBS)	Gibco	21600-069
Phytohaemmagglutinin-L	Sigma	L4144
Red blood cell (RBC) lysis solution	Qiagen	158902/4
RPMI	Sigma	R0883
Sodium Thiocyanate (NaSCN)	Sigma	251410
Staphylococcal enterotoxin B (SEB)	Sigma	S4881
Streptavidin-Alkaline Phosphatase	Mabtech	3420-2A
TNF-α-PE-Cy7 (Mab11)	eBioscience	25-7349-82
Tween-20	Sigma	9005-64-5

Table 2-7. Reagents, buffers and solutions used in the immunological assays.

2.4.1 Interferon-gamma (IFN-γ) enzyme-linked immunospot assay (ELISpot) to ME-TRAP [136]

Blood samples for peripheral blood mononuclear cells (PBMC) separation were collected in lithium heparinised Vacutainer® tubes (Becton Dickinson, UK) and separated on a centrifugation gradient using Lymphoprep® (Axis Shield) within 6 hours of venepuncture. *Ex vivo* (18-20 hour stimulation) ELISpot assays were performed using Multiscreen IP ELISpot plates (Millipore), human IFNγ SA-ALP antibody kits (Mabtech) and BCIP NBT-plus chromogenic substrate (Moss Inc). Cells were cultured in RPMI (Sigma) containing 1% sterile filtered Penicillin-Streptomycin (Sigma), 1% L-Glutamine (Sigma) and 10% heat-inactivated, sterile-filtered foetal calf serum, previously screened for low reactivity (Labtech International). Peptides (NEOBiolab) with purity > 70% were reconstituted in DMSO at 50-200mg/mL. They were designed to cover the length of the TRAP antigen and comprised 20mer peptides overlapping by 10 amino acids, giving a total of 57 peptides. The TT pools are from the TRAP T9/96 strain and the TD pools are from the 3D7 strain. The sequences of the peptides in pool 6 are identical between the two strains; therefore, there is no pool 6 for 3D7. There is also a pool of 20 ME peptides of varying lengths corresponding to the constituents of the multi-epitope string. The plate layout is shown in Figure 2-1.

Medium	Medium	TD 1-10	TD 1-10	Medium	Medium	TD 1-10	TD 1-10	Medium	Medium	TD 1-10	TD 1-10
TT1-10	TT1-10	TD11-20	TD11-20	TT1-10	TT1-10	TD11-20	TD11-20	TT1-10	TT1-10	TD11-20	TD11-20
TT11-20	TT11-20	TD21-30	TD21-30	TT11-20	TT11-20	TD21-30	TD21-30	TT11-20	TT11-20	TD21-30	TD21-30
TT21-30	TT21-30	TD31-40	TD31-40	TT21-30	TT21-30	TD31-40	TD31-40	TT21-30	TT21-30	TD31-40	TD31-40
TT31-40	TT31-40	TD41-50	TD41-50	TT31-40	TT31-40	TD41-50	TD41-50	TT31-40	TT31-40	TD41-50	TD41-50
TT41-50	TT41-50	TT Megapool	TT Megapool	TT41-50	TT41-50	TT Megapool	TT Megapool	TT41-50	TT41-50	TT Megapool	TT Megapool
TT51-57	TT51-57	FEC	FEC	TT51-57	TT51-57	FEC	FEC	TT51-57	TT51-57	FEC	FEC
ME	ME	SEB/ PHA	SEB/ PHA	ME	ME	SEB/ PHA	SEB/ PHA	ME	ME	SEB/ PHA	SEB/ PHA

Figure 2-1. ELISpot plate layout.

Antigens were tested in duplicate, with 250,000 PBMC added to each well of the *ex vivo* ELISpot plate in a final volume of 100 µL. Responses were averaged, responses in unstimulated (negative control) wells were subtracted and responses in individual pools summed. Staphylococcal enterotoxin B (0.02 µg/ ml) and phytohaemmagglutinin-L (10 µg/ml) were combined and used as a positive control. Plates were counted using an AID automated ELISpot counter (AID Diagnostika GmbH, algorithm C), using identical settings for all plates and counts were adjusted only to remove artefacts.

2.4.2 Flow cytometry with intracellular cytokine staining (ICS) [136]

Samples for flow cytometry were stimulated in parallel with the *ex vivo* ELISpot using fresh PBMC. After overnight stimulation, samples were stained and acquired the same day on the Jenner Institute LSR II flow cytometer. Responses to TRAP were assessed using a single pool of peptides. A hierarchical gating strategy was applied for analysis - singlets were identified using forward scatter plots. Dead cells were excluded by violet fluorescent amine-reactive dye staining. Monocytes and B cells

were excluded by CD14 or CD19 expression and T cells identified by CD3 expression. T cells were then subdivided by gating on $CD4^+$ and $CD8^+$ populations. Cytokine expression was quantified by plotting pairs of cytokines against each other and gating positive populations.

Acquisition was performed on the day of staining on a BD LSRII. Data were prepared and analysis performed using FlowJo v9.6.2 (Treestar Inc). Cells were gated on lymphocytes, singlets, live $CD3^+$, $CD8^-CD4^+$ or $CD4^-CD8^+$ and then IFN-γ, IL-2, TNF-α and CD107a. Dead cells ($AQUA^+$), monocytes ($CD14^+$) and B cells ($CD19^+$) were excluded from the analysis. Responses to peptide were determined after subtraction of the response in the unstimulated control for each sample.

2.4.3 Total IgG responses to TRAP [136]

Nunc-Immuno 96 well Enzyme Linked Immunosorbent Assay (ELISA) plates were coated with 0.5µg/mL of recombinant TRAP antigen, which was > 90% pure, in carbonate-bicarbonate coating buffer and left overnight at 4°C. Plates were washed 6x with PBS-Tween (PBS/T), then blocked with 1% BSA in PBS/T for 1 hour at room temperature (RT). Serum was diluted in PBS/T containing 0.2% BSA at concentrations of 1:100, 1:300, or 1:900, and added in triplicate. Plates were incubated at RT for 2 hours then washed. A secondary antibody (goat anti-human whole IgG conjugated to alkaline phosphatase, Sigma) was added at a dilution of 1:1000 in PBS/T 0.2% BSA for 1 hour at RT. After a final wash, plates were developed by adding 4-nitrophenyl phosphate in diethanolamine buffer (Pierce). A positive reference standard (made from pooled TRAP-positive serum) was used on each plate to give a standard curve. It was added in duplicate at an initial dilution of 1:100 (in PBS/T 0.2% BSA) and diluted

2-fold 10 times, starting with an arbitrary value of 20 antibody units. 4 blank wells (zero antibody units) were also designated. The optical density (OD) values were then fitted to a 4 parameter standard curve using SOFTmax PRO software [231]. An internal control was included on every plate in triplicate made up from a 1:400 dilution (in PBS/T 0.2% BSA) of the positive standard. OD was read at 405nm using an ELx800 microplate reader. Test sera antibody units were calculated from their OD values using the parameters estimated from the standard curve.

2.4.4 Total IgG responses to NANP

Nunc 96-well ELISA plates were coated with a synthetic peptide based on the *Pf*CSP repeat region (NANP)6 diluted to 0.2µg/mL in 100µL of Dulbecco's phosphate-buffered saline (dPBS) per well and incubated overnight at room temperature (RT). Plates were washed six times with PBS containing 0.5% Tween-20 (PBS/T) and blocked with casein for 1h at RT. Plates were washed again and serum samples diluted in casein at 1:100, 1:500, 1:1000 or 1:5000, were added for 2h at RT. After washing again, secondary antibody (goat anti-human IgG conjugated to alkaline phosphatase, Sigma) was added at 1:1000 in Casein for 1h at RT. Plates were washed a final time and developed using 4-nitrophenyl phosphate in diethanolamine buffer (Pierce). Optical density (OD) was read at 405nm using an ELx800 microplate reader. A reference pool of positive serum formed a standard curve on each plate and was used to calculate ELISA Units for each. An internal control was included on each plate to standardise between assays.

Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Sample 23	Internal control
Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Sample 23	Internal control
Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	Sample 23	Internal control
St 1:100	St 1:200	St 1:400	St 1:800	St 1:1600	St 1:3200	St 1:6400	St 1:12800	St 1:25600	St 1:51200	Blank	Blank
St 1:100	St 1:200	St 1:400	St 1:800	St 1:1600	St 1:3200	St 1:6400	St 1:12800	St 1:25600	St 1:51200	Blank	Blank

Figure 2-2. ELISA plate layout.

2.4.5 Antibody avidity assays

IgG antibody avidity was assessed by sodium thiocyanate (NaSCN)-displacement ELISA. The assays were conducted as for total IgG ELISAs except that sera were individually diluted in casein to a level calculated to reach an OD405 of 1.0 (using total IgG EUs), and plated at 50uL/well in 16 wells of a 96 well plate. Plates were incubated for 2 hours at RT before chaotropic agent NaSCN was added in duplicate at increasing concentrations down the plate (0 to 7 Molar (M)). Plates were incubated for 15 mins at RT before washing, incubated with secondary antibody and developed with the same conditions as the total IgG assay. The concentration of NaSCN required to reduce the OD405 to 50% of that in wells where no NaSCN (=IC50) was added was used as a measure of avidity.

2.4.6 Inhibition of sporozoite invasion assay

Human hepatoma cells (HC04- hepatoma cell line) cultured in R10 medium were added to 96-well culture plates at 30,000 cells/well and left to settle overnight at 37°C, 5% CO_2. Viable GFP-labelled *Plasmodium berghei* sporozoites expressing *P. falciparum* CSP at the *P. berghei* CSP locus (*P. berghei Pf*CSP@CSP) were obtained by

dissecting infected *Anopheles stephensi* mosquitoes. Salivary glands were pooled into RPMI 1640 medium and homogenized. Sporozoites were counted and diluted to 100,000/mL in RPMI 1640. Culture medium was aspirated from the hepatoma cells then 100µL of serum diluted 1:5 in RPMI supplemented with 10% FCS (R10) and 100µL of sporozoite dilution (10,000 sporozoites, 10% final serum concentration) were added to each well. Samples were tested in duplicate, "hepatoma only" wells and positive control wells containing hepatoma cells and sporozoites but no serum were included. C-1 samples were run for each volunteer. After incubation for 20-26 hours at 37°C media was aspirated and plates were washed with 90µL/well dPBS. Cells were trypsinized, resuspended in 65µL dPBS with 1% BSA and acquired immediately using an LSRII. DAPI stain was added to each sample just before acquisition. Data was analysed in FlowJo software v10.6 (TreeStar Inc.) using the gating strategy shown below.

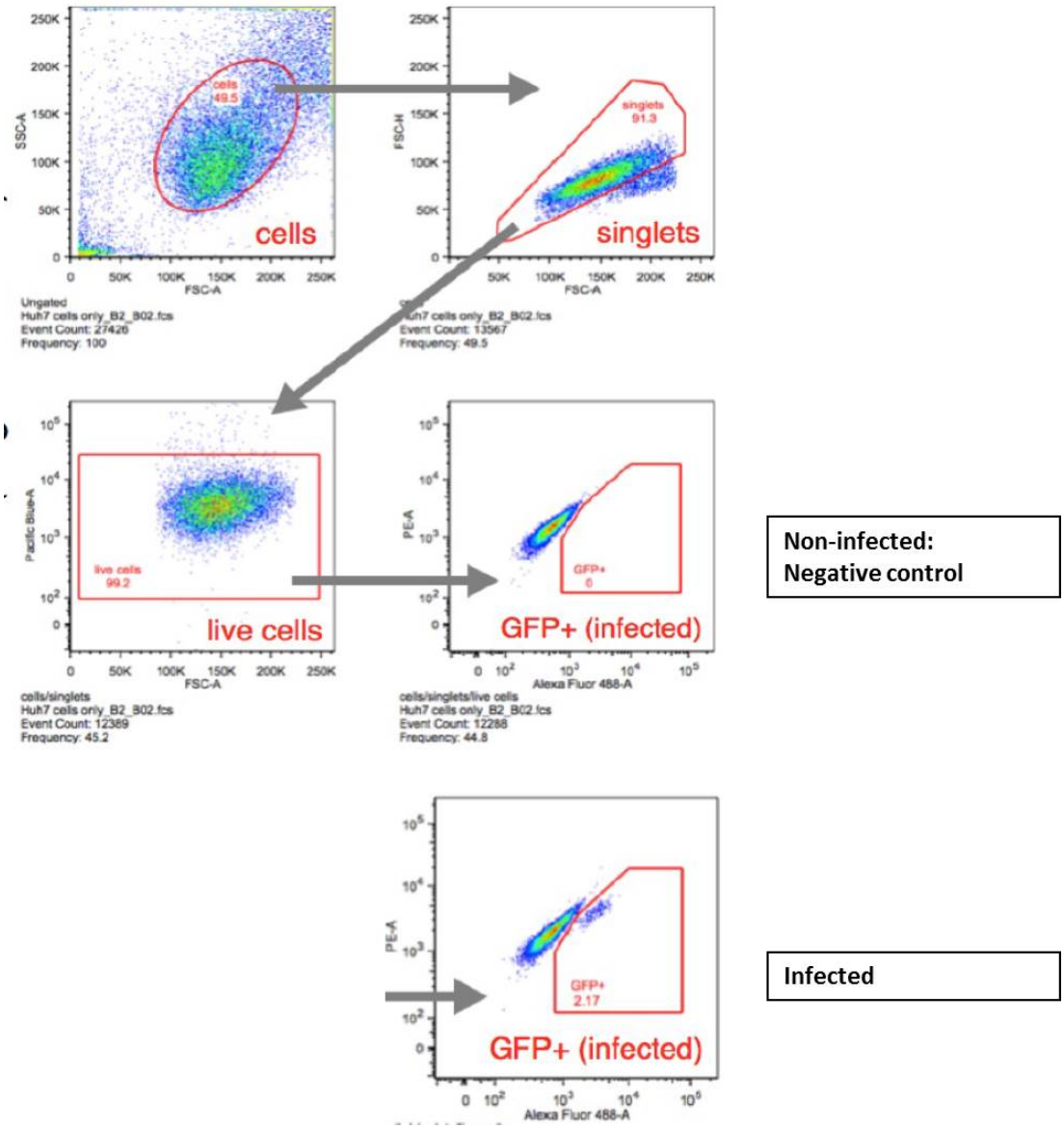


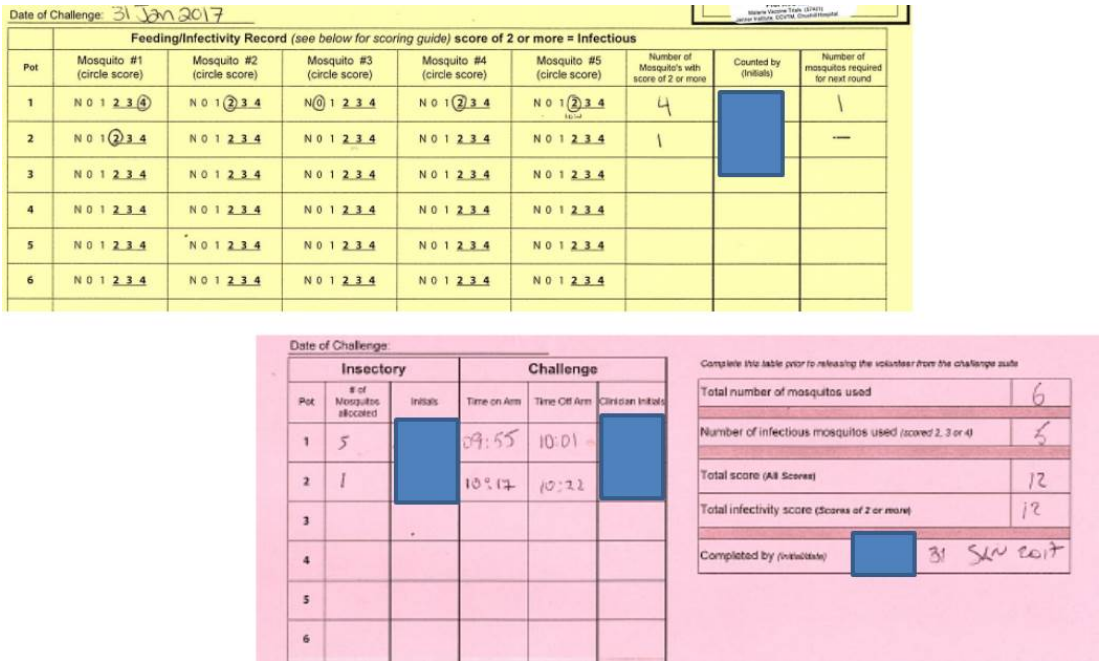
Figure 2-3. Gating strategy to determine percentage of infected hepatocytes.

2.5 Controlled human malaria infection (CHMI)

I was the lead clinician running the malaria sporozoite challenge in VAC065 with a team of physicians, nurses, entomologists and dissectors in the designated CL3 suite within the Sir Alexander Fleming Building at Imperial College, London. The CHMI administered by mosquito bite was performed according to SOP MC011: Mosquito Bite Malaria Sporozoite Challenge. Mosquitoes were infected via a membrane feeder

containing parasite material (fully sensitive 3D7 *Plasmodium falciparum*) plus fresh red cells and AB human serum, 21 days prior to the CHMI, at the Walter Reed Army Institute of Research (WRAIR), USA. The blood products came from volunteers who had been screened according to US Red Cross guidelines (screened for Trypanosoma cruzi, HIV 1 and 2, HBV, HCV, HTLV, West Nile Virus and syphilis). Mosquitoes were supplied directly by a representative of the WRAIR, whereupon an appropriate researcher (fully trained and inducted according to local rules, including the SAF Level 6 CL3 COP) from Imperial College acknowledged receipt of the mosquitoes and transferred them to a secure room within the insectary. Each pot of mosquitoes was kept in a strong transparent plastic storage box, or within a secure Perspex glove box. All manipulation of mosquitoes was performed within a secure Perspex glovebox cabinet. Prior to challenge, an appropriately trained researcher from Imperial College prepared the mosquitoes for challenge as described in the Imperial College SAF Level 6 COP, SOP4. The small pots of mosquitoes brought out for challenge of the volunteers were covered with netting and held in a second pot to minimise any possibility of escape. The challenge was performed in a part of the secure insectary that is isolated by appropriate and approved mechanisms. Volunteers were initially exposed to the bites of five infectious mosquitoes per participant (by placing their forearms over the pot containing mosquitoes) for 5-10 minutes. Fed mosquitoes (as indicated by the presence of a blood meal in the abdomen) were individually dissected and assessed for sporozoite load (graded 0 to +4; a gland rating of +2 or more, representing 10 or more observed sporozoites, qualifies as being infectious). If, by this method the volunteer was found to have been inoculated by less than five infected mosquitoes, further mosquitoes are allowed to feed on the volunteer until

a total of 5 appropriately infected mosquitoes had fed. The bite-challenge procedure continued until the subject had been bitten by 5 infectious mosquitoes.



Date of Challenge: 31 Jan 2017

Pot	Feeding/Infectivity Record (see below for scoring guide) score of 2 or more = Infectious Mosquito #1 (circle score)	Mosquito #2 (circle score)	Mosquito #3 (circle score)	Mosquito #4 (circle score)	Mosquito #5 (circle score)	Number of Mosquito's with score of 2 or more	Counted by (Initials)	Number of mosquitos required for next round
1	N 0 1 2 3 (4)	N 0 1 (2) 3 4	N (0) 1 2 3 4	N 0 1 (2) 3 4	N 0 1 (2) 3 4	4	[redacted]	1
2	N 0 1 (2) 3 4	N 0 1 2 3 4	N 0 1 2 3 4	N 0 1 2 3 4	N 0 1 2 3 4	1		—
3	N 0 1 2 3 4	N 0 1 2 3 4	N 0 1 2 3 4	N 0 1 2 3 4	N 0 1 2 3 4			
4	N 0 1 2 3 4	N 0 1 2 3 4	N 0 1 2 3 4	N 0 1 2 3 4	N 0 1 2 3 4			
5	N 0 1 2 3 4	N 0 1 2 3 4	N 0 1 2 3 4	N 0 1 2 3 4	N 0 1 2 3 4			
6	N 0 1 2 3 4	N 0 1 2 3 4	N 0 1 2 3 4	N 0 1 2 3 4	N 0 1 2 3 4			

Date of Challenge:

Pot	Insectory: # of Mosquitos allocated	Insectory: Initials	Challenge: Time on Arm	Challenge: Time Off Arm	Challenge: Clinician Initials
1	5	[redacted]	09:55	10:01	[redacted]
2	1		10:17	10:22	
3					
4					
5					
6					

Complete this table prior to releasing the volunteer from the challenge suite

Total number of mosquitos used	6
Number of infectious mosquitos used (scored 2, 3 or 4)	5
Total score (All Scores)	12
Total infectivity score (Scores of 2 or more)	12
Completed by (Initials/date)	[redacted] 31 Jan 2017

Figure 2-4. Example biting record during the malaria challenge procedure. *This volunteer required two pots containing 5 mosquitoes and 1 mosquito, respectively to receive the appropriate amount of infectious bites.*

The follow-up procedure post-CHMI is shown in Table 2-8. In summary, volunteers received a daily telephone call for the first 5 days to ensure they were well and contactable. Volunteers were reviewed in clinic once on the evening of Day 6 (C+6.5), then twice daily until the evening of Day 14 (C+14.5). From Day 15 until up to Day 21 (C+15 – C+21) post CHMI, volunteers were reviewed daily each day until the challenge endpoint is passed (i.e. malaria is diagnosed or day 21 post-challenge is reached). All follow-up visits in this intensive post CHMI phase took place at the Centre for Clinical Vaccinology and Tropical Medicine in Oxford. All volunteers were seen by one of the clinical study team at each visit. There were two further final visits at day 35 and day 90 post-challenge. Control volunteers had two additional visits on the evening of day 5 and the morning of day 6.

Timeline (days in relation to CHMI)	0	C+5.5	C+6	C+6.5	C+7	C+8-C+14	C+15-C+21	Post-diagnosis	C+35	C+90
Window (days)	0	0	0	0	0	0	0	0	±7	±14
Medical History	(x)			(x)	(x)	(x)	(x)	(x)	(x)	(x)
Clinical Assessment	(x)			(x)	(x)	(x)	(x)	(x)	(x)	(x)
Urinary β H-CG[£]				(x)	(x)	(x)	(x)	x		
Physical Observations[%]	(X)			X	X	X	X	X	X	X
Medic Alert Card Provided	X									
Local & systemic events				X	X	X	X	X	X	X[€]
Treatment for Malaria				(x)	(x)	(x)	X	X		
Thick Smear / PCR for malaria				3	3 x 2	3 x 14	3 x 7	3 x 2		
Haematology (mL)						2^		2^	2	2
Biochemistry (mL)**						3^		3^	3	3
Immunology						30[$]			30	30
Gene expression profiling[&]		6[&]	6[&]	6[&]	25 + 6[&]	25 x 7[&]			25[&]	
Blood volume per visit(s) (mL) Group 1-3	0	0	0	3	6	77	21	11	35	35
Blood volume per visit (mL) – Control Group	0	6	6	9	37	252	21	11	60	35

Table 2-8. Schedule of Clinic Attendances Post-CHMI. *(Windows refer to time since day of challenge).*
***(x)** = If considered necessary, emphasising any acute complaints.*
[£] Urinary β HCG was performed in female volunteers prior to commencing anti-malaria treatment.
[%] Physical observations included blood pressure, pulse and temperature.
*** Biochemistry included Sodium, Potassium, Urea, Creatinine, Albumin & Liver Function Tests.*
^Blood was drawn for haematology & biochemistry on C+9 and within 12 hours of diagnosis.
[$] Blood was drawn for immunology on the C+14 (30mls) visit or on the day of diagnosis if this is before C+14.
[€] SAEs only were collected on this visit
[&] Control volunteers only

2.5.1 Parasite Quantitative PCR (qPCR) [136]

Quantitative PCR was performed on Ethylenediaminetetraacetic acid (EDTA) blood samples collected from participants after CHMI. This work was led by Nick Edwards, with support from the Jenner laboratory staff and processed as per Jenner SOP ML008: Malaria qPCR. Blood was collected at baseline and at time points shown in

the schedule of attendances. Briefly, blood was filtered to reduce white cell content and DNA was extracted from 0.5ml filtered blood using Qiagen Blood Mini Kit. 5µL of each extraction was used per assay (total eluate volume = 50µl) and was run in triplicate for qPCR (equivalent to 150µl blood directly assessed). Parasites per mL (p/ml) equivalent mean values were generated by a standard Taqman absolute quantitation, against a defined plasmid standard curve. This was conducted on an ABI StepOne Plus machine and v2.3 software using default Universal qPCR and QC settings, apart from the use of 45 cycles and 25µl reaction volume.

2.5.2 Thick blood film preparation and interpretation

Thick blood films were prepared and interpreted according to Jenner Institute SOP ML009: Collection, preparation and slide reading: Malaria Challenge Studies. This was performed by experienced microscopists from UK and Kenya. Briefly, a small drop of blood, collected in an EDTA tube, was prepared on a microscope slide by spreading the drop thinly and allowing it to dry. The slide was initially dipped in Field's stain A for 3 seconds, before being rinsed in clean water for 3 seconds. Subsequently, the slide was dipped in Field's stain B for a further 3 seconds. After drying, the slide was examined and 200 high power fields (1000x) were read. Any trophozoites identified by the microscopist were reviewed by either me or another clinical research fellow for visual confirmation.

2.6 Statistics

Data were analyzed using Microsoft Excel, 2010; GraphPad Prism version 6.0 for Windows and Stata 10.0. Medians with interquartile ranges (IQR) for each group are

described. Significance testing of differences between two groups used Mann-Whitney analysis. A Wilcoxon matched-pairs analysis was used to compare between time points within groups. Kruskal-Wallis analysis with Dunn's test for multiple comparisons was used to compare responses across groups. A chi-squared test was used to compare the safety data between different groups. A statistically significant difference in efficacy between a vaccination regimen and controls was assessed by log rank analysis of Kaplan Meier curves, using a two-tailed log rank test at the different endpoints. A value of $p < 0.05$ was considered significant.

CHAPTER THREE:

A Phase I clinical trial to assess the safety and immunogenicity of ChAd63-MVA ME-TRAP malaria vaccination adjuvanted with Matrix- M™ (VAC048)

3.1 Authorship statement

Dr Nick Anagnostou commenced recruitment and vaccination in October 2012 after the trial received appropriate regulatory and ethical approvals. 12 volunteers had already been enrolled and completed follow-up when I took over the study as lead clinician in November 2013. I screened and enrolled 10 further volunteers in the Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), Oxford. Vaccinations and follow-up visits were conducted by me and the nursing team. Clinical oversight and the analysis of the adverse event data were done by me. Immunogenicity data presented in this chapter are courtesy of the team at the Jenner Institute. All the figures presented in the results section of this chapter were produced by me based on the analysis of the primary safety and immunogenicity data.

3.2 Introduction

Most licensed vaccines including the most advanced malaria vaccine candidate, RTS,S/AS01 predominantly work by inducing antibodies. Malaria immunity is complex, and in addition to humoral responses, cellular responses targeting pre-erythrocytic antigens play a role in natural immunity in malaria endemic populations [232] and can provide protection against malaria in animal models [41, 107, 233], following irradiated sporozoite inoculation [234], and following vaccination in humans [129, 130]. A leading alternative, and potentially complementary strategy to

a sporozoite-based vaccine such as RTS,S is heterologous prime-boost immunization with sequential administration of viral-vectored vaccines chimpanzee adenovirus serotype 63 (ChAd63) and modified vaccinia Ankara (MVA), both encoding ME-TRAP (a multiple epitope string fused to the thrombospondin-related adhesion protein). In addition to eliciting antibody responses, this approach has been shown to elicit potent T cell responses in UK adults [128]. Furthermore, it induces potent cellular responses in adults and infants in malaria endemic regions with an excellent track record of safety and tolerability in these populations [132, 133, 228, 229]. Further details of the development of this heterologous prime-boost regime, with viral-vectored vaccines both encoding the malaria ME-TRAP antigenic sequence, are given in Section 1.6.3.

It is widely accepted that an efficacious malaria vaccine is likely to be a combination of different approaches acting on multiple antigens involved in different stages of its complex life cycle [235]. A recent clinical trial combining the leading malaria vaccine candidate, RTS,S/AS01 with ChAd63-MVA ME-TRAP in the same regime for the first time demonstrated that combining these vaccines in the same regimen was not only safe and tolerable, but also highly immunogenic and efficacious [136]. It is highly likely that an efficacious protein or virus-like particle-based subunit malaria vaccine will require an adjuvant. We therefore assessed whether mixing viral-vectored vaccines with an adjuvant would lead to any deleterious effect on the immunogenicity of the vectored vaccines.

Matrix-M (MM) is an attractive novel candidate vaccine adjuvant for this purpose as it, and other particulate formulations of *Quillaja* saponins, show acceptable safety

[236], and the ability to enhance both cellular and humoral immune responses to a range of vaccines [196, 197, 237-239]. Further details of the MM adjuvant given in combination with a number of vaccines are provided in Section 1.11.1. This study was conducted to evaluate the safety and immunogenicity of the ChAd63-MVA ME-TRAP vaccination regime adjuvanted with MM compared to ChAd63-MVA ME-TRAP given alone.

3.3 Methods

Detailed methods of the recruitment and enrolment of volunteers, as well as the assays used in this trial can be found in Chapter 2.

3.3.1 Study design

This was a Phase I controlled observational clinical trial to assess the safety and immunogenicity of ChAd63 ME-TRAP and MVA ME-TRAP adjuvanted with MM administered in a heterologous prime-boost regimen. Eligible participants were assigned to one of three dose groups: a control group who received ChAd63 ME-TRAP followed by MVA ME-TRAP 8 weeks later and two treatment groups who received ChAd63-MVA ME-TRAP vectors both adjuvanted with MM at doses of 25μg or 50μg, respectively. The first three vaccinations in the treatment groups occurred in a staggered manner and interim safety reviews were conducted prior to progression to the higher dose MM group.

The study was conducted at the CCVTM, University of Oxford. The number of volunteers has been chosen to generate adequate safety and immunogenicity data, whilst minimising the number of volunteers exposed to a new vaccination regimen.

	Day 0	Day 56
Control (n=6)	ChAd63 ME-TRAP	MVA ME-TRAP
Low dose (25μg) Matrix-M (n=8)	ChAd63 ME-TRAP/ 25μg Matrix-M	MVA ME-TRAP/ 25μg Matrix-M
High dose (50μg) Matrix-M (n=8)	ChAd63 ME-TRAP/ 50μg Matrix-M	MVA ME-TRAP/ 50μg Matrix-M

Table 3-1. VAC048 Study groups.

3.3.2 Ethics and regulatory approval

The study protocol and associated documents were reviewed and approved by the UK National Research Ethics Service, Committee South Central–Oxford A (Ref: 12/SC/0036), the Medicines and Healthcare Products Regulatory Agency (Ref: 21584/0299/001-0001), and the Oxford University Clinical Trials and Research Governance team, who independently and externally monitored compliance with Good Clinical Practice guidelines. Vaccine use was authorised by the Genetically Modified Organisms Safety Committee (GMSC) of the Oxford University Hospitals NHS Trust (Reference number GM462.11.66). The trial was registered with ClinicalTrials.gov (Ref: NCT01669512).

3.3.3 Objectives and Endpoints

Primary Objective

To assess the safety of ChAd63-MVA ME-TRAP heterologous prime-boost vaccination adjuvanted with Matrix-M.

Primary Outcome Measures

Analysis of all solicited and unsolicited local and systemic adverse events (AEs) occurring in the 25μg dose Matrix-M and 50μg dose Matrix-M group volunteers. The specific endpoints for safety and reactogenicity were actively and passively collected data on adverse events. The following parameters were assessed for all study groups:

- Occurrence of solicited local and systemic reactogenicity signs and symptoms for 7 days following the vaccination
- Occurrence of unsolicited adverse events during the whole study duration
- Change from baseline for safety laboratory measures
- Occurrence of serious adverse events during the whole study duration

Secondary Objective

To assess the effects of Matrix-M on the immunogenicity of ChAd63-MVA ME-TRAP heterologous prime boost vaccination.

Secondary Outcome Measures

Comparison of immunogenicity of the Matrix-M adjuvanted vaccination regimens versus the control regimen. Measures of immunogenicity included:

- *Ex-vivo* IFN-γ ELISpot responses to ME-TRAP
- Intracellular cytokine staining (ICS) for T-cell populations eg $CD4^+$ and $CD8^+$ T cells and cytokine profiles
- IgG responses to TRAP

3.3.4 Study participants

Participants were healthy adults between the ages of 18 and 50 years, all of whom provided written informed consent. Participant demographics and baseline characteristics are presented in Table 3-2.

Volunteer No.	Age	Sex	BMI*	Country of birth	Smoker
1250	27	Male	26.8	Mexico	No
1256	27	Female	-	United Kingdom	No
1260	18	Male	26.6	United Kingdom	No
1262	20	Female	18.1	United Kingdom	Yes
1252	23	Female	30.1	United Kingdom	No
1251	27	Female	26.6	United Kingdom	No
1254	26	Female	20.0	New Zealand	No
1264	41	Male	31.6	United States	No
1266	29	Male	-	United Kingdom	Yes
1270	26	Male	-	Germany	No
1273	24	Male	-	United Kingdom	No
1277	22	Female	24.4	United Kingdom	Yes
1280	19	Male	33.0	Uganda	No
1282	24	Female	23.1	Canada	No
1284	24	Female	26.5	Germany	No
1292	25	Male	23.7	United Kingdom	No
1294	27	Male	22.0	United Kingdom	No
1297	23	Female	25.5	United Kingdom	No
1296	25	Female	24.5	United Kingdom	Yes
1285	29	Male	25.2	United Kingdom	No
1286	21	Female	18.5	United Kingdom	No
1289	45	Female	28.6	United Kingdom	No
1290	40	Male	27.3	United Kingdom	No

Table 3-2. Demographics and baseline characteristics of volunteers enrolled in VAC048. **BMI (body mass index) was not recorded in 4 volunteers.*

3.3.4.1 Inclusion Criteria

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

- Healthy adult aged 18 to 50 years
- Able and willing (in the Investigator's opinion) to comply with all study requirements

- Willing to allow the investigators to discuss their medical history with their General Practitioner
- For female volunteers, willingness to practice continuous effective contraception during the study
- For male volunteers, must use barrier contraception for 3 months from the day of any administration of Matrix-M
- Agreement to refrain from blood donation during the course of the study and for 6 months after the end of their involvement in the study
- Written informed consent

3.3.4.2 Exclusion Criteria

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

- Participation in another research study involving an investigational product in the 30 days preceding enrolment, or planned use during the study period
- Prior receipt of an investigational malaria vaccine or any other investigational vaccine likely to impact on interpretation of the trial data
- Administration of immunoglobulins and/or any blood products within the three months preceding the planned administration of the vaccine candidate
- Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections; and chronic (more than 14 days) immunosuppressant medication use within the past 6 months (inhaled and topical steroids are allowed)
- Pregnancy, lactation, or intention to become pregnant during the study

- History of allergic disease or reactions likely to be exacerbated by any component of the vaccine, e.g. egg products, Kathon
- History of clinically significant contact dermatitis
- Any history of anaphylaxis or hypersensitivity in relation to vaccination
- History or evidence of pre-existing autoimmune or antibody-mediated disease or laboratory evidence of possible autoimmune disease, defined as a positive antinuclear antibody (ANA) result at screening.
- Any history of malaria
- Travel to a malaria endemic region during the study period or within the six months preceding enrolment in the study with significant risk of malaria infection
- History of serious psychiatric condition that may 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 five years preceding enrolment
- Seropositive for hepatitis B surface antigen (HBsAg)
- Seropositive for hepatitis C virus (antibodies to HCV)
- Any relevant history of cancer (excludes basal cell carcinoma of the skin and cervical carcinoma in situ)
- 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 study data

3.3.5 Study interventions

Vaccine preparation and administration

The study was performed in accordance with Genetically Modified Organisms (GMO) contained use regulations (2000). In order to minimise dissemination of the recombinant vectored vaccine virus into the environment, the inoculation site was covered with a dressing after immunization to absorb any virus that may leak out through the needle track. The dressing was removed from the injection site after 30 minutes and was disposed as GMO waste by autoclaving, in accordance with the relevant Standard Operating Procedure (SOP) and current standard UK practice. Vials of ChAd63 ME-TRAP and MVA ME-TRAP were stored in a locked -80^{O}C freezer. Vials of Matrix-M were stored in a locked refrigerator at 2 to 8^{o}C, and not frozen at any time. On vaccination day, ChAd63 ME-TRAP and MVA ME-TRAP were thawed to room temperature and were administered intramuscularly within 1 hour of removal from the freezer, either alone, or mixed with Matrix-M.

ChAd63 ME-TRAP (Batch no: 01S11-01)

ChAd63 ME-TRAP was manufactured under Good Manufacturing Practice conditions by the Clinical Biomanufacturing Facility (CBF), Churchill Hospital, Oxford. ChAd63 ME-TRAP is supplied as a sterile 0.5-1.0 ml liquid in 2.0ml glass vials. The dose of

ChAd63 ME-TRAP used in this study was 5 x 10^{10} vp, which was administered intramuscularly.

MVA ME-TRAP (Batch no: 0080312)

MVA ME-TRAP was manufactured under Good Manufacturing Practice conditions by IDT Biologika GmbH, Germany. MVA ME-TRAP is supplied as a sterile liquid in 2.0 mL clear glass injection vials. The dose of MVA ME-TRAP used in this study was 2 × 10^8 pfu to be given intramuscularly. The first two volunteers received a dose of 1.44 × 10^8 pfu – this was due to an error in the dilution of the vaccines during mixing, prior to administration. Final batch certification of the vaccines and associated labelling was performed by a Qualified person (QP) at the Clinical Biomanufacturing facility (CBF).

Matrix-M (Batch no: 12-104)

Matrix-M was manufactured in compliance with cGMP by Apoteket Produktion & Laboratorier AB (APL) Formvägen 5B, SE-903 03 Umeå, Sweden. It was supplied as a sterile 1mg/ml solution in 2ml glass vials. Matrix-M was mixed with the ChAd63 ME-TRAP and MVA ME-TRAP at the bedside immediately prior to administration and administered intramuscularly within one hour of thawing of the vaccines.

3.3.6 Assessment of safety

Volunteers were observed for 45 minutes following vaccine administration. Follow-up visits were scheduled for days 1, 7, 14, 28, 57, 63, 70, 84, 112 and 168, with an additional visit at day 3 post-vaccination for the first three volunteers in the treatment groups. All participants were issued with a paper diary card to record their temperature and any local and systemic adverse events for 7 days post-vaccination.

A review of solicited and unsolicited adverse events occurred at each follow-up visit. Safety bloods including full blood count, renal function and liver function tests were done on visits at day 0, 7, 28, 56, 63, 84 and 168.

3.3.7 Assessment of immunogenicity

T-cell immunity was assessed using an *ex vivo* IFN-γ enzyme-linked immunospot (ELISpot) assay and flow cytometry with intracellular cytokine staining (ICS). *Ex vivo* IFN-γ ELISpot assays were performed on samples from days 0, 14, 28, 56, 63, 84, 112 and 168. Cytokine production in response to stimulation with a pool of TRAP peptides was analysed at day 28 and day 63 for each group, using flow cytometry. Antibody responses measured by anti-TRAP IgG ELISA were performed on samples from days 0, 14, 28, 56, 63, 84, 112 and 168. The full methods used for these immunological assays are included in Chapter 2.

3.4 Results

3.4.1 Participant flow

From 1st October, 2012 to 23rd June, 2014, a total of 23 volunteers (11 males, 12 females) of the 48 who were screened for eligibility were enrolled. Participant flow and study design is summarised in Figure 3-1. One volunteer in the low dose group withdrew from the study 7 days after receiving their first vaccination for reasons unrelated to their vaccination and was subsequently replaced. This volunteer was asymptomatic apart from mild fatigue when reviewed at 7 days post-vaccination. Safety bloods done at this time point were also normal. All the remaining 22 volunteers completed the study and were followed up until Day 168.

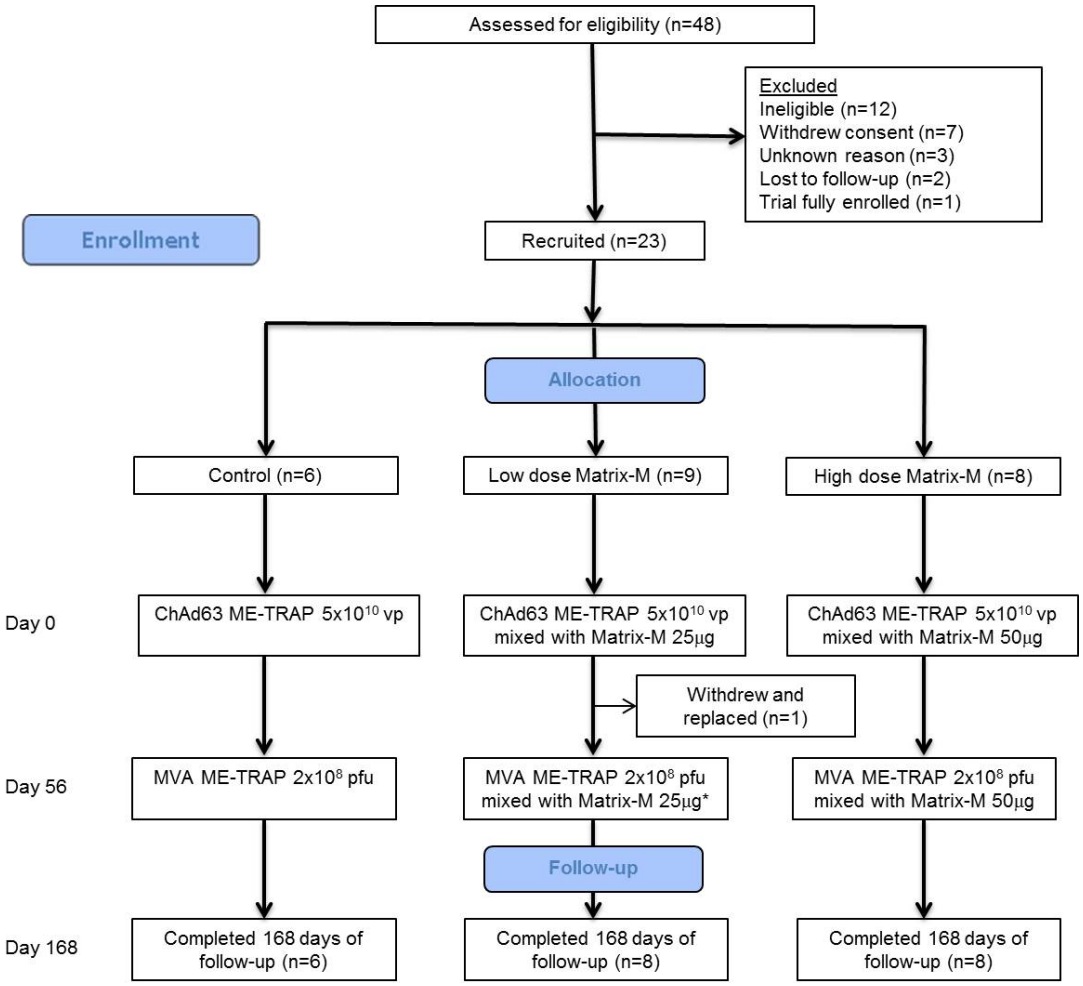


Figure 3-1. VAC048 flow chart of study design and volunteer recruitment. *Abbreviations: ChAd63, chimpanzee adenovirus serotype 63; ME-TRAP, multiple epitope string fused to the thrombospondin-related adhesion protein; MVA, modified vaccinia Ankara; pfu, plaque forming units; vp, viral particles. A total of 48 volunteers were screened and 23 were enrolled in total. One volunteer in the low dose group withdrew consent after their first vaccination and was subsequently replaced. A total of 22 volunteers completed the study. *The first two volunteers in the low dose group received MVA ME-TRAP at a dose of 1.44 x 10^8 pfu instead of 2 x 10^8 pfu.*

3.4.2 Vaccine safety and reactogenicity

The safety profile of heterologous prime-boost immunization with ChAd63-MVA ME-TRAP has been previously described [128, 129, 229]. There were no serious adverse events (SAEs) or suspected unexpected serious adverse reactions (SUSARs) in this

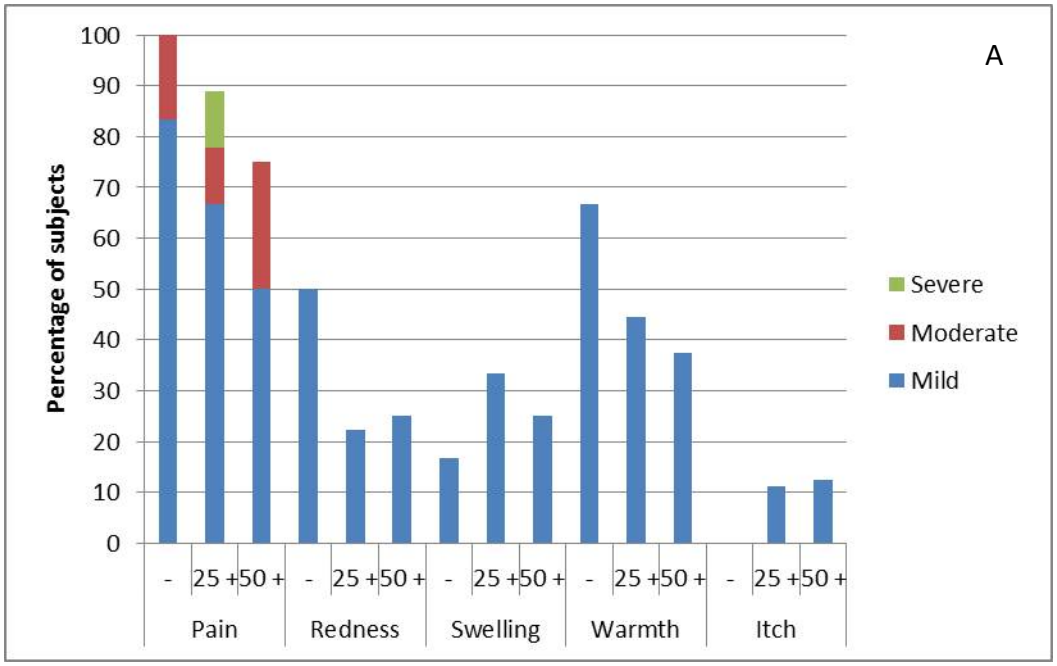
trial. The majority of adverse events (AEs) reported were self-limiting and mild in severity.

Local and systemic AEs related to the ChAd63 ME-TRAP vaccination either administered alone or mixed with MM are shown in Figures 3-2A and 3-2B, respectively. The majority of local and systemic adverse events were mild in nature. Vaccine site pain was the most common local adverse event and was predominantly mild in nature. One volunteer in the 25μg MM group reported severe pain, which resolved within 48 hours. Five volunteers reported a fever; an episode of severe fever, characterized as documented fever > 39°C was reported by two of these volunteers (one in the 25μg MM group and one in the 50μg MM group). All episodes of fever occurred within the first day post-vaccination and did not persist for more than 24 hours. Severe flu-like symptoms were reported by one volunteer each in the control group and 50μg MM group. One volunteer in the 25μg MM group reported a severe headache. Overall, there was no significant increase in reactogenicity with the addition of MM to the priming vaccination (p=0.2), with no clear pattern of attribution to the adjuvant.

Local and systemic AEs related to MVA ME-TRAP vaccination either administered alone or mixed with MM are shown in Figures 3-3A and 3-3B, respectively. The majority of local and systemic AEs were mild in nature. Local reactogenicity was more pronounced compared to vaccination with ChAd63 ME-TRAP (3.6 AEs/volunteer vs 2.3 AEs/volunteer). All volunteers receiving MVA ME-TRAP reported vaccine site pain. Three volunteers in the control group and two volunteers in the 25μg MM group reported severe pain. Eight volunteers (three in the control group, two in the 25μg

MM group and three in the 50μg MM group) reported a fever. Two of these volunteers (one in the control group and one in the 50μg MM group) had an episode of severe fever. All episodes of fever occurred within the first day post-vaccination and did not persist for more than 24 hours. Severe systemic symptoms were reported by two volunteers each in the control group, 25μg Matrix-M group and 50μg Matrix-M group. Overall, there was no significant increase in the reactogenicity with the addition of MM to the MVA ME-TRAP vaccination (p=0.08), with no clear pattern of attribution to the adjuvant.

Unsolicited adverse events collected for 28 days after each vaccination deemed definitely, possibly or probably related to vaccination were predominantly mild in nature and are coded according to the MedDRA classification and tabulated in Table 3-3. One volunteer in the control group developed a mild transient leukopaenia at day 7 post-ChAd63 ME-TRAP vaccination, which resolved spontaneously. One volunteer each in the low dose group and the high dose group had a mild leukopaenia associated with neutropaenia present prior to their first vaccination and persisted intermittently through the course of the trial. There were no other biochemical or haematological abnormalities associated with vaccination.



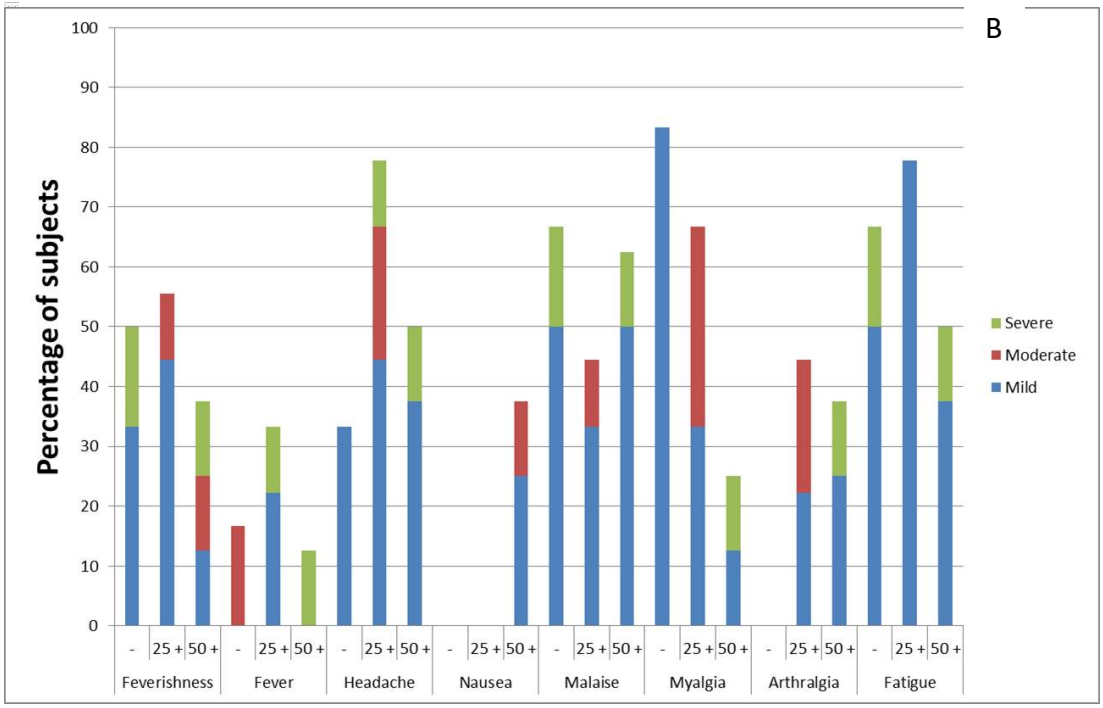
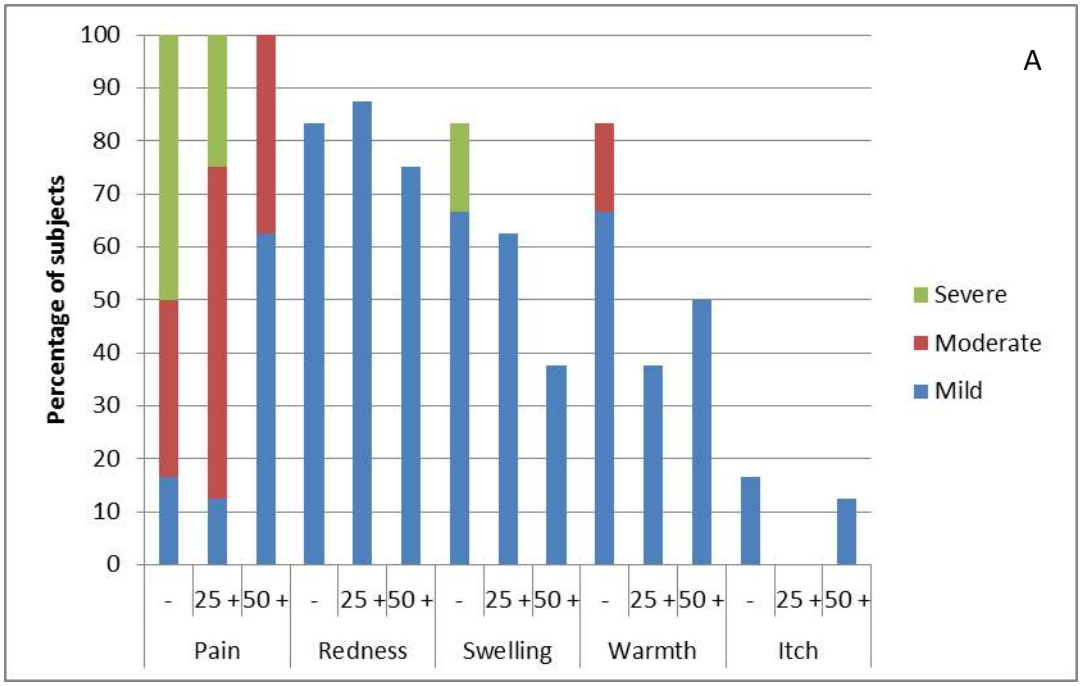


Figure 3-2. VAC048 - Local (A) and systemic (B) adverse events (AE) deemed definitely, probably or possibly related to ChAd63 ME-TRAP vaccination. *ChAd63 ME-TRAP (5 x 10^{10} vp) was either administered alone (-) or mixed with Matrix-M given at a dose of 25μg (25+) or 50μg (50+). Only the highest intensity of each AE per subject is listed.*



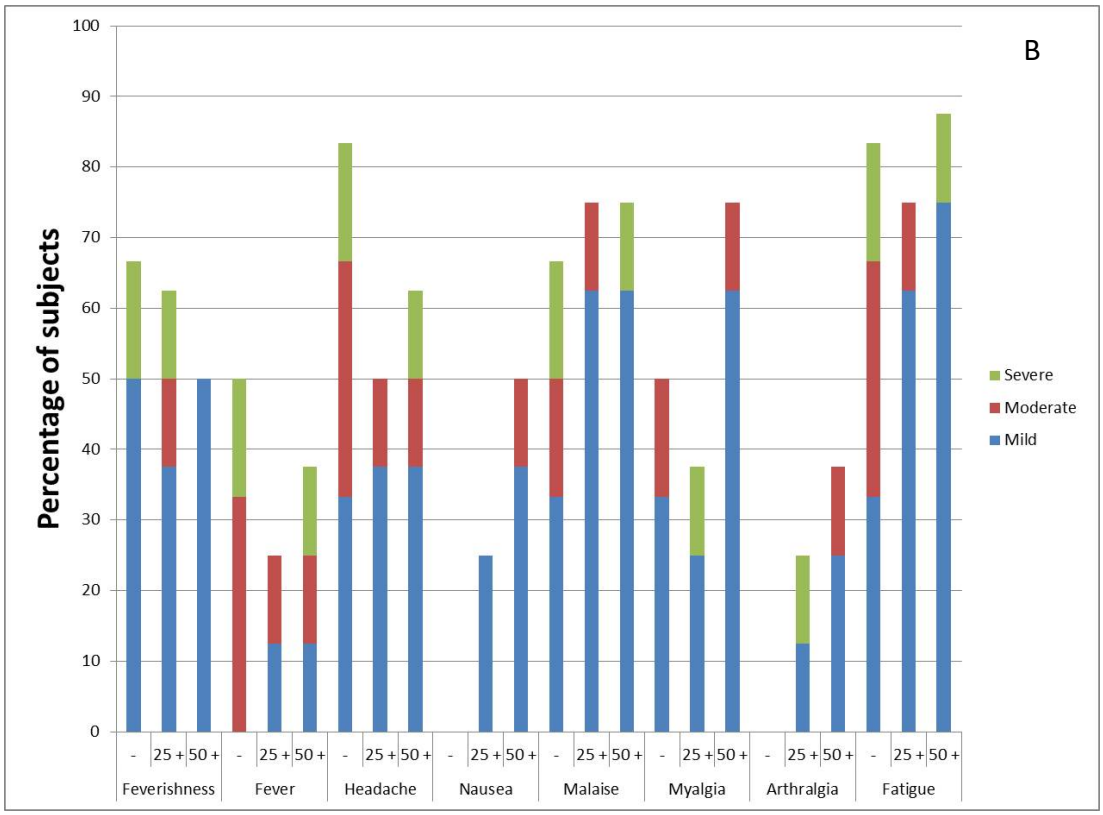


Figure 3-3. VAC048 - Local (A) and systemic (B) adverse events (AE) deemed definitely, probably or possibly related to MVA ME-TRAP vaccination. *MVA ME-TRAP (2 x 10^8 pfu) was either administered alone (-) or mixed with Matrix-M given at a dose of 25μg (25+) or 50μg (50+). The first two volunteers in the* 25μg *dose group received MVA ME-TRAP at a dose of 1.44 x 10^8 pfu instead of 2 x 10^8 pfu. Only the highest intensity of each AE per subject is listed.*

Symptom	MedDRA code	Intensity	Day of onset	Last day	Vaccination no.	Causality
ChAd63-MVA ME-TRAP/25μg Matrix-M						
Bruise	10006502	Mild	3	7	1	Definite
Dizziness	10013573	Moderate	1	1	1	Possible
Heart rate increased	10019303	Mild	0	0	2	Probable
Light headedness	10024492	Severe	0	0	1	Possible
Rigor	10039175	Severe	0	0	2	Probable
Rigor	10039175	Moderate	0	0	1	Probable
Shaky feelings	10040531	Mild	0	0	2	Probable
ChAd63-MVA ME-TRAP/50μg Matrix-M						
Abdominal pain	10000081	Moderate	1	1	2	Possible
Backache	10003993	Mild	1	2	2	Probable
Backache	10003993	Mild	1	3	2	Probable
Backache	10003993	Mild	7	8	1	Possible
Backache	10003993	Moderate	0	0	1	Probable
Bruise	10006502	Mild	5	9	2	Definite
Bruise	10006502	Mild	8	10	2	Probable
Chills	10008531	Moderate	1	1	2	Definite
Difficulty sleeping	10012804	Moderate	0	0	1	Probable
Dizziness	10013573	Moderate	1	1	2	Probable
Dry cough	10013773	Mild	7	11	1	Possible
Rigor	10039175	Moderate	1	1	2	Definite
Rigor	10039175	Severe	0	0	1	Probable
Runny nose	10039296	Mild	6	21	1	Possible
Shaky feelings	10040531	Moderate	1	1	2	Probable
Shoulder pain	10040617	Mild	7	9	1	Possible
Sore throat	10041367	Mild	13	19	1	Possible
Sore throat	10041367	Mild	6	9	1	Possible
Sputum	10041802	Mild	13	19	1	Possible
Vomiting	10047700	Moderate	1	1	1	Probable

Table 3-3 (A). VAC048 - Unsolicited adverse events (AEs) deemed definitely, probably and possibly related to vaccination reported in the 28 days following vaccination with ChAd63-MVA ME-TRAP adjuvanted with Matrix-M. *The maximum severity for each AE is shown. AEs have been classified according to the Medical Dictionary for Regulatory Activities (MedDRA) at the Lower Level (LLT) Term level.*

Symptom	MedDRA code	Intensity	Day of onset	Last day	Vaccination no.	Causality
ChAd63-MVA ME-TRAP						
Abdominal cramps	10000057	Mild	0	1	2	Possible
Back pain	10003988	Moderate	1	2	2	Possible
Back pain	10003988	Mild	0	1	1	Probable
Bruise	10006502	Mild	0	9	1	Definite
Burning sensation	10006784	Severe	0	0	2	Probable
Dizziness	10013573	Severe	15	17	1	Possible
Hyperaesthesia	10020568	Mild	0	1	1	Possible

Table 3-3 (B). Unsolicited AEs deemed definitely, probably and possibly related to vaccination reported in the 28 days following vaccination with ChAd63-MVA ME-TRAP. *The maximum severity for each AE is shown. AEs have been classified according to the Medical Dictionary for Regulatory Activities (MedDRA) at the Lower Level (LLT) Term level.*

3.4.3 Immunogenicity

3.4.3.1 Cell-mediated immunity

ME-TRAP-specific ELISpot responses were induced by ChAd63-ME-TRAP vaccination and peaked at day 14 in the no MM and 25μg MM group – median (IQR) spot forming cells per million PBMC (SFC/10^6 PBMC) 1013 (406 – 1402.5) and 205 (176.5-316.5), respectively. The responses peaked at day 28 in the 50μg MM group – median (IQR) SFC/10^6 PBMC 472 (169-1066). The cellular responses significantly increased after MVA boost in all groups ($p < 0.0001$), peaking 1 week after boost [day 63 median (IQR) SFC/10^6 PBMC: no MM 3424 (3163-4326), 25μg MM 2992 (2463 – 3790), 50μg MM 2290 (1185 – 4675)]. There were no significant differences in the peak ELISpot responses post-MVA boost in the adjuvanted groups in comparison to the control group ($p=0.45$). T cell responses were well maintained at 4 months post-vaccination, where they remained at levels significantly above baseline (Figure 3-4; $p<0.0001$) and

comparable to levels at peak post-prime. There were significantly lower durable responses seen at day 168 in the 25μg MM dose group compared to the control (p=0.048) and the 50μg MM (p=0.02) dose group (Figure 3-5).

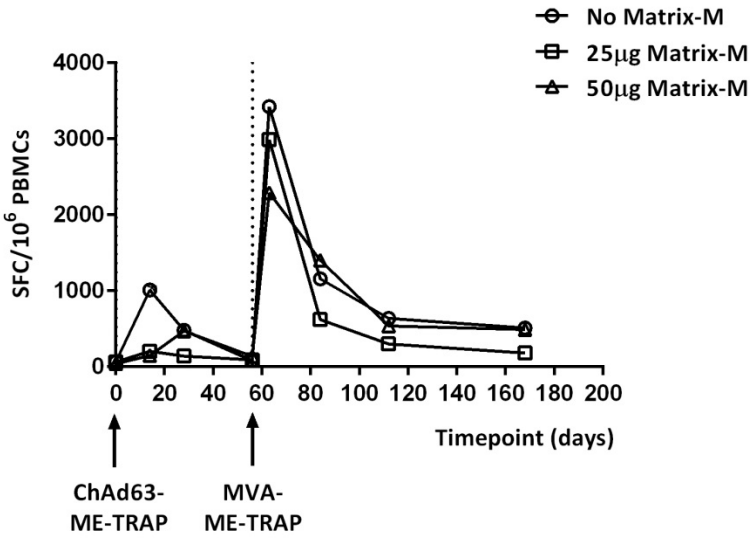


Figure 3-4. VAC048 - *Ex vivo* IFN-γ ELISpot responses to vaccination. *(A) ME-TRAP IFN-γ ELISPOT time courses. Lines show medians. G1: no Matrix-M, n=6, G2: 25μg Matrix-M, n=8, G3: 50μg Matrix-M, n=8.*

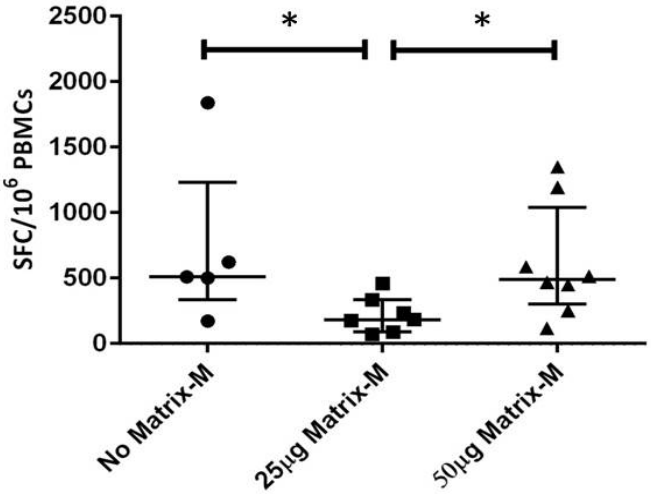


Figure 3-5. VAC048 - Durability of ME-TRAP-specific T cell responses, measured by IFN-γ ELISpot at D168. *Medians with interquartile range are shown. G1: no Matrix-M, n=6, G2: 25μg Matrix-M, n=8, G3: 50μg Matrix-M, n=8. *p<0.05*

3.4.3.2 Flow cytometry for T-cell phenotypes and their cytokine profile

The proportion of total $CD4^+$ and $CD8^+$ T cells producing cytokine (IFN-γ, IL-2, TNF-α), or expressing CD107a (a marker of potential cytotoxic activity) was not significantly different between groups at day 28 or day 63. However, only the group that received 50μg of Matrix-M had a significant increase in cytokine production from day 28 to day 63 ($CD4^+$ - Figure 3-6A; p=0.02; $CD8^+$ - Figure 3-6B; p=0.03). The percentage of $CD4^+$ T cells producing IFN-γ and/or IL-2 increased from post-prime to post-boost in both the unadjuvanted and 50μg dose MM groups but remained low in the 25μg dose group (Figure 3-6C). The percentage of $CD8^+$ T cells producing IFN-γ increased from post-prime to post-boost in both the adjuvanted MM groups but not in the unadjuvanted group (Figure 3-6D).

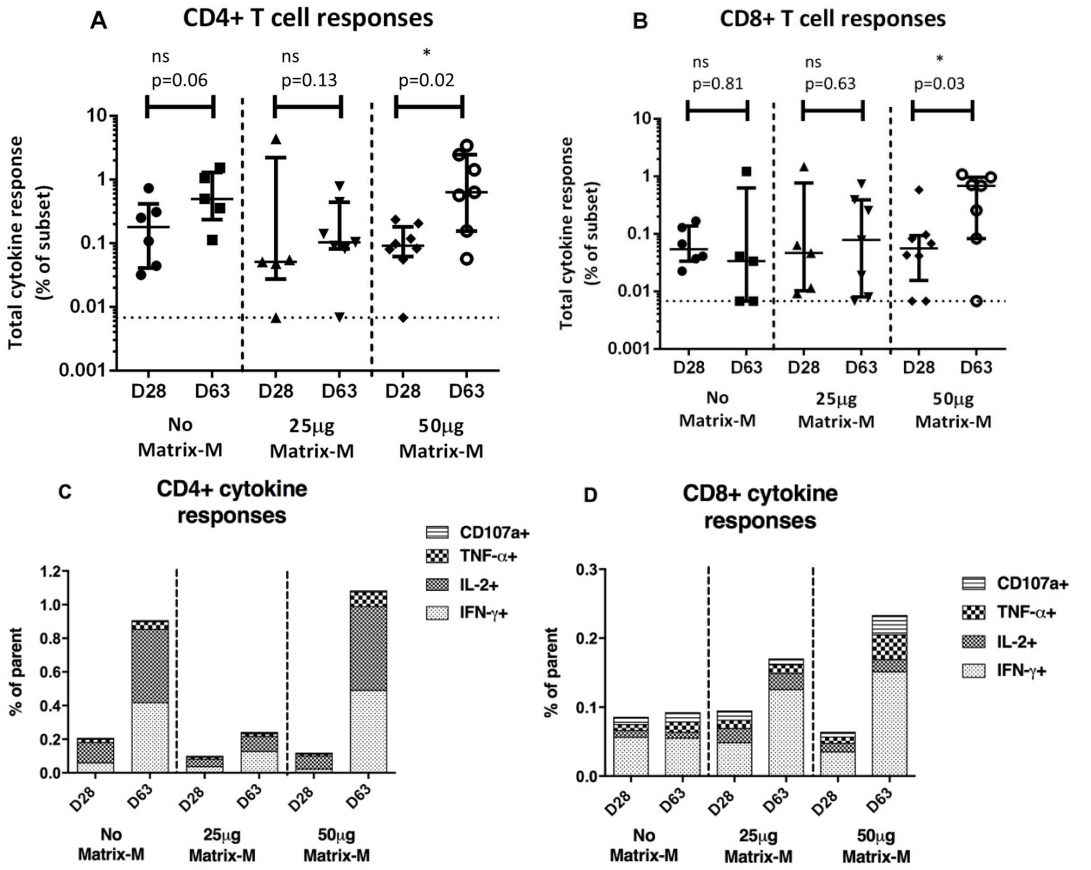


Figure 3-6. VAC048 - Total cytokine responses in each group at day 28 (D28) and peak post-boost, day 63 (D63). *Percentage of (A) CD4⁺ or (B) CD8⁺ T cells producing IFN-γ, IL-2, TNF-α or expressing CD107a. Medians with interquartile ranges are shown. Individual points are PBMC from individual vaccinees. Dotted lines indicate the lower limits of detection.* ***Distribution of cytokine responses at*** **day 28 (D28) and peak post-boost, day 63 (D63)** ***in each group.*** *Percentage of (C) CD4⁺ or (D) CD8⁺ T cells producing each cytokine within each group; ns = non-significant, * P<0.05.*

3.4.3.3 Humoral immunity

Antibody responses to the TRAP antigen were induced by ChAd63-ME-TRAP vaccination in all volunteers. The peak response was seen at day 14 in the control group [median (IQR): 102.5 (55-112.5) ELISA units] and at day 28 in the MM-adjuvanted groups [median (IQR): 25µg MM group – 91.5 (50.8-120.3) ELISA units, 50µg MM group - 128 (55-192.8) ELISA units]. MVA ME-TRAP boosted titres by over 10-fold and the peak response was seen at day 63 in the control group [median (IQR): 1332.5 (889.5-2046.3) ELISA units] and at day 84 in the MM-adjuvanted groups

[median (IQR): 25μg MM group – 643.5 (512-1068.8) ELISA units, 50μg MM group – 1323.5 (811.5-1764.3) ELISA units]. Titres were maintained at levels significantly above baseline at 4 months after boost (Figure 3-7; p<0.0001). There were no significant differences in the peak post-prime (p=0.49) or post-boost (p=0.14) anti-TRAP IgG titres between the three groups.

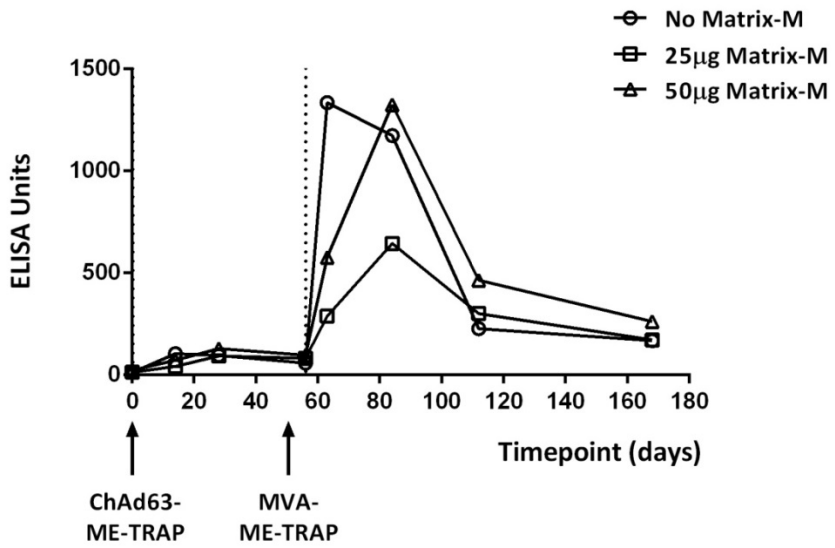


Figure 3-7. VAC048 - Antibody responses to vaccination. *Anti-TRAP IgG time courses. Lines show medians. G1: no Matrix-M, n=6, G2: 25μg Matrix-M, n=8, G3: 50μg Matrix-M, n=8.*

3.5 Discussion

I report here on the safety and immunogenicity of the first-in-human administration of the heterologous prime-boost regimen with ChAd63-MVA ME-TRAP administered with Matrix-M. I have shown that this vaccine approach was safe and well tolerated with AEs being predominantly mild in nature. In keeping with previous studies, the MVA ME-TRAP vaccine was more reactogenic in comparison to the ChAd63 ME-TRAP priming vaccination [129, 136, 159, 173, 229, 240]. The addition of Matrix-M to the ChAd63 ME-TRAP vaccine did not result in increased local reactogenicity. However,

moderate to severe systemic adverse events were reported more frequently by volunteers in the Matrix-M adjuvanted groups in comparison to the control group (0.8 AEs/volunteer vs 1.6 AEs/volunteer).

Similarly, the addition of Matrix-M to the MVA ME-TRAP vaccine did not increase the local reactogenicity. Though volunteers in the Matrix-M adjuvanted groups experienced increased systemic adverse events such as nausea, myalgia and arthralgia in comparison to the control group (0.5 AEs/volunteer vs 1.25 AEs/volunteer), there was no overall increase in moderate or severe AEs in the groups receiving Matrix-M in comparison to the control group.

Matrix-M has been tested as an adjuvant to vaccines for other diseases, including influenza. It has been shown that addition of Matrix-M does result in increased local and systemic reactogenicity compared to controls who receive unadjuvanted vaccine [196]. I did not observe a similar increase in the local reactogenicity. Though there was a trend towards increased moderate and severe systemic AEs with the adjuvanted ChAd63 ME-TRAP; overall, the safety profile of the adjuvanted vaccines was very reassuring with the majority of adverse events being mild in nature.

The need to induce cell-mediated immunity in addition to humoral immunity is widely recognized to be an important feature of vaccines being developed against a number of diseases, including malaria. The ability of heterologous prime-boost immunisation regimes to elicit high levels of T cell responses is well documented [128, 129, 229]. In keeping with this, we showed that priming with ChAd63 ME-TRAP followed by a boost vaccination with MVA ME-TRAP given 8 weeks later resulted in potent T cell responses (> 2500 SFC/10^6 PBMC). As reported previously, median T-cell

ELISpot responses peaked at 7-days post boost vaccination with MVA ME-TRAP in all three groups [128, 129, 229]. The addition of MM did not alter T-cell responses and durable responses that were well maintained at 6 months were observed. The dose of Matrix-M may have some effect on the durability of responses, with T-cell responses comparatively lower at day 168 in the group receiving 25μg of Matrix-M; the reasons for this are unclear.

The proportion of total $CD4^+$ and $CD8^+$ T cells producing cytokines (IFN-γ, IL-2, TNF-α), or expressing CD107a was not significantly different between groups at day 28 or day 63. The higher dose of the adjuvant was required to induce a significant increase in cytokine production by $CD4^+$ and $CD8^+$ T cells from day 28 to day 63. Interestingly, the addition of the adjuvant resulted in an increased percentage of $CD8^+$ T cells producing IFN-γ from post-prime to post-boost. In the first malaria sporozoite challenge study testing ChAd63-MVA ME-TRAP, it was shown that the frequency of monofunctional IFN-γ producing $CD8^+$ T cells, but not antibodies, correlated with efficacy. RTS,S/AS01 the leading malaria vaccine candidate induces potent antibody responses to circumsporozoite protein, but low level $CD4^+$ and no $CD8^+$ T-cell responses [241]. In this study, there were no differences in the peak post-prime or post-boost anti-TRAP IgG titres between the three groups.

In conclusion, I showed that heterologous prime-boost immunization with viral-vectored vaccines adjuvanted with Matrix-M was safe and well tolerated. There was no evidence of reduction in vectored vaccine-induced immunogenicity when used in combination with an adjuvant. It is highly likely that an efficacious protein or virus-like particle-based malaria vaccine will require an adjuvant and our data suggest that

Matrix-M could be used mixed with viral vectors as part of this combination regime without leading to any deleterious effect on the immunogenicity of the vectored vaccines. I will be describing the assessment of the safety, immunogenicity and efficacy of a novel virus-like particle malaria vaccine, R21 administered alone, with two different adjuvants and in combination with the ChAd63-MVA ME-TRAP vaccine regime in Chapters 4 and 5.

CHAPTER FOUR:

Phase I clinical trials to assess the safety and immunogenicity of a novel protein particle malaria vaccine candidate, R21, administered alone and with Matrix-M™ (VAC053) and AS01$_B$ (VAC056) in healthy UK volunteers

4.1 Authorship statement

I set up the two Phase I clinical trials, VAC053 and VAC056 with the assistance of Alison Lawrie, Rachel Roberts, Ian Poulton and Adrian Hill. This involved preparation of the study documents with submission for ethical and regulatory approval for all sites, and R&D department approval for NHS sites (in Southampton and Imperial College, London).

I screened and enrolled volunteers for the trial in Oxford. Vaccinations and follow-up visits were conducted by me and by the nursing team at the Centre for clinical vaccinology and tropical medicine. Volunteers were also enrolled at the NIHR Wellcome Trust clinical research facility in Imperial College, London and Southampton for the trials VAC053 and VAC056, respectively. Enrolment, vaccination and follow-up of volunteers at the NHS sites were conducted by local study staff. The local PI at the site was David Lewis in Imperial College and Saul Faust in Southampton. Adrian Hill was the chief investigator for both these trials.

Laboratory assays were carried out in Oxford by Georgina Bowyer, Jonathan Powlson and me.

4.2 Introduction

The most advanced malaria vaccine candidate, RTS,S/AS01 has completed Phase III testing in a multicentre study across several African sites and demonstrates low-level (~30%) efficacy against clinical malaria in the target population of African infants aged 6-12 weeks after a three dose schedule [93-96]. This efficacy wanes rapidly over time after the first 12 months [21] and there remain safety concerns for this vaccine schedule. It was associated with an increased incidence of meningitis of varying aetiology in the 5-17 month age group [96, 242]. Furthermore, more recently, it was shown that RTS,S/AS01 was associated with higher all-cause mortality in girls in both age groups in which the vaccine was tested [243]. Therefore, there is a significant need to improve on RTS,S/AS01 to achieve the goals set down by the Malaria Vaccine Technology Roadmap [14]. RTS,S/AS01 predominantly elicits antibodies to the pre-erythrocytic circumsporozoite protein (CSP), which is pivotal in sporozoite development and invasion of hepatocytes, and has long been considered as one of the leading pre-erythrocytic antigens for the development of a malaria vaccine.

R21 has been developed at the Jenner Institute, University of Oxford. This is an improved RTS,S construct without the excess of unfused HBsAg protein found in RTS,S (Figure 4-1), which was required to allow it to form a particle in *Saccharomyces cerevisiae* [226]. The CSP comprises less than 20% of the total protein content of RTS,S [57] and a large proportion of the antibody response induced by RTS,S is towards the HBsAg [244]. In contrast, R21 comprises only fusion protein moieties, i.e. as 100% of its molecules, which increases the density of CSP antigen on the VLP surface. This was made possible by expressing R21 in the better expressing yeast

Pichia pastoris, rather than in *Saccharomyces cerevisiae* and it spontaneously forms a particle just like RTS,S [226]. At the C-terminus of R21, a four amino acid sequence has been added, EPEA, which is required for efficient immunochromatographic purification of R21. This very short sequence is found many times in the proteome of malaria parasites and humans but has not, to our knowledge, been used previously as a vaccine component.

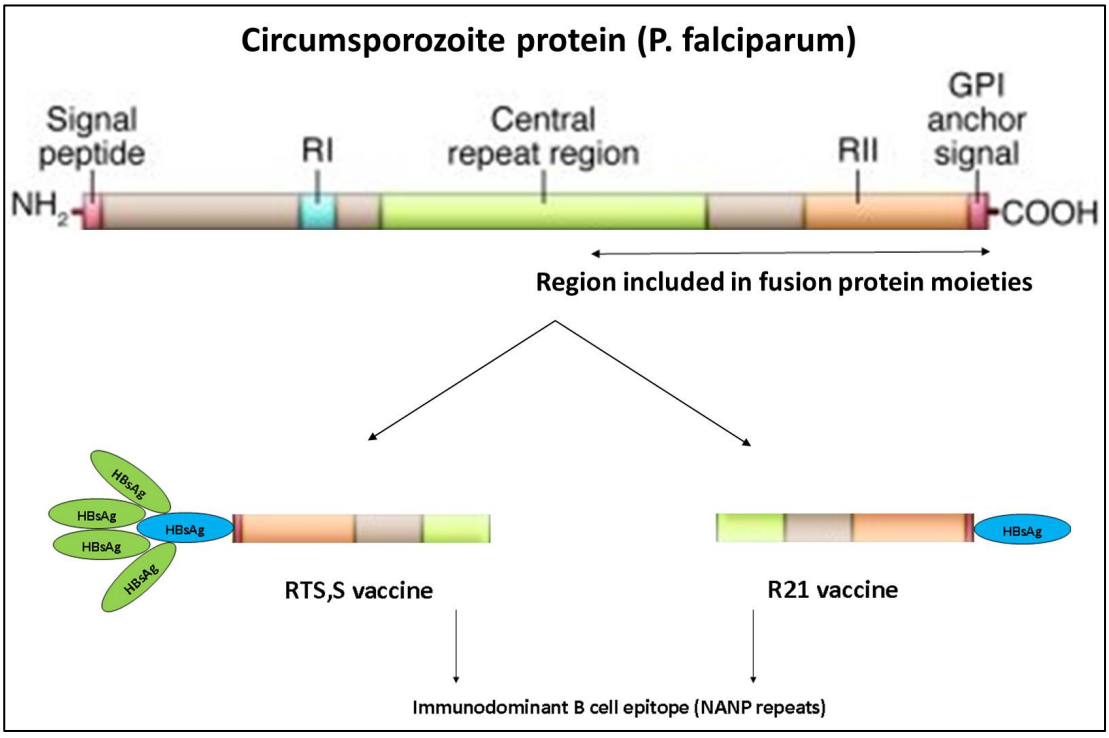


Figure 4-1. Schematic diagram showing RTS,S and R21 fusion proteins *(Adapted from Crompton et al., 2010 [245]). Both RTS,S and R21 include fusion protein moieties of hepatitis B surface antigen fused to a portion of the circumsporozoite protein (CSP). The portion of CSP used contains 19 NANP repeats from the central repeat region, which are known to be a protective B cell epitope, and the C-terminal region which contains some important T cell epitopes. RTS,S requires expression of excess of the unfused HBsAg to allow it to form hybrid particles (resulting ratio, RTS:S=1:4). R21, expressed in a different yeast, does not require the excess HBsAg to spontaneously form a virus-like particle.*

R21 predominantly induces antibodies to malaria rather than hepatitis proteins in pre-clinical studies. It has been shown to be safe, non-toxic and highly immunogenic with comparable immunogenicity and similar high level efficacy to RTS,S. Sporozoite

challenge (1000 sporozoites per mouse injected intravenously) using transgenic *P. berghei* parasites (expressing the *Plasmodium falciparum* CSP homologue) performed in BALB/c mice showed that R21 adjuvanted with Matrix-M (MM) elicited 87.5% sterile protection [226].

Protein or virus-like particle-based vaccines, such as R21 usually require an adjuvant to be immunogenic. Therefore, we initially conducted a Phase I, first-in-human, open-label clinical trial to assess the safety and immunogenicity of R21 administered alone and with the same saponin-based adjuvant used in VAC048, MM in healthy UK volunteers. We tested the doses of 50μg, which is the same adult dose as RTS,S and 10μg, which would have a similar amount of malaria protein exposed on the VLP surface as RTS,S. On the basis of an encouraging safety profile and comparable humoral immunogenicity to RTS,S at the lower dose of 10μg, we extended the study to include a group where we tested an even lower dose of 2μg R21. VAC056 was the second Phase I trial to assess the safety and immunogenicity of R21 at the doses of 10μg and 50μg administered with $AS01_B$, which is the same adjuvant that is used in combination with RTS,S.

4.3 Methods

Detailed methods of the recruitment and enrolment of volunteers, as well as the assays used in this trial can be found in Chapter 2.

4.3.1 Study design

These Phase I studies were observational, open-label, first-in-human clinical trials. Eligible participants were assigned to one of the dose groups as shown in Tables 4-1 & 4-2 for VAC053 and VAC056, respectively. The first three vaccinations in Groups 1-3 in VAC053 and both groups in VAC056 occurred in a staggered manner and interim safety reviews were conducted by the local safety monitor prior to progression to the higher dose group.

VAC053 was conducted at the CCVTM at the University of Oxford and the NIHR Wellcome Trust CRF in Imperial College, London. VAC056 was conducted at the CCVTM at the University of Oxford and the NIHR Wellcome Trust CRF in the University of Southampton. The number of volunteers has been chosen to generate adequate safety and immunogenicity data, whilst minimising the number of volunteers exposed to a new vaccination regimen.

	Day 0	**Day 28**	**Day 56**
Group 1 (n=10)	10μgR21/ 50μg Matrix-M	10μgR21/ 50μg Matrix-M	10μgR21/ 50μg Matrix-M
Group 2 (n=4)	50μg R21	50μg R21	50μg R21
Group 3 (n=10)	50μgR21/ 50μg Matrix-M	50μgR21/ 50μg Matrix-M	50μgR21/ 50μg Matrix-M
ADDITIONAL GROUP			
Group 4 (n=6)	2μgR21/ 50μg Matrix-M	2μgR21/ 50μg Matrix-M	2μgR21/ 50μg Matrix-M

Table 4-1. VAC053 study groups.

	Day 0	**Day 28**	**Day 56**
Group 1 (n=10)	10μgR21/ 50μg AS01	10μgR21/ 50μg AS01	10μgR21/ 50μg AS01
Group 2 (n=10)	50μgR21/ 50μg AS01	50μgR21/ 50μg AS01	50μgR21/ 50μg AS01

Table 4-2. VAC056 study groups.

4.3.2 VAC053 ethics and regulatory approval

The study protocol and associated documents were reviewed and approved by the UK National Research Ethics Service, Committee South Central–Berkshire B (Ref: 15/SC/0386), the Medicines and Healthcare Products Regulatory Agency (Ref: 21584/0352/001-0001), and the Oxford University Clinical Trials and Research Governance team, who independently and externally monitored compliance with Good Clinical Practice guidelines. The trial was registered with ClinicalTrials.gov (Ref: NCT02572388) and an independent local safety monitor provided safety oversight.

4.3.3 VAC056 ethics and regulatory approval

The study protocol and associated documents were reviewed and approved by the UK National Research Ethics Service, Committee South Central–Berkshire B (Ref: 15/LO/1353), the Medicines and Healthcare Products Regulatory Agency (Ref: 21584/0355/001-0001), and the Oxford University Clinical Trials and Research Governance team, who independently and externally monitored compliance with Good Clinical Practice guidelines. The trial was registered with ClinicalTrials.gov (Ref: NCT02600975) and an independent local safety monitor provided safety oversight.

4.3.4 Objectives and Endpoints

Primary Objective

VAC053 - To assess the safety and tolerability of R21 with and without adjuvant Matrix-M in healthy volunteers.

VAC056 - To assess the safety and tolerability of R21 with adjuvant $AS01_B$ in healthy volunteers.

Primary Outcome Measures

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

The following parameters were assessed for all study groups

- Occurrence of solicited local reactogenicity signs and symptoms for 7 days following the vaccination
- Occurrence of solicited systemic reactogenicity signs and symptoms for 7 days following the vaccination
- Occurrence of unsolicited adverse events for 28 days following the vaccination
- Change from baseline for safety laboratory measures
- Occurrence of serious adverse events during the whole study duration

Secondary Objective

To assess the humoral and cellular immunogenicity of R21 in humans with and without adjuvant Matrix-M in healthy volunteers.

To assess the humoral immunogenicity of R21 in humans with adjuvant $AS01_B$ in healthy volunteers.

Secondary Outcome Measures

Measures of immunogenicity included:

- ELISA to quantify antibodies to NANP
- Sodium thiocyanate displacement ELISA to measure avidity of antibodies
- ELISpot to enumerate IFN-γ producing T-cells to the CSP
- Antibodies to HBsAg

4.3.5 Study participants

Participants were healthy adults between the ages of 18 and 50 years all of whom provided written informed consent. Patient demographics and baseline characteristics are presented in Tables 4-3 and 4-4 for VAC053 and VAC056, respectively.

Volunteer number	Age	Sex	BMI	Country of birth	Smoker
1002	24	Male	19.7	South Africa	No
1003	28	Male	25.9	United Kingdom	No
1004	29	Female	21.2	United Kingdom	No
1005	28	Female	22.7	United Kingdom	No
1006	21	Male	21.1	United Kingdom	No
1009	26	Male	26.0	Spain	Yes
1012	22	Female	25.8	Malaysia	No
1014	32	Male	23.2	United Kingdom	Yes
1015	28	Female	45.6	United Kingdom	No
1017	20	Male	23.2	United Kingdom	No
1020	41	Male	26.3	United Kingdom	No
1021	37	Male	21.7	Belgium	No
1025	25	Female	22.4	Spain	No
1026	29	Male	26.4	United Kingdom	No
1031	28	Female	20.2	United Kingdom	No
1039	37	Male	23.4	United Kingdom	No
1040	47	Female	28.0	United Kingdom	No
1041	49	Female	24.8	United Kingdom	Yes
1043	36	Male	31.0	Pakistan	No
1048	25	Male	26.0	United Kingdom	No
1050	26	Male	26.7	Namibia	No
3303	26	Female	21.3	United Kingdom	Yes
3307	28	Female	22.7	Greece	No
3308	45	Male	28.8	Brazil	No
3309	19	Male	32.4	China	No
3310	26	Female	27.0	United Kingdom	No
3314	21	Female	27.3	India	No
3316	23	Female	21.0	Malaysia	No
3317	19	Female	20.1	United Kingdom	Yes
3319	21	Female	32.5	United Kingdom	No
3321	39	Female	20.4	South Africa	No

Table 4-3. Demographics and baseline characteristics of volunteers enrolled in the VAC053 trial. *BMI - body mass index*

Volunteer number	Age	Sex	BMI	Country of birth	Smoker
1001	23	Female	25.2	Canada	No
1002	22	Male	23.8	Ireland	No
1005	24	Female	25.6	Sweden	No
1006	22	Male	26.0	United Kingdom	No
1007	25	Female	25.0	Zimbabwe	No
1008	33	Male	25.9	United Kingdom	No
1009	27	Male	22.3	Japan	No
1011	44	Female	27.5	United Kingdom	No
1013	22	Male	28.4	United Kingdom	No
1014	27	Male	23.5	United Kingdom	No
1015	23	Female	22.0	United Kingdom	No
2201	24	Male	26.1	United Kingdom	No
2202	27	Male	19.9	United Kingdom	Yes
2203	35	Male	29.1	United Kingdom	No
2206	22	Female	21.6	United Kingdom	No
2208	20	Female	22.2	United Kingdom	No
2209	24	Female	24.0	United Kingdom	Yes
2210	28	Female	21.7	United Kingdom	No
2212	26	Female	36.1	United Kingdom	No
2217	39	Female	18.5	United Kingdom	No

Table 4-4. Demographics and baseline characteristics of volunteers enrolled in the VAC056 trial. *BMI - body mass index*

4.3.5.1 Inclusion Criteria

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

- Healthy adults aged 18 to 50 years
- Able and willing (in the Investigator's opinion) to comply with all study requirements
- Willing to allow the investigators to discuss the volunteer's medical history with their General Practitioner

- For females only, willingness to practice continuous effective contraception during the study and a negative pregnancy test on the day of screening and vaccination
- Agreement to refrain from blood donation during the course of the study
- Provide written informed consent

4.3.5.2 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
- Administration of immunoglobulins and/or any blood products within the three months preceding the planned administration of the vaccine candidate
- Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days) immunosuppressant medication within the past 6 months (inhaled and topical steroids are allowed)
- 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)
- History of clinical malaria (any species)
- 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

4.3.6 Study Interventions

Vaccine manufacture, preparation and administration

R21 (Batch no: 01015-01) was formulated and vialed under Good Manufacturing Practice conditions at the CBF, University of Oxford. R21 was formulated in formulation buffer at a target concentration to allow the extraction of 25μg from each vial. The drug product was filled into 2mL glass vials with a 13 mm grey bromobutyl rubber freeze-dry stopper (CE Marked, supplied by Adelphi Tubes) and a 13 mm complete tear, clear lacquered aluminum seal. R21 was stored in a locked -80^{O}C freezer.

Manufacture and formulation of MM (Batch No: 14-170) has been described in Section 3.3.5. The AS01$_B$ (Batch No: DA01A058A) adjuvant system has been developed and manufactured by GSK Biologicals and is formulated as a 0.5ml liquid solution containing 50μg of MPL and 50μg of Stimulon QS21. The drug product is filled into sterile monodose glass vials. Vials of MM and AS01$_B$ were stored in a locked refrigerator at 2-8^{O}C, and not frozen at any time.

At the CBF the R21 and the adjuvants were certified and labelled for both these trials by a Qualified Person (QP) before transfer to the clinical site. On vaccination day, R21 was thawed to room temperature and administered intramuscularly within 1 hour of

removal from the freezer, either alone, or mixed with an adjuvant at the bedside, immediately prior to administration.

4.3.7 Assessment of safety

Volunteers were observed for 60 minutes following vaccine administration in case of immediate adverse events. Follow-up visits were scheduled for days 1, 7, 14, 29, 35, 42, 57, 63, 70, 84 and 238, with an additional visit at day 3 post-vaccination for the first three volunteers in Groups 1-3 in VAC053 and both groups in VAC056. All participants recorded their temperature and any solicited local and systemic adverse events for 7 days post-vaccination and unsolicited adverse events for 28 days post-vaccination using an electronic diary. A review of solicited and unsolicited adverse events occurred at each follow-up visit. Safety bloods including full blood count, renal function and liver function tests were done on visits at day 0, 7, 28, 35, 56, 63 and 84. Group holding and individual stopping safety rules (See Section 2.3.4) were used in these studies to ensure volunteer safety as they were both first-in-human, dose-escalation studies.

4.3.8 Assessment of immunogenicity

Antibody responses measured by anti-NANP IgG ELISA were performed on samples from days 0, 7, 14, 28, 42, 56, 70, 84 and 238. Functional assessment assessing the avidity of the antibody responses was undertaken using a sodium thiocyanate

displacement assay. Cellular immunogenicity was assessed using IFN-γ ELISpot assay on samples from days 0, 42 and 84. The full methods used for these immunological assays are included in Section 2.4. Humoral responses to the HBsAg were measured at the Oxford University Hospitals' NHS Trust laboratories.

4.4 Results

4.4.1 VAC053 participant flow

From 15th October, 2015 to 3rd January, 2017, a total of 31 volunteers of the 75 who were screened for eligibility were enrolled in the study. One volunteer in Group 1 withdrew after their first vaccination due to a change in personal circumstances and was replaced. One Group 2 volunteer withdrew after their third vaccination as they were no longer able to commit to the schedule of attendances. Two volunteers in Group 3 withdrew after their first vaccination – one was due to personal logistical difficulties and the second was due to difficulties in venipuncture. These two volunteers were not replaced. None of the withdrawals were related to the vaccination and there were no ongoing AEs and safety bloods were normal. All of the remaining 21 volunteers in Groups 1-3 completed follow-up until day 238. The additional 6 volunteers in Group 4 have all completed follow-up until day 84 and 4 of these volunteers have completed follow-up until day 238. The trial flow diagram is shown in Figure 4-2.

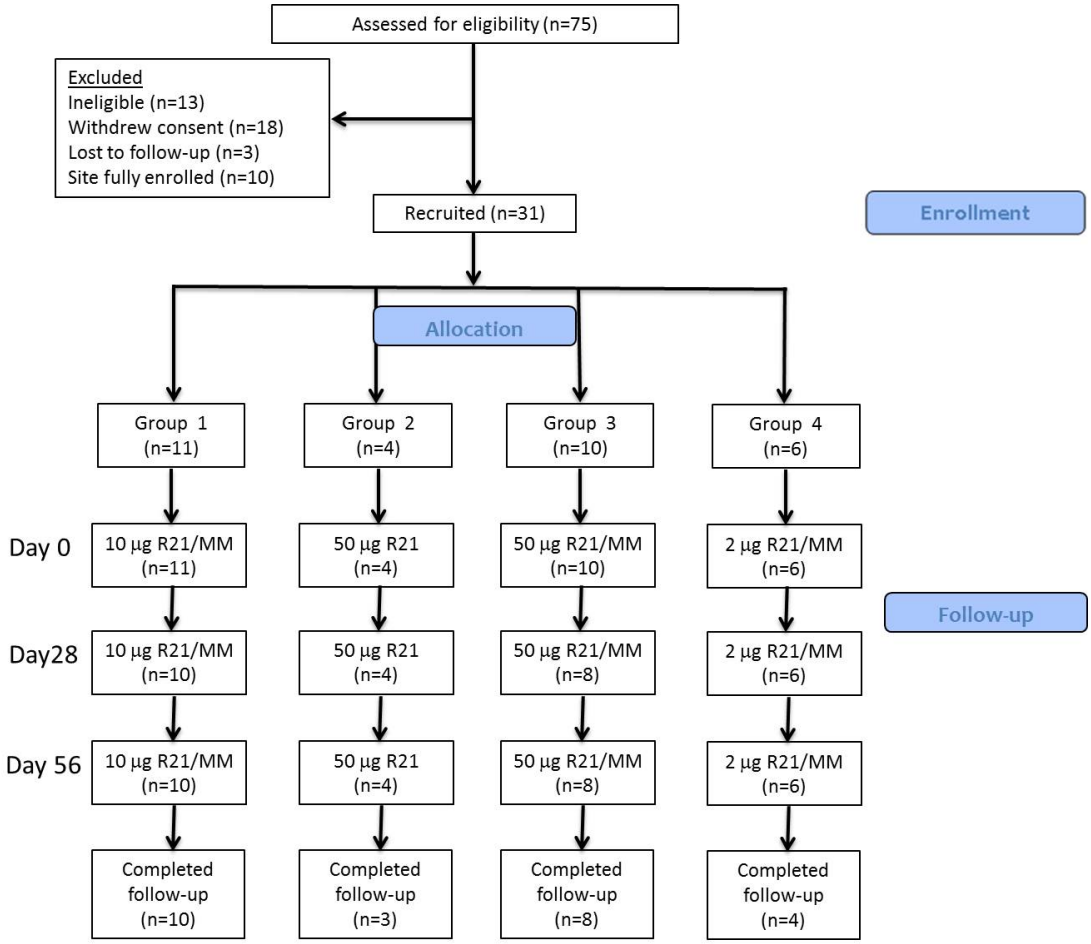


Figure 4-2. VAC053 flow chart of study design and volunteer recruitment. *Abbreviations: MM, Matrix-M. A total of 75 volunteers were screened and 31 were enrolled in total. One Group 1 volunteer withdrew consent after their first vaccination and was subsequently replaced. One Group 2 volunteer withdrew after their third vaccination and two Group 3 volunteers withdrew after their first vaccination and were not replaced. A total of 25 volunteers have completed the study.*

4.4.2 VAC053 Vaccine safety and reactogenicity

No serious adverse reactions (SARs) or suspected unexpected serious adverse reactions (SUSARs) occurred. The majority of adverse events (AEs) reported were self-limiting and mild in severity. Solicited local and systemic adverse events in the first 7 days after each vaccination related to R21 vaccination either administered alone or mixed with MM are shown in Figures 4-3 to 4-5. The addition of MM increased reactogenicity at the 50µg dose, particularly the number of moderate and

severe adverse events reported. However, there was minimal reactogenicity when R21 was administered at the very low dose of 2μg and there was a significant trend for more reactogenicity in the higher dose groups than in the 2μg group ($p<0.0001$). Vaccine site pain was the most common local adverse event and was predominantly mild in severity. One volunteer in Group 1 reported a mild fever (37.7^{O}C) and another volunteer in Group 1 reported a moderate fever (38.1^{O}C). Both these episodes occurred after the 2nd vaccination and resolved within 24 hours. One volunteer in Group 2 reported a fever of 39^{O}C associated with a constellation of flu-like symptoms, 8 days after their first vaccination, which resolved within 24 hours. Short-lived severe adverse events were only reported by three volunteers who were all in the 50μg R21/MM group.

Unsolicited adverse events collected for 28 days after each vaccination deemed definitely, possibly or probably related to vaccination were predominantly mild in nature and are coded according to the MedDRA classification and tabulated in Table 4-5. Laboratory adverse events were all Grade 1 according to the FDA toxicity grading scale apart from one volunteer who developed Grade 2 anaemia 28 days post-vaccination 3 of R21 administered with Matrix M, which was thought unlikely to be related to vaccination. All laboratory AEs are tabulated in Table 4-6.

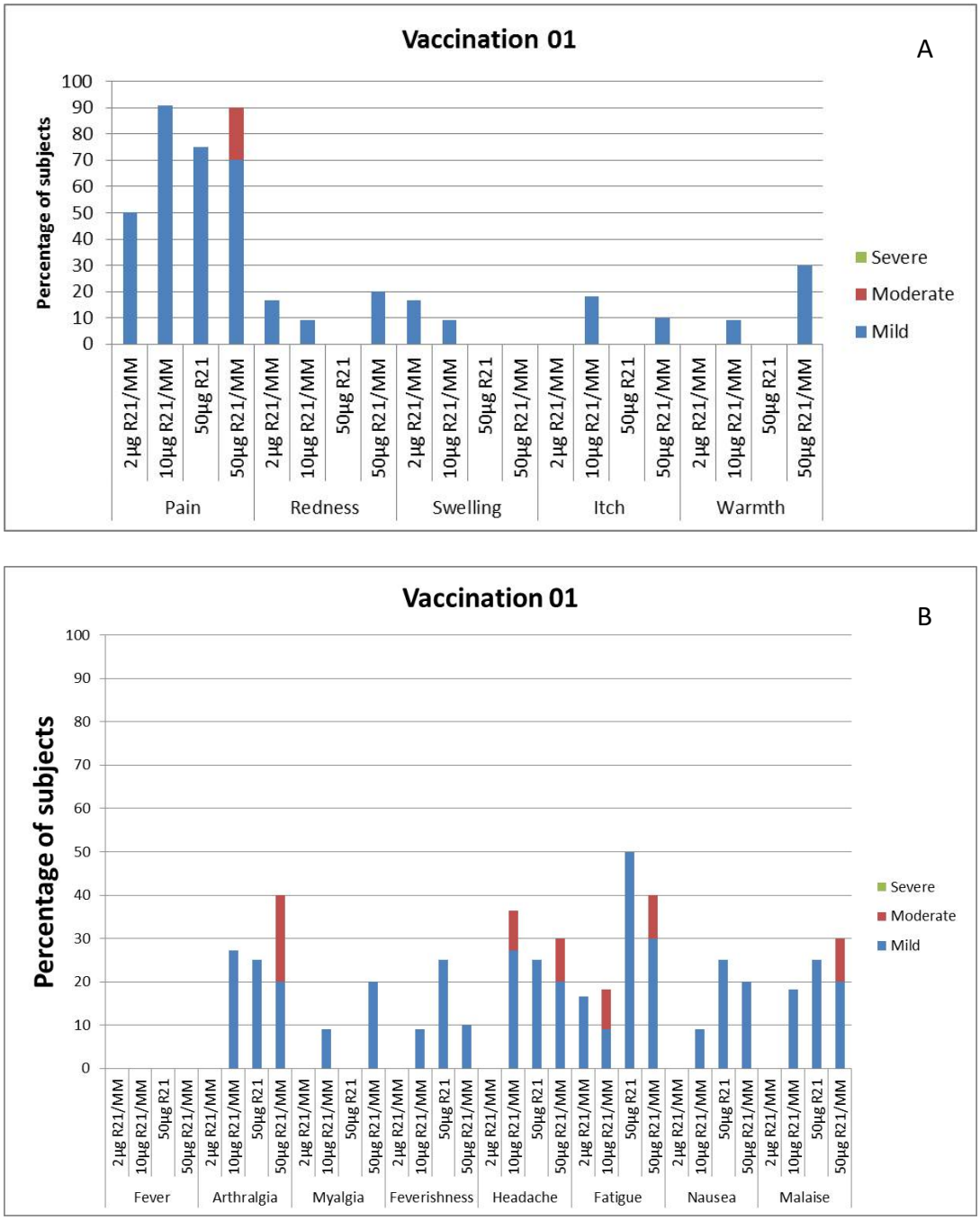


Figure 4-3. VAC053 - Local (A) and systemic (B) solicited adverse events (AE) reported in the electronic diary card during the first seven days related to the first vaccination. *R21 was either administered alone (n=4) or mixed with Matrix-M at different doses of 2µg (n=6), 10µg (n=11) and 50µg (n=10). Only the highest intensity of each AE per subject is listed. Data are combined for all AEs for all volunteers receiving the same vaccine at the stated time point.*

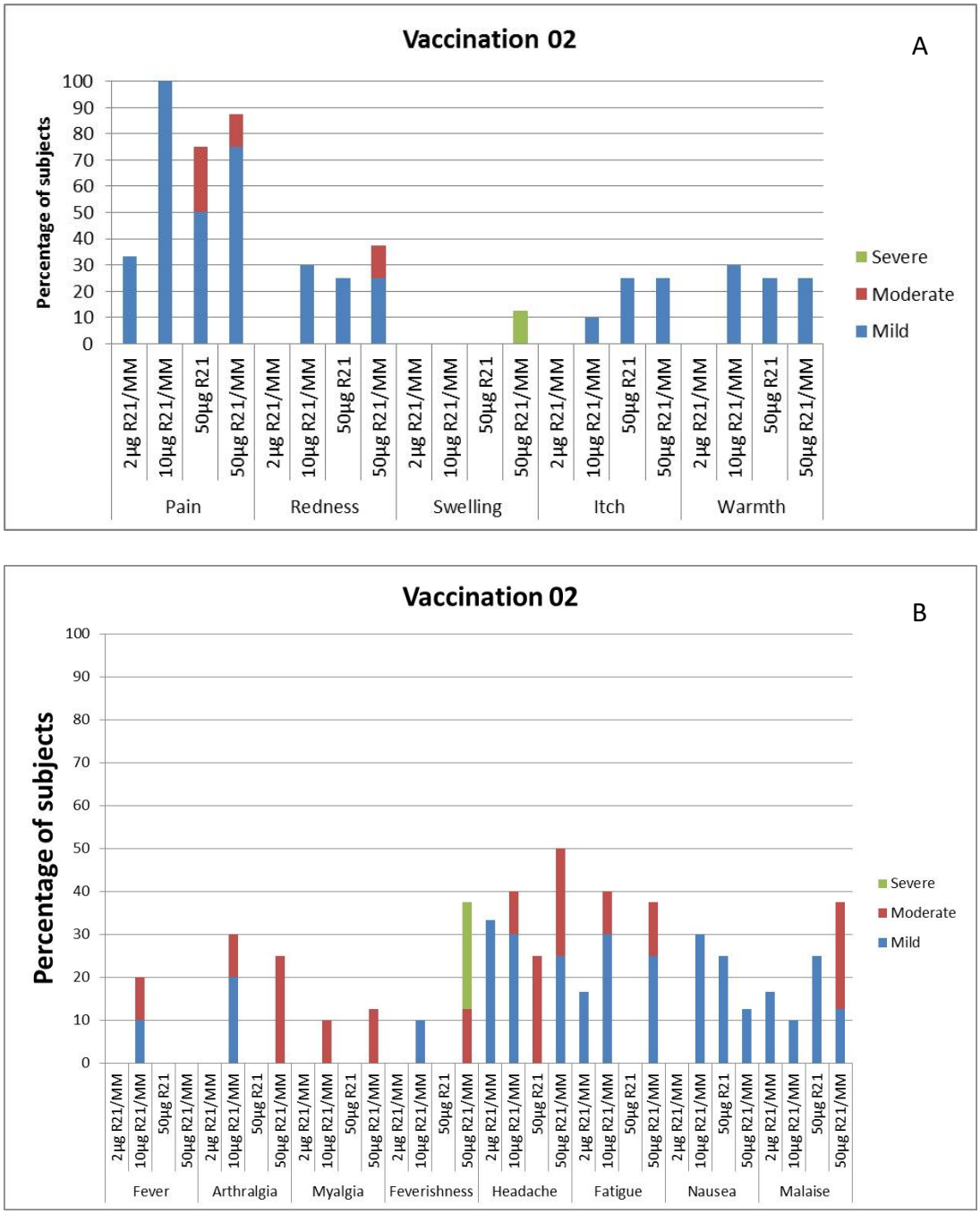


Figure 4-4. VAC053 - Local (A) and systemic (B) solicited adverse events (AE) reported in the electronic diary card during the first seven days related to the second vaccination (4 weeks). *R21 was either administered alone (n=4) or mixed with Matrix-M at different doses of 2µg (n=6), 10µg (n=10) and 50µg (n=8). Only the highest intensity of each AE per subject is listed. Data are combined for all AEs for all volunteers receiving the same vaccine at the stated time point.*

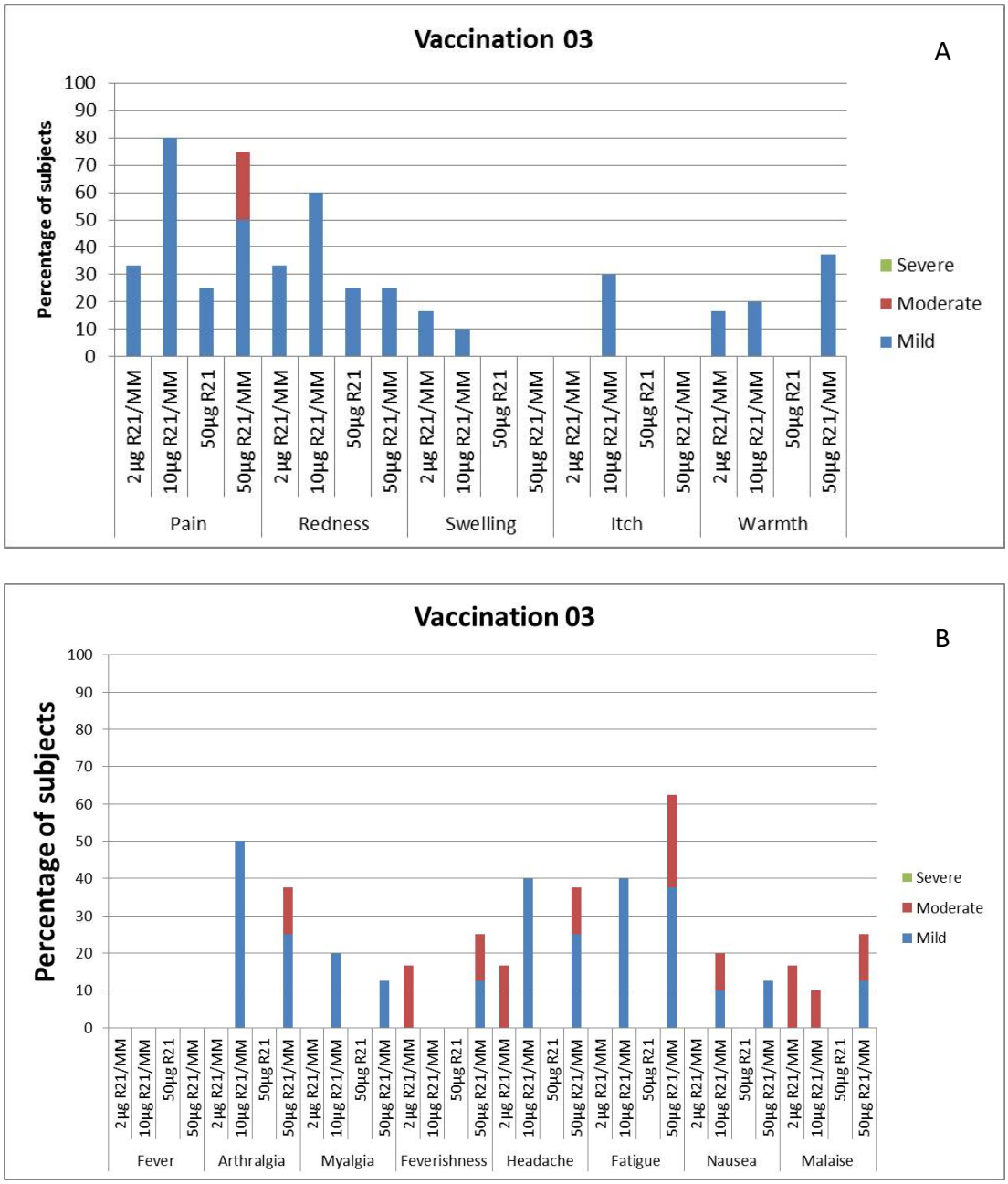


Figure 4-5. VAC053 - Local (A) and systemic (B) solicited adverse events (AE) reported in the electronic diary card during the first seven days related to the third vaccination (8 weeks). *R21 was either administered alone (n=4) or mixed with Matrix-M at different doses of 2µg (n=6), 10µg (n=10) and 50µg (n=8). Only the highest intensity of each AE per subject is listed. Data are combined for all AEs for all volunteers receiving the same vaccine at the stated time point.*

Symptom	MedDRA code	Intensity	Day of onset	Last day	Vaccination no.	Causality
2μgR21/50μg Matrix-M						
Axillary pain	10048750	Mild	13	14	3	Possible
Headache	10019211	Mild	7	7	3	Possible
Headache	10019211	Mild	7	7	2	Possible
Lump feeling in throat	10051196	Mild	0	4	1	Possible
10μgR21/50μg Matrix-M						
Cough	10011224	Severe	7	9	3	Possible
Intestinal cramps	10049900	Moderate	6	7	3	Possible
Itchy	10023089	Mild	11	11	1	Possible
Itchy	10023089	Mild	2	2	2	Possible
Light headedness	10024492	Mild	1	1	1	Possible
Muscle spasm	10028333	Mild	0	0	3	Probable
Pain in jaw	10033433	Mild	2	4	1	Possible
Shivers	10040559	Mild	6	6	3	Possible
Shoulder pain	10040617	Mild	0	0	1	Definite
Sore throat	10041367	Mild	0	0	1	Possible
Sore throat	10041367	Severe	7	9	3	Possible
Sore throat	10041367	Mild	0	3	3	Possible
Stomach cramps	10049901	Mild	6	6	2	Possible
50μgR21/50μg Matrix-M						
Chills	10008531	Severe	1	1	2	Definite
Injection site pain	10022086	Mild	18	20	3	Probable
Itchy	10023089	Mild	1	4	1	Possible
Localised rash	10062705	Mild	2	4	1	Probable
Lump feeling in throat	10051196	Mild	6	6	1	Possible
Rash	10037844	Moderate	1	3	2	Definite
Shivers	10040559	Mild	1	1	2	Definite
Stomach cramps	10049901	Mild	0	0	1	Possible

Table 4-5 (A). VAC053 - Unsolicited AEs deemed definitely, probably and possibly related to vaccination reported in the 28 days following vaccination with R21 adjuvanted with Matrix-M. *The maximum severity for each AE is shown. AEs have been classified according to the Medical Dictionary for Regulatory Activities (MedDRA) at the Lower Level (LLT) Term level.*

Symptom	MedDRA code	Intensity	Day of onset	Last day	Vaccination no.	Causality
50μg R21 alone						
Stomach ache	10042076	Moderate	0	0	3	Possible
Itchy throat	10053560	Mild	1	1	3	Possible
Sore throat	10041367	Mild	5	8	2	Possible

Table 4-5 (B). VAC053 - Unsolicited AEs deemed definitely, probably and possibly related to vaccination reported in the 28 days following vaccination with R21 alone. *The maximum severity for each AE is shown. AEs have been classified according to the Medical Dictionary for Regulatory Activities (MedDRA) at the Lower Level (LLT) Term level.*

Lab AE and intensity	Number (% of volunteers)		
2μg R21/MM	**Vaccination 01 (n=6)**	**Vaccination 02 (n=6)**	**Vaccination 03 (n=6)**
Anaemia Grade 1	1 (16.7)	0	0
Lymphopaenia Grade 1	0	2 (33.3)	1 (16.7)
10μg R21/MM	**Vaccination 01 (n=11)**	**Vaccination 02 (n=10)**	**Vaccination 03 (n=10)**
Anaemia Grade 1	0	2 (20)	0
Neutropaenia Grade 1	0	1 (10)	2 (20)
Hypokalaemia Grade 1	0	1 (10)	2 (20)
50μg R21 alone	**Vaccination 01 (n=4)**	**Vaccination 02 (n=4)**	**Vaccination 03 (n=4)**
Elevated ALT Grade 1	1 (25)	0	0
50μg R21/MM	**Vaccination 01 (n=10)**	**Vaccination 02 (n=8)**	**Vaccination 03 (n=8)**
Anaemia Grade 1 Grade 2	0 0	2 (25) 0	0 1 (12.5)
Low WBC Grade 1	1 (10)	0	1 (12.5)
Lymphopaenia Grade 1	1 (10)	0	0
Elevated Urea Grade 1	0	0	1 (12.5)

Table 4-6. VAC053 - Frequency of all laboratory adverse events following vaccination with R21 alone or adjuvanted with Matrix-M. *Adverse events are defined as a deviation from the baseline result collected pre-vaccination on Day 0.*

4.4.3 VAC053 humoral immunogenicity

The primary immunological response of interest in this study is the antibody response to the NANP repeat region of the CSP. Potent humoral responses were seen in both the groups that received R21 adjuvanted with MM, which peaked at day 70 [median (IQR): 1399.5 (831.3-2031.8) ELISA units] and day 42 [median (IQR): 1260 (494.8-2687.8) ELISA units] in Group 1 and 3, respectively (Figure 4-6). As expected, there was no significant antibody response elicited in the R21 alone group (Figure 4-6). Antibody titres were well maintained in the adjuvanted groups and remained significantly above baseline at 6 months after the third vaccination and durability was significantly improved in the 10μg dose group (Figure 4-7; p=0.02). There was no difference in the avidity of the induced antibodies between the adjuvanted groups as assessed by a sodium thiocyanate displacement assay at day 84 and 238 (Figure 4-8). The lowest dose of 2μg of R21 adjuvanted with MM also elicited an antibody response to NANP; although there was a significant difference in the humoral response at 28 days after the first and second vaccination, the antibody response at day 84 was comparable across all the three adjuvanted groups (Figure 4-9).

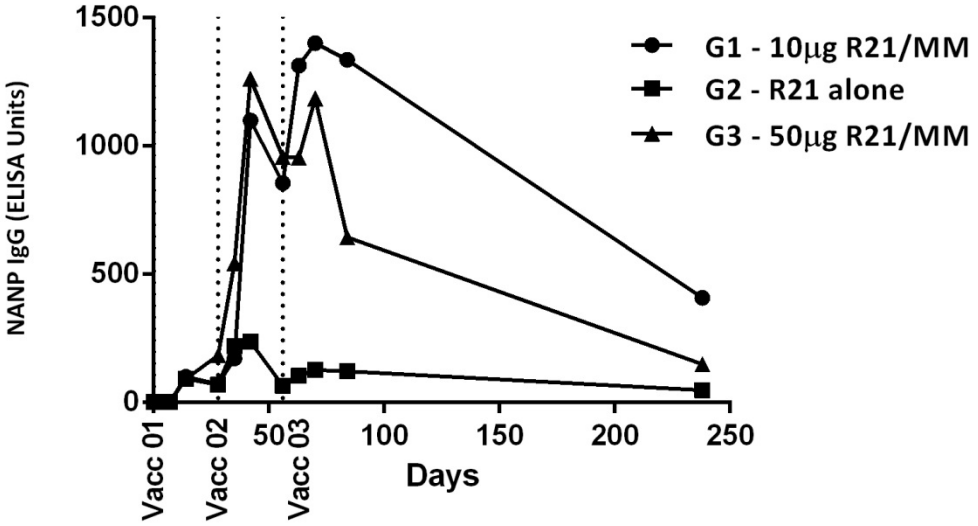


Figure 4-6. VAC053 - Median NANP IgG time courses in response to vaccination. *Abbreviations: MM, Matrix-M. Lines show medians. G1: 10μg R21/MM, n=10, G2: 50μg R21 alone, n=3, G3: 50μg R21/MM, n=8.*

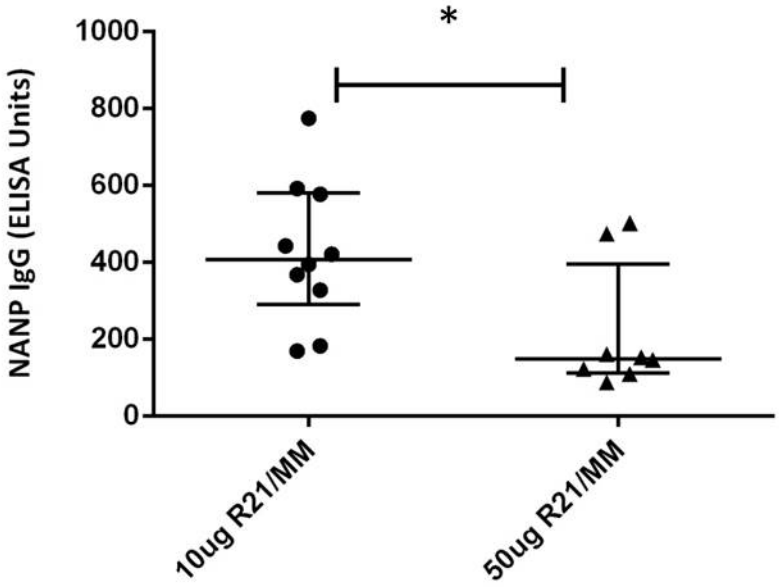


Figure 4-7. VAC053 - Individual NANP IgG responses to vaccination at day 238. *Abbreviations: MM, Matrix-M. Median and interquartile ranges are shown. *p=0.02.*

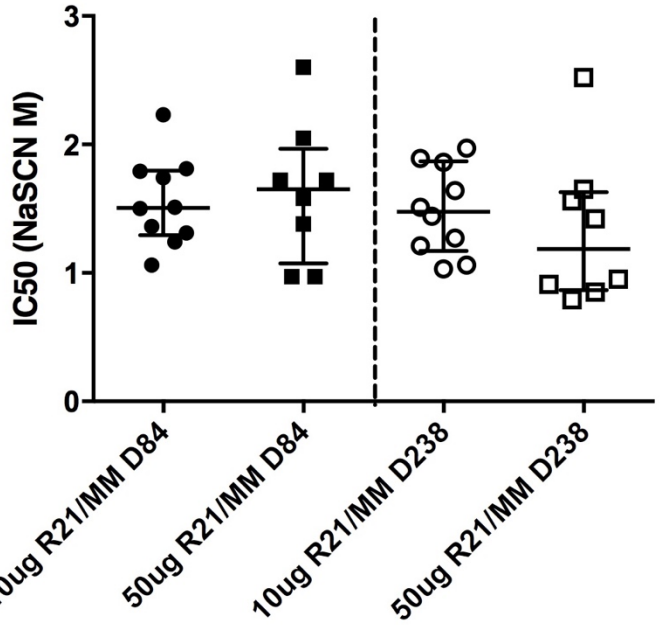


Figure 4-8. VAC053 - Avidity of NANP IgG responses at day 84 and day 238 in the adjuvanted groups measured by inhibition using sodium thiocyanate. *Abbreviations: MM, Matrix-M. Median and interquartile ranges are shown.*

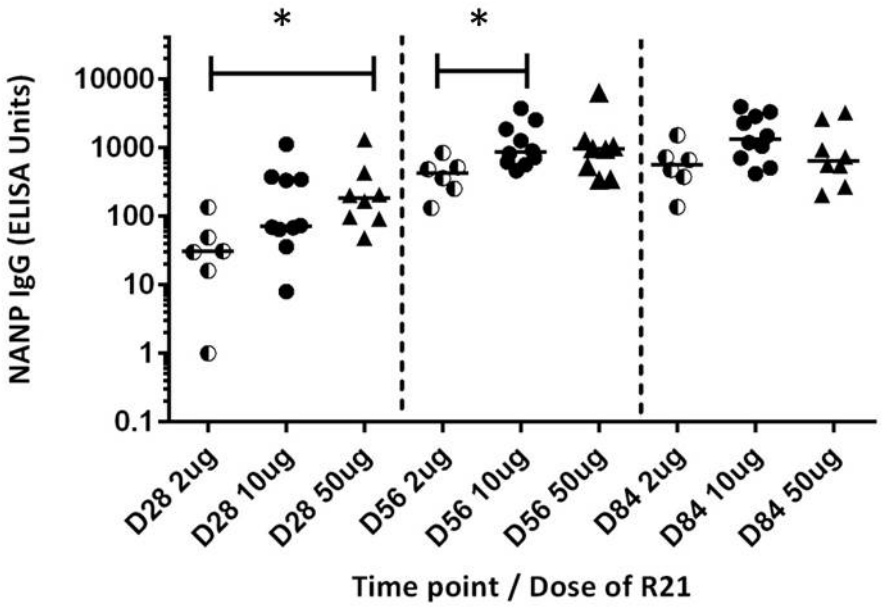


Figure 4-9. VAC053 - Individual NANP IgG responses to vaccination in volunteers vaccinated with 2μg, 10μg or 50μg R21/MM. **p=0.02, p=0.04 and p=0.12 at D28, D56 and D84, respectively.*

4.4.4 VAC053 cellular immunogenicity

T cell responses to the CSP as enumerated by IFNγ ELISPOT were weak and were undetectable in the unadjuvanted group.

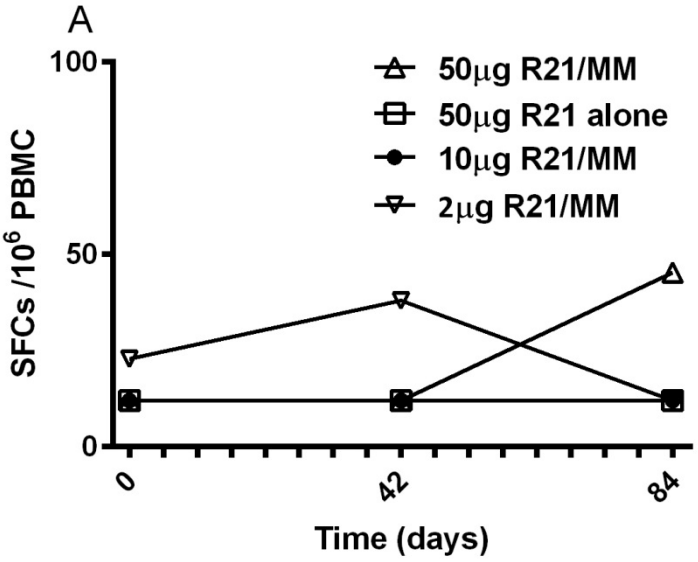


Figure 4-10. VAC053 - Median *ex vivo* IFN-γ ELISPOT responses to vaccination. *Abbreviations: MM, Matrix-M. Lines show medians. G1: 10μg R21/MM, n=10, G2: 50μg R21 alone, n=3, G3: 50μg R21/MM, n=8, G4: 2μg R21/MM.*

4.4.5 VAC056 participant flow

From 8th December, 2015 to 26th May, 2016, a total of 20 volunteers of the 30 who were screened for eligibility were enrolled in the study. There were no withdrawals and all volunteers completed follow-up until Day 238. The trial flow diagram is shown in Figure 4-11.

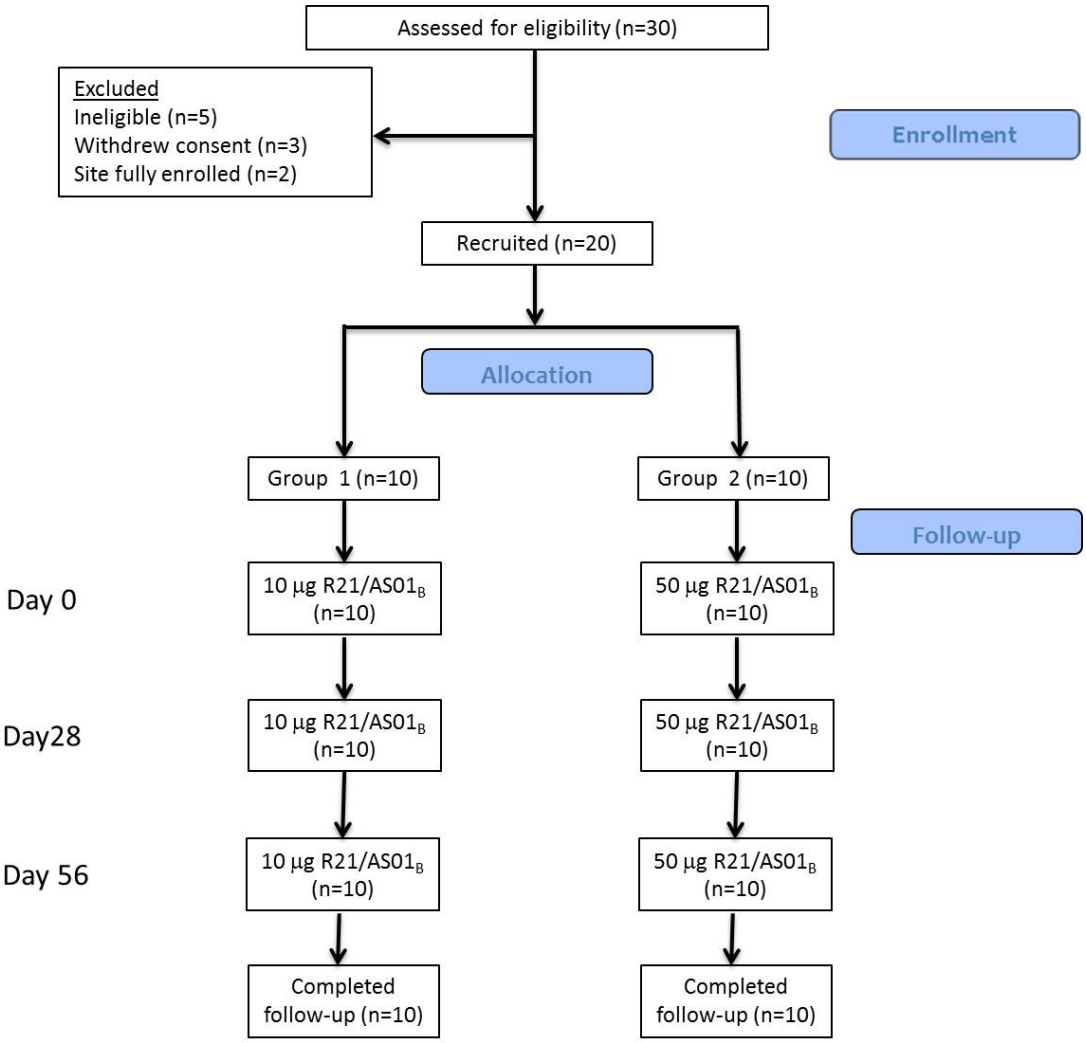


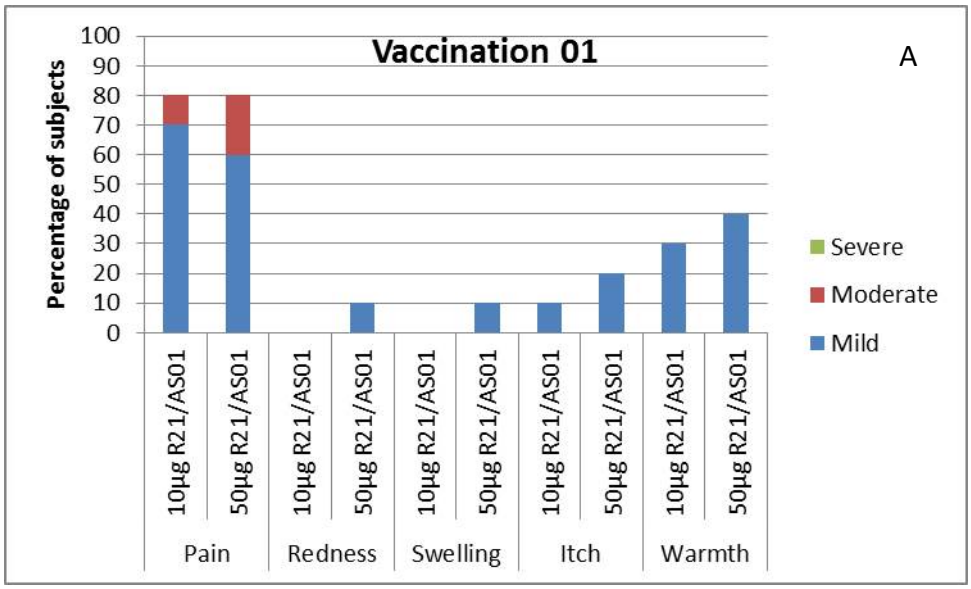
Figure 4-11. VAC056 flow chart of study design and volunteer recruitment. *A total of 30 volunteers were screened and 20 were enrolled in total. All volunteers completed the study.*

4.4.6 VAC056 Vaccine safety and reactogenicity

No serious adverse reactions (SARs) or suspected unexpected serious adverse reactions (SUSARs) occurred. Solicited local and systemic adverse events in the first 7 days after each vaccination related to R21 at doses of 10μg and 50μg administered with $AS01_B$ are shown in Figures 4-12 to 4-14. The majority of adverse events (AEs) reported were self-limiting and mild in severity. Vaccine site pain was the most common local adverse event and was predominantly mild in severity. There was no significant difference in the adverse event profile of the 10μg and 50μg R21 groups (p=0.24). The second and third vaccinations were more reactogenic compared to the first vaccination (p=0.0002) with a higher percentage of moderate and severe AEs reported. Mild temperature (< 38.1^{O}C), which resolved within 24 hours was reported by three volunteers in Group 1 and two volunteers in Group 2. In addition, one volunteer in Group 1 reported a moderate fever (38.1^{O}C) after their second vaccination, and one volunteer in Group 2 reported a severe fever of 39.3^{O}C after their second and third vaccination, which resolved within 24 hours. Short-lived severe adverse events were only reported by three volunteers who were all in the 50μg R21/$AS01_B$ group.

Unsolicited adverse events collected for 28 days after each vaccination deemed definitely, possibly or probably related to vaccination were predominantly mild in nature and are coded according to the MedDRA classification and tabulated in Table 4-7. Laboratory adverse events were all grade 1 apart from one volunteer who developed a grade 2 bilirubin on their day 56 safety bloods. They had a grade 1 bilirubin prior to receiving any vaccinations and the remainder of their liver function

tests were all normal and the bilirubin normalised during the course of the trial. All laboratory AEs are tabulated in Table 4-8.



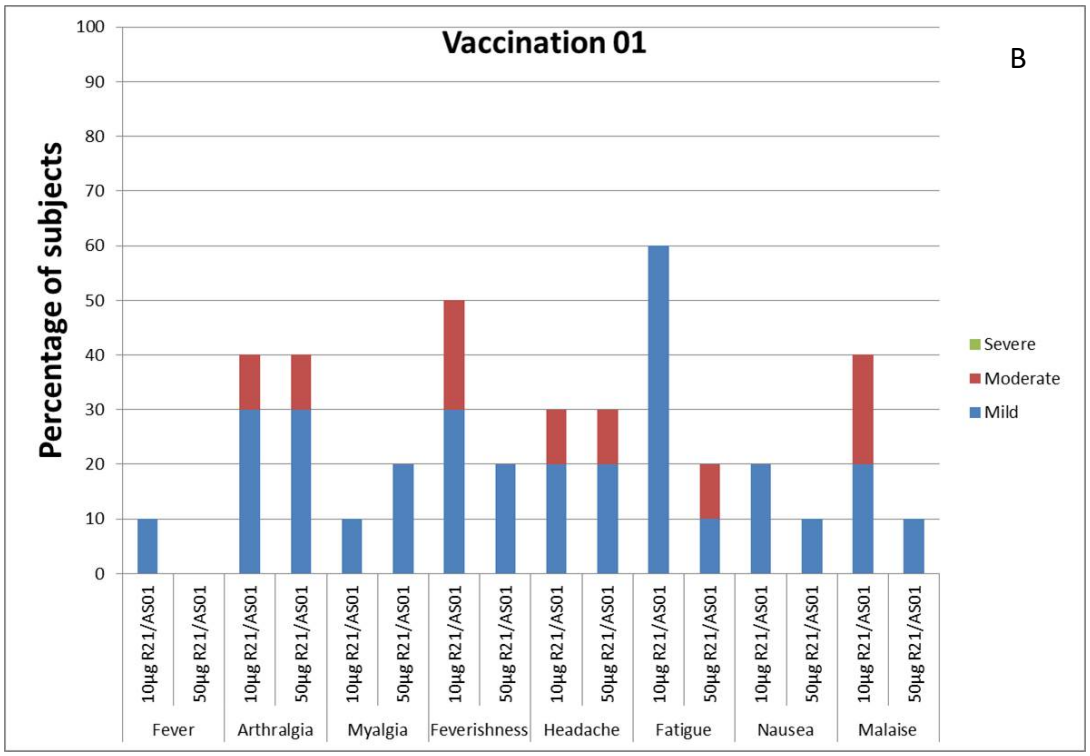
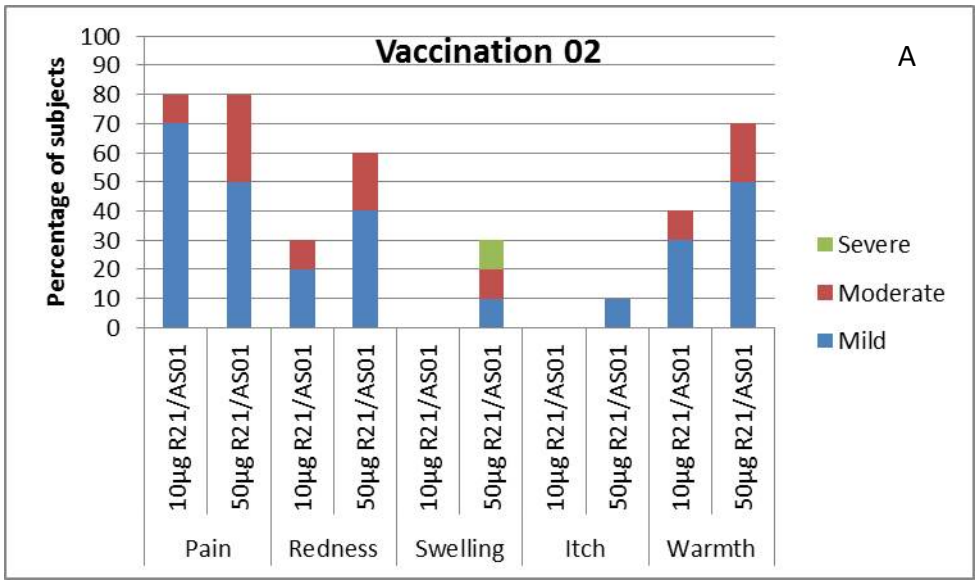


Figure 4-12. VAC056 - Local (A) and systemic (B) solicited adverse events (AE) reported in the electronic diary card during the first seven days related to the first vaccination. *R21 was administered mixed with AS01$_B$ at two different doses of 10µg (n=10) and 50µg (n=10). Only the highest intensity of each AE per subject is listed. Data are combined for all AEs for all volunteers receiving the same vaccine at the stated time point.*



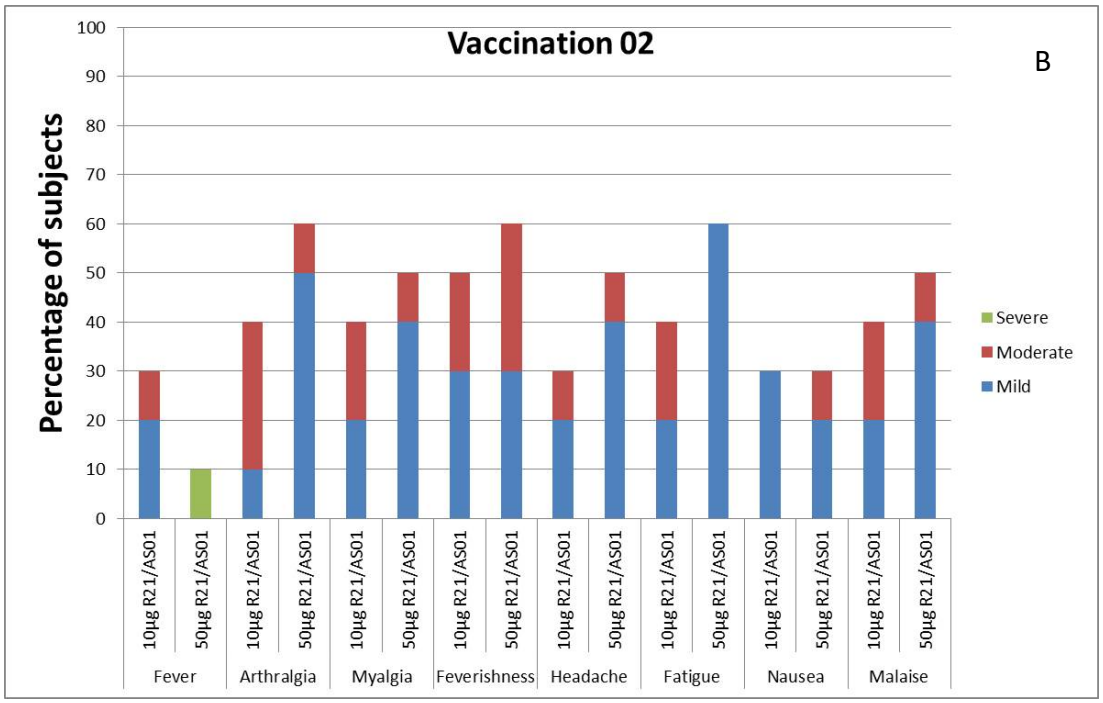
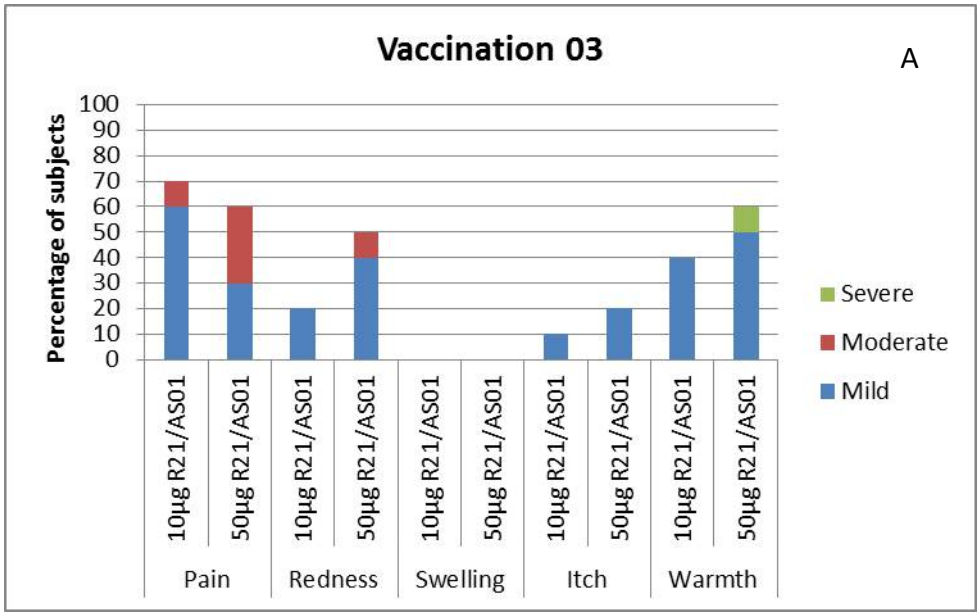


Figure 4-13. VAC056 - Local (A) and systemic (B) solicited adverse events (AE) reported in the electronic diary card during the first seven days related to the second vaccination (4 weeks). *R21 was administered mixed with AS01$_B$ at two different doses of 10µg (n=10) and 50µg (n=10). Only the highest intensity of each AE per subject is listed. Data are combined for all AEs for all volunteers receiving the same vaccine at the stated time point.*



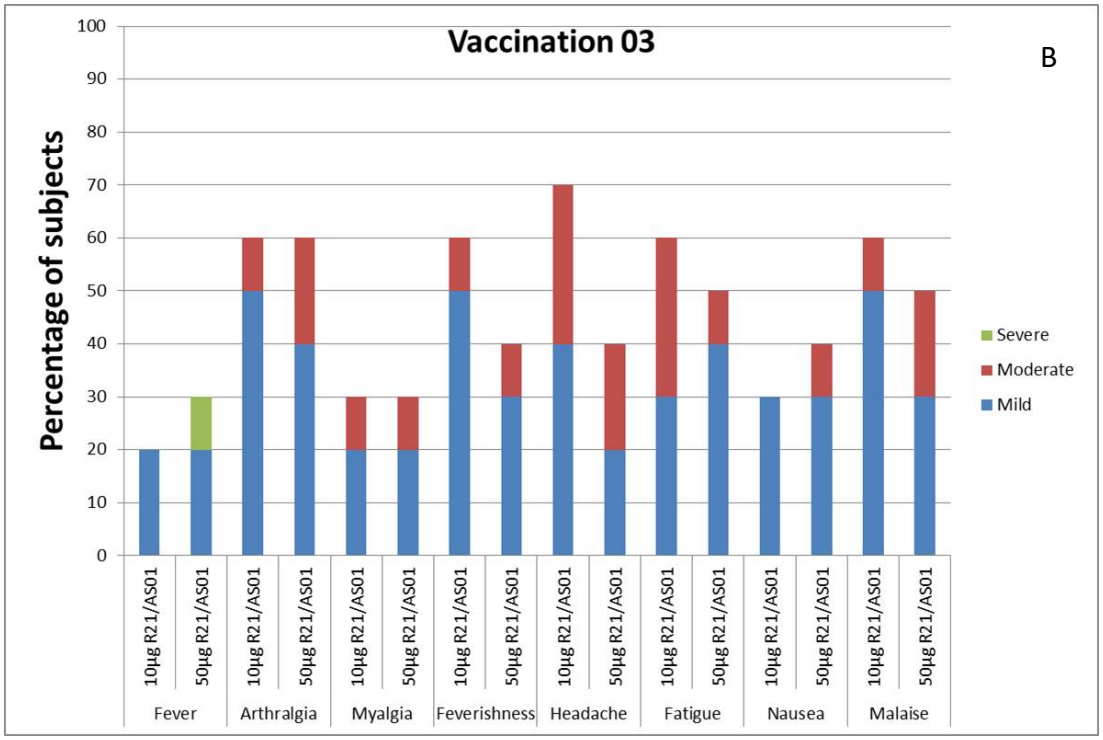


Figure 4-14. VAC056 - Local (A) and systemic (B) solicited adverse events (AE) reported in the electronic diary card during the first seven days related to the third vaccination (8 weeks). *R21 was administered mixed with AS01$_B$ at two different doses of 10µg (n=10) and 50µg (n=10). Only the highest intensity of each AE per subject is listed. Data are combined for all AEs for all volunteers receiving the same vaccine at the stated time point.*

Symptom	MedDRA code	Intensity	Day of onset	Last day	Vaccination no.	Causality
10µgR21/50µg AS01$_B$						
Chills	10008531	Severe	1	1	1	Possible
Fatigue	10016256	Moderate	7	7	3	Possible
Headache	10019211	Moderate	7	7	3	Possible
Headache	10019211	Mild	12	12	2	Possible
Nasal stuffiness	10028771	Mild	1	1	2	Possible
Redness	10038198	Mild	2	2	2	Probable
Shivering	10040558	Mild	0	1	2	Probable
50µgR21/50µg AS01$_B$						
Axillary pain	10048750	Moderate	0	4	3	Possible
Axillary pain	10048750	Mild	4	8	2	Possible
Axillary pain	10048750	Mild	13	27	2	Possible
Breast pain	10006298	Mild	5	27	3	Possible
Breast pain	10006298	Mild	4	8	2	Possible
Breast pain	10006298	Mild	13	27	2	Possible
Chills	10008531	Mild	0	0	1	Probable
Diarrhoea	10012735	Moderate	5	5	3	Possible
Increased skin sensitivity	10021681	Moderate	1	1	2	Possible
Injection site pain	10022086	Mild	21	21	1	Possible
Light headedness	10024492	Moderate	1	1	2	Possible
Rash	10037844	Mild	1	2	2	Probable
Shivering	10040558	Mild	1	1	3	Possible

Table 4-7. VAC056 - Unsolicited AEs deemed definitely, probably and possibly related to vaccination reported in the 28 days following vaccination with R21 adjuvanted with AS01$_B$. *The maximum severity for each AE is shown. AEs have been classified according to the Medical Dictionary for Regulatory Activities (MedDRA) at the Lower Level (LLT) Term level.*

Lab AE and intensity	Number (% of volunteers)		
10μg R21/AS01$_B$	**Vaccination 01 (n=10)**	**Vaccination 02 (n=10)**	**Vaccination 03 (n=10)**
Eosinophilia Grade 1	1 (10)	0	1 (10)
Elevated Urea Grade 1	0	1 (10)	1 (16.7)
Hyperbilirubinaemia Grade 1	1 (10)	0	0
Hypoalbuminaemia Grade 1	0	0	1 (10)
50μg R21/AS01$_B$	**Vaccination 01 (n=10)**	**Vaccination 02 (n=10)**	**Vaccination 03 (n=10)**
Lymphopaenia Grade 1	0	0	1 (10)
Hyperbilirubinaemia Grade 2	0	1 (10)	0

Table 4-8. VAC056 - Frequency of all laboratory adverse events following vaccination with R21 adjuvanted with AS01$_B$. *Adverse events are defined as a deviation from the baseline result collected pre-vaccination on Day 0.*

4.4.7 VAC056 humoral immunogenicity

R21 administered at both the doses of 10μg and 50μg adjuvanted with AS01$_B$ elicited comparable humoral immune responses (Figure 4-15). The peak response was seen at day 70 [median (IQR): 1525 (888.5-2072) ELISA units] and day 84 [median (IQR): 2115 (1762-4353.1) ELISA units] for the 10μg and 50μg dose groups, respectively. The antibody response was maintained at 6 months after the final vaccination with no differences in durability observed between the two groups.

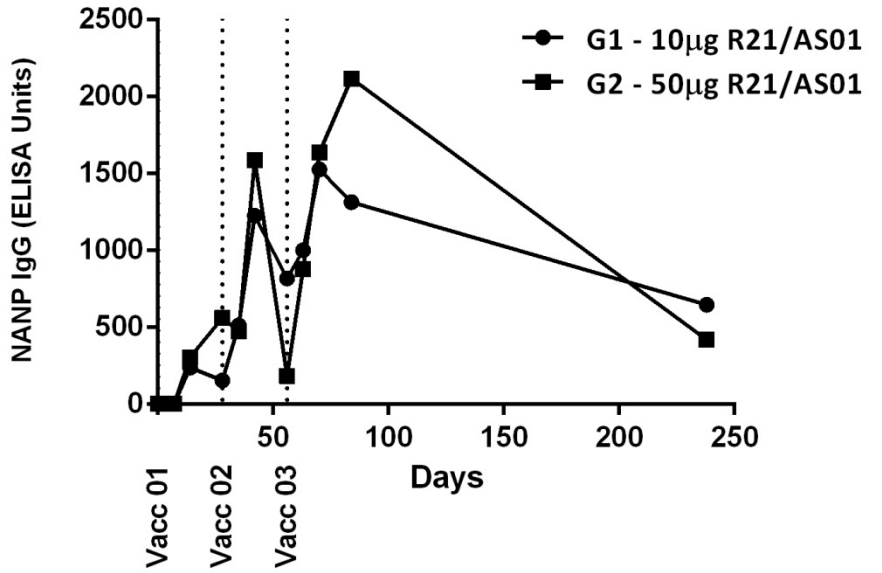


Figure 4-15. VAC056 - Median NANP IgG time courses in response to vaccination. *Lines show medians. G1: 10μg R21/AS01$_B$, n=10, G2: 50μg R21/AS01$_B$, n=10.*

4.4.8 Comparison with RTS,S/AS01$_B$

R21 administered at a dose of 10μg was more reactogenic when administered with AS01$_B$ in comparison to when administered with MM ($p<0.0001$) with a higher percentage of volunteers reporting moderate and severe AEs (Table 4-9). There was no significant difference in the AE profile ($p=0.07$) of 50μg R21 administered with either adjuvant. I also compared the adverse event profile in VAC053 and VAC056 with data from a previous clinical trial conducted in our centre (VAC055) where volunteers received three doses of 50μg RTS,S/AS01$_B$ given four weeks apart [136]. 50μg R21 administered with either adjuvant had significantly reduced reactogenicity compared to RTS,S/AS01$_B$ (R21 with MM - $p < 0.0001$; R21 with AS01$_B$ – $p = 0.017$), which is mainly due to a significant improvement in the systemic adverse event profile. There was also a higher percentage of moderate and severe adverse events reported by volunteers receiving RTS,S/AS01$_B$ (Table 4-10).

Vaccination 01		Vaccination 02		Vaccination 03	
10μgR21/ MM	10μgR21/ AS01$_B$	10μgR21/ MM	10μgR21/ AS01$_B$	10μgR21/ MM	10μgR21/ AS01$_B$
18.2%	30%	20%	40%	10%	50%
50μgR21/ MM	50μgR21/ AS01$_B$	50μgR21/ MM	50μgR21/ AS01$_B$	50μgR21/ MM	50μgR21/ AS01$_B$
40%	20%	62.5%	50%	50%	40%

Table 4-9. Percentage of volunteers reporting moderate or severe adverse events after vaccination with R21/MM and R21/AS01$_B$. *R21 was administered at doses of 10μg and 50μg adjuvanted with either MM or AS01$_B$.*

LOCAL AE's ALL VACCINATION			
	50 μg R21/MM	50 μg RTS,S/AS01$_B$	50 μg R21/ AS01$_B$
None	89	145	92
Mild	34	83	42
Moderate	6	17	14
Severe	1	5	2
SYSTEMIC AE's ALL VACCINATION			
	50 μg R21/MM	50 μg RTS,S/ AS01$_B$	50 μg R21/ AS01$_B$
None	155	233	154
Mild	31	83	63
Moderate	20	66	21
Severe	2	18	2
ALL AE's ALL VACCINATION			
	50 μg R21/MM	50 μg RTS,S/ AS01$_B$	50 μg R21/ AS01$_B$
None	244	378	246
Mild	65	166	105
Moderate	26	83	35
Severe	3	23	4
Total possible AEs	338	650	390
% mild	19.2	25.5	26.9
% moderate	7.7	12.8	9.0
% severe	0.9	3.5	1.0
Total %	27.8	41.8	36.9

Table 4-10. Comparison of the local and systemic adverse event profile between 50μg R21/MM, 50μg R21/AS01$_B$ and 50μg RTS,S/AS01$_B$. *The total number of local and systemic adverse events reported by all volunteers following all vaccinations of each vaccine regime is shown.*

Humoral responses to the NANP repeat region of the CSP antigen were compared to those in VAC055. Though these assays were performed on different dates, the ELISA was run using the same protocol in the same laboratory by the same operator. The magnitude and dynamics of the antibody response at both the doses of 10μg and 50μg R21 were comparable to 50μg RTS,S/AS01$_B$ with maximal boosting of the response seen after the second vaccination (Figure 4-16). There were no statistically significant differences between the groups at day 84.

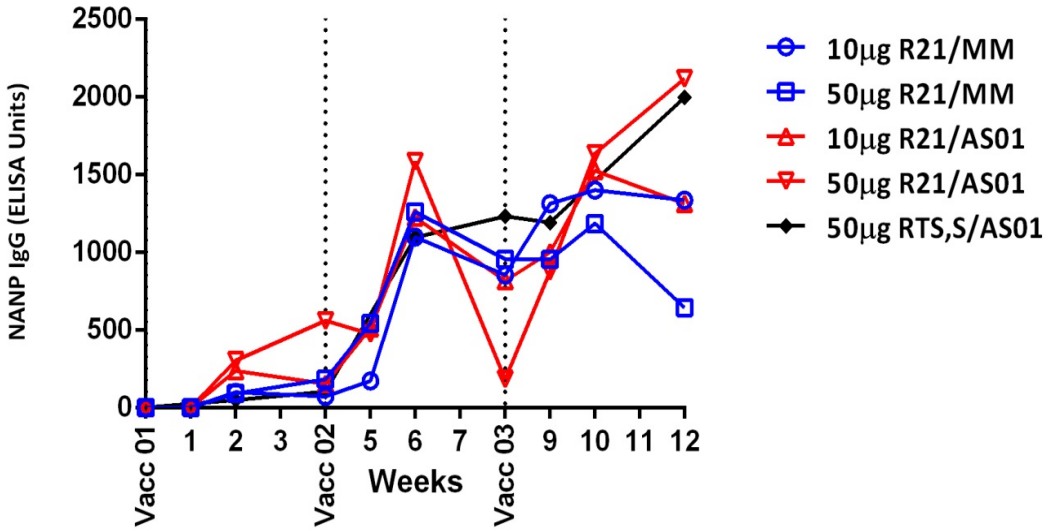


Figure 4-16. Median NANP IgG time courses in response to vaccination with R21/MM, R21/AS01$_B$ and RTS,S/AS01$_B$. *Lines show medians. Volunteers received three doses given four weeks apart of either 10μg R21/MM (n=10), or 50μg R21/MM (n=8), or 10μg R21/AS01 (n=10), or 50μg R21/AS01 (n=10) or 50μg RTS,S/AS01$_B$.*

The main compositional difference between RTS,S and R21 is the density of CSP present on the VLP surface, which is approximately 5-fold higher in R21. It is well known that most volunteers receiving RTS,S seroconvert to the HBsAg irrespective of pre-existing immunity, which may result in reduction in the magnitude and breadth of the immune response elicited against the CSP [57, 72]. We assessed humoral responses to the HBsAg pre-vaccination and at 28 days after the third vaccination.

Prior to R21 vaccination, ~50% and 60% of participants were seropositive to HBsAg, usually due to vaccination against Hepatitis B. Of those that were seronegative at vaccination, 0, 17% and 50% of volunteers seroconverted for HbsAg after 3 doses of R21/MM in the 2μg, 10μg and 50μg dose groups, respectively. Similarly, 33% and 40% of volunteers seroconverted after 3 doses of R21/AS01$_B$ in the 10μg and 50μg dose groups, respectively. This is in contrast to data from a previous clinical trial conducted in our centre (Rampling et al., personal communication), where vaccination with 3 doses of RTS,S/AS01$_B$ induced seroconversion in 100% of seronegative vaccines.

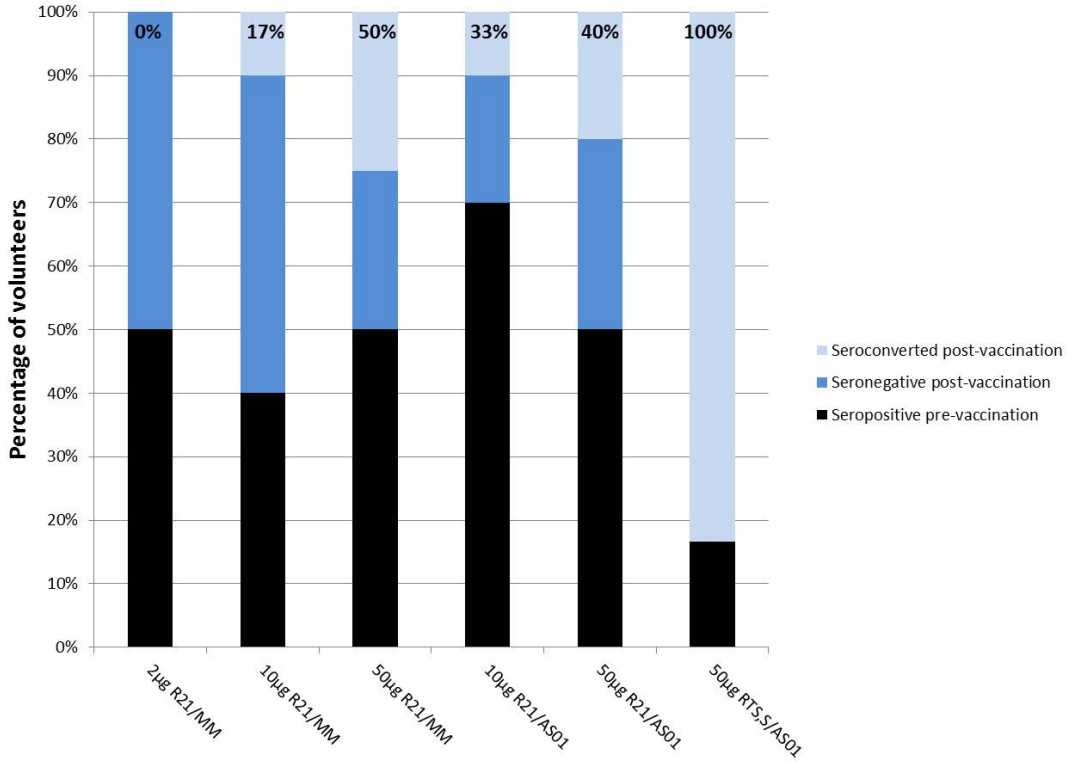


Figure 4-17. Rates of seroconversion to HBsAg after R21/MM (2μg, 10μg, 50μg), R21/AS01$_B$ (10μg, 50μg) and 50μg RTS,S/AS01. *Percentages denote number of seronegative volunteers who seroconverted after their vaccination schedule.*

4.5 Discussion

In this Chapter, I have described the safety and immunogenicity of the first-in-human administration of the novel malaria vaccine candidate, R21 administered with two different adjuvants in UK adults. R21 was safe and well tolerated with adverse events being predominantly mild in nature. In VAC053, the addition of MM to the 50μg dose resulted in increased reactogenicity, especially moderate and severe adverse events. However, the reactogenicity of R21 was markedly reduced, even when administered with MM, when the dose of the antigen is reduced to 2μg. It has been previously shown that addition of Matrix-M does result in increased local and systemic reactogenicity compared to controls who receive unadjuvanted vaccine [196]. I reported in Chapter 3 that addition of MM to ChAd63 ME-TRAP resulted in increased reporting of moderate and severe systemic AEs, however there was no clear pattern of increased local reactogenicity attributable to the adjuvant. Similarly, the addition of MM to MVA ME-TRAP did not result in increased local reactogenicity.

After the initial safety review of the 10μg R21/MM dose in VAC053, we also commenced a similar trial testing the same vaccine regime in Burkinabe adults. Volunteers reported significantly less adverse events with minimal systemic effects at a dose of 10μg R21/MM (A Tiono; personal communication), which makes it more acceptable for deployment. We have also observed reduced reactogenicity in African populations compared to UK volunteers in clinical trials assessing ChAd63-MVA ME-TRAP [129, 131, 229]. Furthermore, the reactogenicity profile of 50μg R21 administered with either adjuvant was significantly improved compared to 50μg RTS,S/AS01$_B$ in healthy adult UK volunteers [136]. However, this must be interpreted

with caution due to the small numbers of participants in these trials and further evaluation of R21 in adults, children and infants would be valuable in making a better powered comparison.

R21 is a protein particle subunit vaccine with a similar composition to the leading malaria vaccine candidate, RTS,S/AS01$_B$. It was tested in these two clinical trials with an adjuvant as it is well recognized that these vaccines are poorly immunogenic when administered alone. In keeping with this, R21 administered alone elicited a poor humoral response in VAC053. The humoral response to the conserved central NANP repeat region is thought to be the key mechanism of efficacy of RTS,S/AS01$_B$. I showed that R21 administered with either adjuvant, at doses of both 10μg and 50μg, elicited comparable antibody responses to RTS,S/AS01$_B$. It is also very encouraging that comparable humoral responses are elicited even at the very low dose of 2μg at 28 days after the third vaccination. This will have significant dose-sparing and cost-saving implications for production of the vaccine.

R21 administered with either adjuvant at doses of 10μg and 50μg did not result in significant boosting of the humoral response after the third vaccination compared to the peak response after the second vaccination. Though RTS,S is currently given in a three dose schedule, given 4 weeks apart, preliminary studies with RTS,S assessed regimes where the third dose was delayed to 24-28 weeks [57, 72]. Though these studies did not show significant boosting of the humoral response with a delayed third dose, high level efficacy of 86% was observed in malaria-naïve adults who received RTS,S/AS02 given at 0, 1 and 7 months [72]. However, these volunteers had also received one-fifth of the vaccine and adjuvant due to earlier reactogenicity

concerns. Studies in African adults living in malaria endemic regions have shown that there is boosting of the immune response after the third dose with a 0, 1 and 6 month schedule [74, 76]. More recently, Regules et al. revisited a delayed fractional dose with RTS,S/AS01$_B$ where high level efficacy was again observed, which was associated with increased somatic hypermutation and antibody avidity, rather than the magnitude of the humoral response [246]. In addition, Kester et al. demonstrated that 2 doses of RTS,S/ASO2 elicited similar protective efficacy to 3 doses in a malaria sporozoite challenge study [247].

Durable antibody responses were observed at 6 months after the final vaccination with both adjuvants. In VAC053, the 10μg dose elicited significantly higher titres compared to the 50μg dose at 6 months. R21 at both doses of 10μg and 50μg adjuvanted with AS01$_B$ also elicited comparable responses to 10μg R21 adjuvanted with Matrix-M at 6 months. Though some of the malaria vaccine approaches mentioned in Chapter 1 have demonstrated short-term efficacy in malaria-naïve and endemic populations, durable protective immunity is a hitherto unmet goal. It is well recognized that the antibody responses to the CSP elicited by RTS,S/AS01$_B$ decay over time, which is reflected in a reduction of vaccine efficacy [73, 75, 248]. The half-life of induced antibodies may differ between pathogens following natural infection, between vaccine-induced antibodies for different pathogens and can also vary between individual antigens within a single pathogen. The waning of antibody responses over 6 months seen in the two trials discussed in this chapter is not unexpected as the response is against the same antigen that has been associated with immunity rendered by RTS,S/AS01$_B$. It is also worth considering that the

magnitude of the antibody response may not directly reflect protective immunity, which may be influenced by a number of other factors such as affinity maturation of the antibodies due to somatic hypermutation and isotype switching of the antibodies.

It is of particular interest that the lower dose of R21/MM induces the more durable response. In general, higher priming antigen doses favour production of antigen-specific plasma cells that only have a short lifespan, whereas lower doses can preferentially drive the induction of immune memory [249, 250]. A few of these plasma cells differentiate into long-lived plasma cells (LLPC) and in the absence of subsequent antigen exposure; the proportion of LLPC generated by a vaccine is predictive of the durability of the antibody response. Antibody titres at 6-12 months after immunisation would reflect this as the initial short-term plasma cell response would have declined. Follicular helper T cells (T_{fh}) play a critical role in germinal centres by facilitating B cell differentiation into affinity-matured long-lived plasma cells and memory B cells through expression of a series of costimulatory molecules and chemokines [251]. Recent work undertaken in our laboratory (data not presented) showed that a lower dose (10μg) of R21/MM induced higher proportion of a subset of T_{fh} cells (Th2-like), which correlated with the memory B cell response and the durability of the response (G Bowyer, personal communication).

We detected minimal T cell responses by IFN-γ ELISpot to this vaccine regimen as would be expected for this type of vaccine. Similarly, RTS,S/$AS01_B$ induces potent antibody responses to circumsporozoite protein, but low level $CD4^+$ and no $CD8^+$ T-cell responses [241]. It is likely that an efficacious pre-erythrocytic malaria vaccine

would benefit from robust $CD4^+$ T-cell responses to enhance innate and humoral immunity and facilitate killing of infected hepatocytes. To date, the viral-vectored vaccines detailed in Chapter 3 are the most advanced candidates that elicit potent cellular immunity and could be used as a complementary strategy in a multi-stage vaccine.

In conclusion, these Phase I clinical trials showed that the novel malaria vaccine candidate, R21 adjuvanted with MM and $AS01_B$ was safe and well tolerated in malaria-naïve subjects. This was the first administration of R21 in humans and immunogenicity profiles observed are encouraging, with comparable antibody responses to RTS,S/$AS01_B$ at even one-fifth of the dose (10μg). I have also shown that R21 administered with MM resulted in a favourable adverse event profile in comparison to R21/$AS01_B$, especially after the second and third vaccination. The use of the novel saponin-based adjuvant MM instead of AS01 will also result in reduced cost of production of the vaccine. These data provide strong evidence to assess the efficacy of R21 administered with MM in malaria-naïve volunteers using controlled human malaria infection to provide proof-of-concept before initiating testing in malaria endemic populations. This formed the basis for the VAC065 trial, which is described in Chapter 5.

CHAPTER FIVE:

A Phase I/II sporozoite challenge study to assess the safety and protective efficacy of adjuvanted R21 at different doses and the combination malaria vaccine candidate regimen of adjuvanted R21 + ChAd63-MVA ME-TRAP

5.1 Authorship statement

I set up the VAC065 clinical trial with the assistance of Alison Lawrie, Rachel Roberts, Ian Poulton and Adrian Hill. This involved preparation of the study documents with submission for ethical and regulatory approval for all sites, and R&D department approval for NHS sites (in Southampton and Imperial College, London). Screening, enrolment, vaccinations and follow-up visits in Oxford were conducted by me, Daniel Silman, Pedro Folegatti and by the nursing team. Volunteers were also enrolled at the NIHR WTCRF in Southampton. Enrolment, vaccination and follow-up of volunteers at the NHS sites were conducted by local study staff. The local PI at the site was David Lewis in Imperial College and Saul Faust in Southampton. Adrian Hill was the chief investigator for this trial.

Laboratory assays in Oxford were carried out by Georgina Bowyer, Jonathan Powlson, Katie Ewer and me. I was the lead clinician responsible for running the CHMI, which was conducted in collaboration with the team at Imperial College, London. The *Pf*-infected mosquitoes were provided by the Walter Reed Army Institute of Research, USA. Thick blood films were read in real-time by experienced microscopists. qPCR was carried out by Nick Edwards and a team of laboratory staff at the Jenner Institute.

5.2 Introduction

The promising safety and immunogenicity observed in the two Phase I clinical trials presented in Chapter 4, which is comparable to the most advanced malaria vaccine candidate, RTS,S/AS01, provided the basis to assess efficacy using the well-established CHMI model using a mosquito-bite sporozoite challenge that mimics natural infection. Though the antibody responses observed with both adjuvants were comparable, Matrix-M was chosen as the adjuvant to be used in this efficacy study due to the improved reactogenicity profile. Furthermore, I described in Chapter 3 that Matrix-M could be used safely with the ChAd63-MVA ME-TRAP regime without any immunological interference and a recent study demonstrated that combining these viral-vectored vaccines with RTS,S/AS01 in the same regimen was safe, highly immunogenic and efficacious [136].

As mentioned in Chapter 4, there has been an increased recent interest in revisiting the use of a fractional ($1/5^{th}$) third dose in RTS,S/AS01 regimes [246]. The exact mechanisms for the additional efficacy obtained by this dose reduction are unclear but we recently showed that this may be related to a significant increase in the functional ability of the induced antibodies to inhibit sporozoite invasion rather than the magnitude of the response. (Rampling et al., Manuscript in preparation) This is thought to be the predominant mechanism that is responsible for the partial short-lived field efficacy of RTS,S/AS01. We therefore undertook this study in healthy UK volunteers to assess the efficacy against malaria sporozoite challenge of R21/MM in different dose schedules and in combination with ChAd63-MVA ME-TRAP.

5.3 Methods

Detailed methods of the recruitment and enrolment of volunteers, the assays used in this trial and the CHMI procedure can be found in Chapter 2.

5.3.1 Study design

This was a Phase I/II sporozoite challenge study in healthy malaria-naïve adult males and non-pregnant females between the ages of 18 and 45 years. Recruitment and vaccinations were conducted at the CCVTM at the University of Oxford and the Wellcome Trust CRF in Southampton and Imperial College, London. This trial consisted of 4 cohorts and the sample sizes reflect a balance between several considerations: practical limitations on volunteer recruitment, ethical considerations limiting the number of volunteers that should receive a vaccine regimen without prior evidence of efficacy, ethical considerations limiting the number of volunteers that should undergo CHMI and the desire to describe the efficacy of the immunisation regimes. Allocation to study group was undertaken by me prior to enrolment based on subject preference. Group 1 received 3 vaccinations (R21/MM 10µg at 0, 4 and 8 weeks); Group 2 received 3 vaccinations (R21/MM 50µg at 0 and 4 weeks and R21/MM 10µg at 8 weeks); Group 3 received 5 vaccinations (R21/MM 10µg at 0, 4 and 8 weeks and ChAd63 ME-TRAP 5 x 10^{10} vp at 1 week, and MVA ME-TRAP 2 x 10^{8} pfu at 9 weeks) and Group 4 (n=6) received no vaccinations. All subjects underwent CHMI by mosquito bite at the same time over a two day period (week 12 after first vaccination for vaccinated subjects).

The volunteers were infected using five infectious bites from *P. falciparum* 3D7-strain infected *Anopheles stephensi* mosquitoes at Imperial College, London. All subjects were infected with a single batch of mosquitoes supplied by the Department of Entomology, Walter Reed Army Institute of Research, Washington DC, USA. All participants gave written informed consent prior to participation, and the study was conducted according to the principles of the Declaration of Helsinki and in accordance with Good Clinical Practice (GCP).

	Day 0	**Day 7**	**Day 28**	**Day 56**	Day 63	Day 84
Group 1 (n=12)	10μgR21/50μg Matrix-M	-	10μgR21/50μg Matrix-M	10μgR21/50μg Matrix-M	-	CHMI
Group 2 (n=12)	50μgR21/50μg Matrix-M	-	50μgR21/50μg Matrix-M	10μgR21/50μg Matrix-M	-	CHMI
Group 3 (n=12)	10μgR21/50μg Matrix-M	ChAd63 ME-TRAP	10μgR21/50μg Matrix-M	10μgR21/50μg Matrix-M	MVA ME-TRAP	CHMI
Group 4 (n=6)	-	-	-	-	-	CHMI

Table 5-1. VAC065 Study groups.

5.3.2 Ethics and regulatory approval

The study was approved by the UK National Research Ethics Service, Committee South Central–Berkshire (Ref: 16/SC/0261), the Medicines and Healthcare Products Regulatory Agency (Ref: 21584/0360/001-0001), and the Oxford University Clinical Trials and Research Governance team, who independently and externally monitored compliance with Good Clinical Practice guidelines. Viral-vectored vaccine use was authorised by the Genetically Modified Organisms Safety Committee (GMSC) of the Oxford University Hospitals NHS Trust (Reference number GM 462.16.88). The trial was registered with ClinicalTrials.gov (Ref: NCT02905019) and an independent local safety monitor provided safety oversight.

5.3.3 Objectives and Endpoints

Primary Objectives

To assess the efficacy (occurrence of *P. falciparum* parasitaemia defined by a composite of symptoms, blood film result and parasitaemia, as shown in Table 5-4) of R21/MM at two different doses and the combination malaria vaccine candidate regimen of R21/MM and ChAd63-MVA encoding ME-TRAP, against malaria sporozoite challenge, in healthy malaria-naïve volunteers.

To assess the safety of adjuvanted R21/MM at two different doses and the combination malaria vaccine candidate regimen of adjuvanted R21 + ChAd63 and MVA encoding ME-TRAP, in healthy malaria-naïve volunteers.

The following parameters were assessed for all study groups:

- Occurrence of solicited local reactogenicity signs and symptoms for 7 days following the vaccination
- Occurrence of solicited systemic reactogenicity signs and symptoms for 7 days following the vaccination
- Occurrence of unsolicited adverse events for 28 days following the vaccination
- Change from baseline for safety laboratory measures
- Occurrence of serious adverse events during the whole study duration

Secondary Objectives

To assess immunogenicity generated in malaria naïve individuals of R21/MM at two different doses and the combination malaria vaccine candidate regimen of adjuvanted R21 + ChAd63 and MVA encoding ME-TRAP.

Measures of immunogenicity included:

- ELISA to quantify antibodies to NANP
- ELISpot to enumerate IFN-γ producing T cells
- Functional assays to assess inhibition of sporozoite invasion

To assess the efficacy (measured as time to *P. falciparum* parasitaemia assessed by PCR dynamics) of adjuvanted R21/MM and the combination malaria vaccine candidate regimen of adjuvanted R21 + ChAd63 and MVA encoding ME-TRAP, against malaria sporozoite challenge, in healthy malaria-naïve volunteers.

5.3.4 Study participants

Volunteers were recruited and vaccinated at two UK study sites, Oxford and Southampton. Patient demographics and baseline characteristics are presented in Tables 5-2 and 5-3 for Oxford and Southampton volunteers, respectively. All participants provided written informed consent. The CHMI was carried out over two days at Imperial College, London and there were three unvaccinated control volunteers on each day. The intensive post-CHMI follow-up was carried out at the CCVTM, University of Oxford.

Volunteer number	Age	Sex	BMI	Country of birth	Smoker
1002	30	Female	24.1	Australia	No
1003	25	Female	20.6	United kingdom	No
1004	22	Female	25.5	United kingdom	No
1005	24	Male	25.0	United kingdom	No
1007	32	Male	22.9	United kingdom	No
1010	26	Female	24.1	Australia	No
1011	26	Female	23.9	United kingdom	No
1012	35	Male	24.8	United kingdom	No
1013	28	Female	31.8	United kingdom	No
1014	36	Female	41.1	United kingdom	No
1015	39	Female	34.6	United kingdom	No
1019	24	Male	24.7	Canada	No
1022	43	Female	26.8	United States of America	No
1024	39	Female	38.2	United kingdom	No
1027	25	Female	28.7	Faroe island	No
1032	24	Female	20.3	United kingdom	No
1033	18	Female	17.8	United kingdom	No
1035	20	Female	23.3	Austria	Yes
1036	19	Male	23.5	United kingdom	No
1037	27	Female	21.5	United states	No
1039	22	Male	24.0	United kingdom	No
1040	23	Male	25.4	United kingdom	No
1041	34	Female	18.7	United kingdom	No
1042	20	Male	24.1	United kingdom	No
1046	39	Female	23.9	United kingdom	No
1049	19	Female	24.4	United kingdom	No
1050	22	Male	22.3	United kingdom	Yes
1053	21	Female	21.5	United kingdom	No
1056	23	Female	27.2	United kingdom	Yes
1060	21	Male	26.1	United kingdom	No
1061	24	Female	20.7	United kingdom	No
1062	21	Female	31.1	United kingdom	No
1065	22	Male	29.8	United kingdom	No
1067	23	Male	24.1	United kingdom	No
1068	22	Female	25.9	United kingdom	Yes
1072	24	Male	34.7	China	No
1073	37	Female	30.1	United kingdom	No
1074	43	Female	20.4	United kingdom	No
1076	30	Male	26.8	South Africa	No

Table 5-2. Demographics and baseline characteristics of Oxford volunteers enrolled in the VAC065 trial. *BMI - body mass index*

Volunteer number	Age	Sex	BMI	Country of birth	Smoker
2201	38	Female	35.8	Latvia	No
2202	27	Male	24.5	United kingdom	No
2204	23	Female	25.8	United kingdom	Yes
2205	23	Female	23.3	United kingdom	No

Table 5-3. Demographics and baseline characteristics of Southampton volunteers enrolled in the VAC065 trial. *BMI - body mass index*

5.3.5.1 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.
- Women only: Must practice continuous effective contraception for the duration of the study.
- Agreement to refrain from blood donation during the course of the study and for at least 3 years after the end of their involvement in the study.
- Written informed consent to participate in the trial.
- Reachable (24/7) by mobile phone during the period between CHMI and completion of antimalarial treatment.
- Willingness to take a curative anti-malaria regimen following CHMI.

- For volunteers not living in Oxford: agreement to stay in a hotel room close to the trial centre during a part of the study (from at least day 6.5 post mosquito bite until anti-malarial treatment is completed).
- Answer all questions on the informed consent quiz correctly.

5.3.5.2 Exclusion Criteria

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

- History of clinical malaria (any species).
- Travel to a clearly malaria endemic locality during the study period or within the preceding six months
- Use of systemic antibiotics with known antimalarial activity within 30 days of CHMI (e.g. trimethoprim-sulfamethoxazole, doxycycline, tetracycline, clindamycin, erythromycin, fluoroquinolones and azithromycin)
- Receipt of an investigational product in the 30 days preceding enrolment, or planned receipt 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 3 volunteers only: prior receipt of a non-malaria MVA or non-malaria adenovirus vectored experimental vaccine
- Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days) immunosuppressant medication within the past 6 months (inhaled and topical steroids are allowed).

- Use of immunoglobulins or blood products within 3 months prior to enrolment.
- History of allergic disease or reactions likely to be exacerbated by any component of the vaccine (e.g. egg products, Kathon) or malaria infection.
- Any history of anaphylaxis post vaccination.
- History of clinically significant contact dermatitis.
- History of sickle cell anaemia, sickle cell trait, thalassaemia or thalassaemia trait or any haematological condition that could affect susceptibility to malaria infection.
- Pregnancy, lactation or intention to become pregnant during the study.
- 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
- Any clinical condition known to prolong the QT interval
- History of cardiac arrhythmia, including clinically relevant bradycardia
- Disturbances of electrolyte balance, eg, hypokalaemia or hypomagnesaemia
- Family history of congenital QT prolongation or sudden death
- Contraindications to the use of all three proposed anti-malarial medications; Riamet, Malarone and Chloroquine.
- History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ).

- History of serious psychiatric condition that may 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 standard UK units every week.
- Suspected or known injecting drug abuse in the 5 years preceding enrolment.
- Hepatitis B surface antigen (HBsAg) detected in serum.
- Seropositive for hepatitis C virus (antibodies to HCV) at screening
- An estimated, ten year risk of fatal cardiovascular disease of ≥5%, as estimated by the Systematic Coronary Risk Evaluation (SCORE) system.
- Positive family history in 1st and 2nd degree relatives < 50 years old for cardiac disease.
- Volunteers unable to be closely followed for social, geographic or psychological reasons.
- Any clinically significant abnormal finding on biochemistry or haematology blood tests, urinalysis or clinical examination.
- 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.

5.3.6 Study Interventions

Vaccine manufacture, preparation and administration

R21 (Batch no: 01015-01) was manufactured and vialed under Good Manufacturing Practice conditions at the Clinical Biomanufacturing Facility, University of Oxford: the production, manufacture and storage of this product have been described in Section 4.3.6. Generation, manufacture and storage of the ChAd63 ME-TRAP (Batch no: 01S11-01) and MVA ME-TRAP (Batch no: 0091013) vaccines and the Matrix-M Batch no: M1-103) adjuvant has been described in Section 3.3.5. At the CBF the R21 and the adjuvants were certified and labelled for both these trials by a Qualified Person (QP) before transfer to the clinical site.

On the day of vaccination, R21 was thawed to room temperature and was administered intramuscularly into the deltoid of the non-dominant arm within 1 hour of removal from the freezer, mixed with Matrix-M. The viral vectored vaccines were administered intramuscularly within 1 hour of thawing into the deltoid of the dominant arm (Contralateral arm to R21 administration).

5.3.7 Assessment of safety

All volunteers were observed in the unit for 1 hour after vaccination. Volunteers were provided with an electronic diary card to record their temperature and any solicited local and systemic adverse events for 7 days post-vaccination and unsolicited adverse events for 28 days post-vaccination. An independent Safety Monitoring Committee provided safety oversight during the course of the trial. Safety bloods including full blood count, renal function and liver function tests were done on visits at day 0, 7, 28, 35, 56, 63 and 83 (day before CHMI) in all vaccinated volunteers. Additional safety bloods were done on day 14 and 70 for volunteers in Group 3. Safety bloods were also performed on days 9, 35 and 90 post-CHMI.

5.3.8 Assessment of immunogenicity

Antibody responses measured by anti-NANP IgG ELISA were performed on samples from days 0, 7, 14, 28, 35, 42, 56, 63, 70 and 83. *Ex vivo* IFN-γ ELISpot responses to ME-TRAP were assessed on samples from day 0, 28, 70 and 83 in Group 3 volunteers. The inhibition of sporozoite invasion assay was done on samples from the day before CHMI (day 83).

5.3.9 Diagnosis of malaria

The CHMI procedure and the follow up visit schedule are detailed in Section 2.5. Following CHMI, a diagnosis of blood stage malaria infection was made in subjects with symptoms suggestive of malaria and positive thick film microscopy, or qPCR result >500 parasites/ml if either the thick film was negative, or symptoms were absent (Table 5-4). Vaccinated subjects who had not developed blood stage malaria by day 21 after CHMI were deemed to exhibit sterile protection.

MALARIAL SYMPTOMS	THICK FILM MICROSCOPY	
	Positive	Negative

Symptomatic	Successfully Infected	Successfully infected if any available PCR result is > 500 parasites/ml
Asymptomatic	Successfully infected if any available PCR result is > 500 parasites/ml (Otherwise delay treatment)	Not infected

Table 5-4. Diagnostic criteria for primary endpoint evaluation.

5.3.10 Management of malaria

The first-line treatment for all volunteers diagnosed with malaria was Riamet, which is a combination drug consisting of 20mg artemether and 120mg lumefantrine per tablet. In addition, all volunteers who had not developed malaria at day 21 after the mosquito bite were treated with a full course of Riamet. A treatment course of Riamet consisted of 6 doses of 4 tablets. The first 4 tablets were given in our clinic when a diagnosis of malaria was made and blood was taken for safety bloods. In addition, volunteers also attended clinic at 24 and 48 hours after initiation of treatment for directly observed therapy of the third and fifth dose. During these two visits, volunteers were also reviewed to ensure there were no clinical concerns and blood was taken for thick film microscopy and qPCR. Volunteers were discharged from the intensive post-CHMI follow up if they were clinically improved and had two consecutive negative thick blood films. Volunteers who had a contraindication to Riamet (for eg. concomitant use of QT prolonging agents) were treated with Malarone, which is a combination of 250mg atovaquone and 100mg proguanil hydrochloride. A treatment course consisted of 3 doses of 4 tablets and all doses were observed in clinic. Volunteers were also provided with antihistamine cream, cyclizine and paracetamol for symptomatic management.

5.4 Results

5.4.1 Participant flow

From 7th November, 2016 to 31st January, 2017, a total of 43 volunteers of the 88 who were screened for eligibility were enrolled into this study. One volunteer in Group 1 withdrew after their first vaccination due to a change in personal circumstances and was replaced. Another Group 1 volunteer withdrew after their second vaccination due to a change in personal circumstances. One Group 2 volunteer withdrew after their first vaccination as they were no longer able to commit to the schedule of attendances. Two volunteers in Group 3 withdrew after their third vaccination due to a change in personal circumstances. Another Group 3 volunteer withdrew after their fifth vaccination due to apprehensions to undergo CHMI. None of the withdrawals were related to the vaccination and there were no ongoing AEs and safety bloods were normal. 37 volunteers (11 Group 1 volunteers, 11 Group 2 volunteers, 9 Group 3 volunteers and 6 unvaccinated controls) underwent CHMI on the 30th and 31st January 2017. Participant flow and study design is summarised in Figure 5-1.

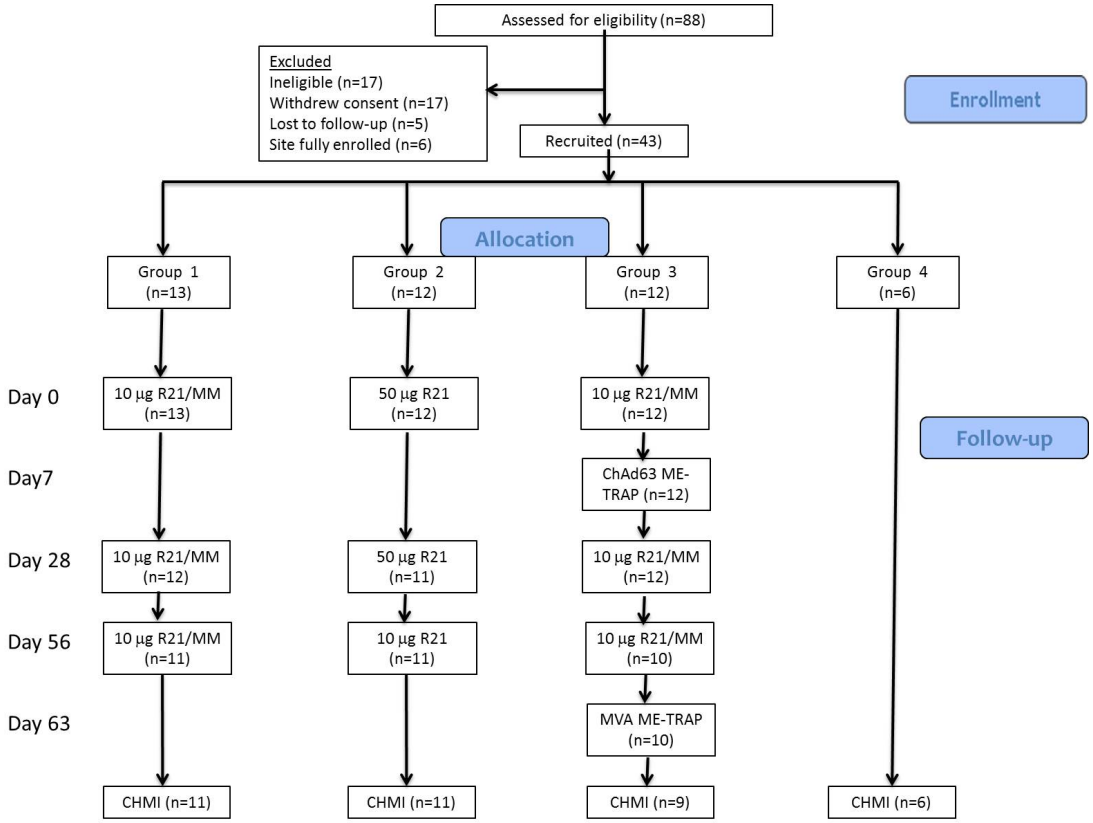


Figure 5-1. VAC065 flow chart of study design and volunteer recruitment. *Abbreviations: MM, Matrix-M. Abbreviations: ChAd63, chimpanzee adenovirus serotype 63; ME-TRAP, multiple epitope string fused to the thrombospondin-related adhesion protein; MVA, modified vaccinia Ankara; ChAd63 ME-TRAP was administered at 5 x 10^{10} viral particles and MVA ME-TRAP was administered at 2 x 10^{8} plaque forming units. A total of 88 volunteers were screened and 43 were enrolled in total. One volunteer in Group 1 withdrew after their first vaccination and was replaced and another Group 1 volunteer withdrew after their second vaccination. One Group 2 volunteer withdrew after their first vaccination. Two volunteers in Group 3 withdrew after their third vaccination. Another Group 3 volunteer withdrew after their fifth vaccination.*

5.4.2 Vaccine efficacy

There were 9/11 Group 1 volunteers, 7/11 Group 2 volunteers and 6/9 Group 3 volunteers who hadn't developed malaria at 21 days post-CHMI resulting in a sterile efficacy of 81.8% (p=0.0009), 63.6% (p=0.004) and 66.7% (p=0.006), respectively (Figure 5-2A). Five out of six control volunteers developed malaria with a mean time to diagnosis of 11.3 days (Median 11, range 11-12, SD 0.45). A significant delay in

patency was observed in all three groups with a mean time to diagnosis of 15.3 days (Median 15.3, range 14.5-16, SD 1.1), 16.4 days (Median 16.5, range 14.5-16, SD 1.5) and 15.3 days (Median 16, range 14-16, SD 1.2) in Groups 1, 2 and 3, respectively (Table 5-5).

	n=6	**n=11**	**n=11**	**n=9**
Time point of diagnosis for each volunteer	**Day of diagnosis**			
	Controls	**Group 1**	**Group 2**	**Group 3**
	11	14.5	14.5	14
	11	16	16	16
	11		17	16
	11.5		18	
	12			
Mean Diagnosis Time point	**11.3**	**15.3**	**16.4**	**15.3**
Standard deviation	0.45	1.1	1.5	1.2

Table 5-5. VAC065 - Comparison of days post-CHMI at diagnosis between control and vaccinated volunteers.

There was a significant difference in secondary efficacy endpoints measured using qPCR (Figure 5-2B & C). The significant delay in being diagnosed with malaria in the vaccinated volunteers compared to the controls was also reflected in the parasite density dynamics measured by the sensitive quantitative PCR assay. The mean delay in reaching the secondary endpoint of qPCR > 500 parasites/ml was 3.5 days, 3.3 days and 2.3 days in Groups 1, 2 and 3, respectively. I compared the mean delay in qPCR > 500 between the 10/10/10μg R21/MM in VAC065 with previous efficacy data on three doses of RTS,S/AS01 from our trial centre using the same PCR method

performed by the same operator. This showed that there was a longer delay in patency with R21/MM in comparison to RTS,S/AS01 (3.5 days vs 2 days).

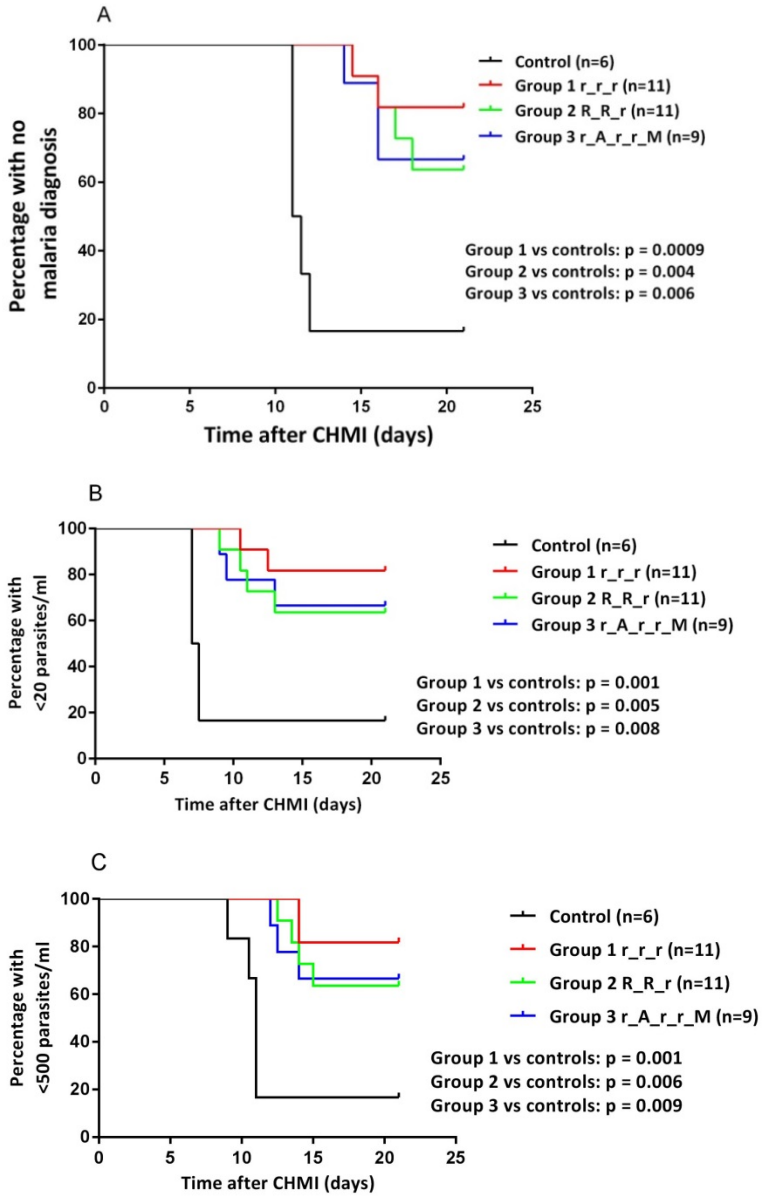


Figure 5-2. VAC065 - Efficacy of R21 adjuvanted with Matrix-M given alone and in combination with ChAd63-MVA ME-TRAP following *Plasmodium falciparum* 3D7 sporozoite challenge. *Group 1 received 10/10/10μg R21/MM; Group 2 received 50/50/10μg R21/MM and Group 3 received 10/10/10μg R21/MM with ChAd63-MVA ME-TRAP. The control group did not receive any vaccinations and underwent CHMI. (A) Kaplan–Meier survival analysis of the time to treatment following initial CHMI. (B) Kaplan–Meier survival analysis of the time to the first sample with >20 parasites/mL detected by quantitative polymerase chain reaction (qPCR). (C) Kaplan–Meier survival analysis of the time to the first sample with > 500 parasites/mL detected by qPCR. Significance testing was performed by the log-rank test.*

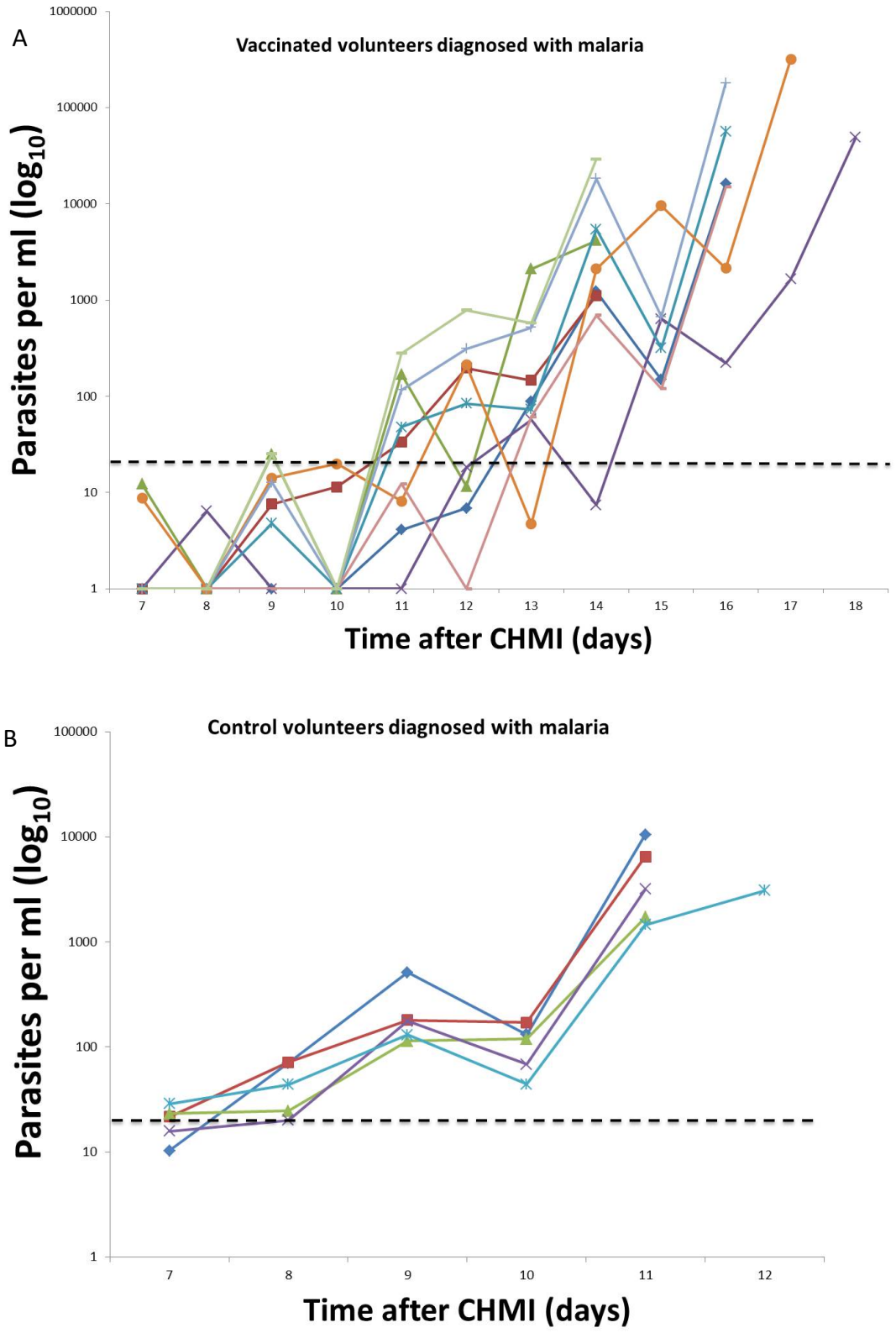


Figure 5-3. VAC065 - Parasite densities measured by quantitative polymerase chain reaction. *A) Vaccinated volunteers who developed blood stage malaria before day 21 after CHMI (n=9). B) Control volunteers who developed blood stage malaria before day 21 after CHMI (n=5). Individual qPCR data are shown. The lower limit of quantification is indicated by the dotted line at 20 parasites/mL.*

5.4.3 Vaccine safety and reactogenicity

No serious adverse reactions (SARs) or suspected unexpected serious adverse reactions (SUSARs) occurred. There were no withdrawals due to safety concerns and no pre-defined study stopping or holding rules were activated. The majority of adverse events (AEs) reported were self-limiting and mild in severity. The reactogenicity profile of R21 adjuvanted with Matrix-M observed was similar to that seen in VAC053. Solicited local and systemic adverse events in the first 7 days after each R21 vaccination are shown in Figures 5-4 to 5-6. Vaccine site pain was the most common local adverse event and was predominantly mild in severity. The addition of ChAd63 ME-TRAP to the 10μg R21/MM dose did not result in increased reactogenicity of the second and third vaccination with R21/MM. The local and systemic adverse events reported after ChAd63 ME-TRAP and MVA ME-TRAP are shown in Figure 5-7. Unsolicited adverse events collected for 28 days after each vaccination deemed definitely, possibly or probably related to vaccination were predominantly mild in nature and are coded according to the MedDRA classification and tabulated in Table 5-6. Laboratory adverse events were predominantly Grade 1 according to the FDA toxicity grading scale; they are tabulated in Table 5-7 and 5-8.

In view of the fact that high level efficacy was observed in Group 1 volunteers, I compared the reactogenicity profile of the 10/10/10μg R21/MM (VAC053 and VAC065 combined) with the adverse event profile of 50μg RTS,S/AS01 in a previous clinical trial conducted in our centre (VAC055) [136]. The adverse event profile was significantly improved with 10μg R21/MM after all three doses in comparison to RTS,S/AS01 (Vaccination 1- $p<0.0001$, Vaccination 2- $p<0.0001$ and Vaccination 3-

p=0.005). There was also a considerably higher percentage of moderate and severe adverse events reported by volunteers receiving RTS,S/AS01 (Table 5-9).

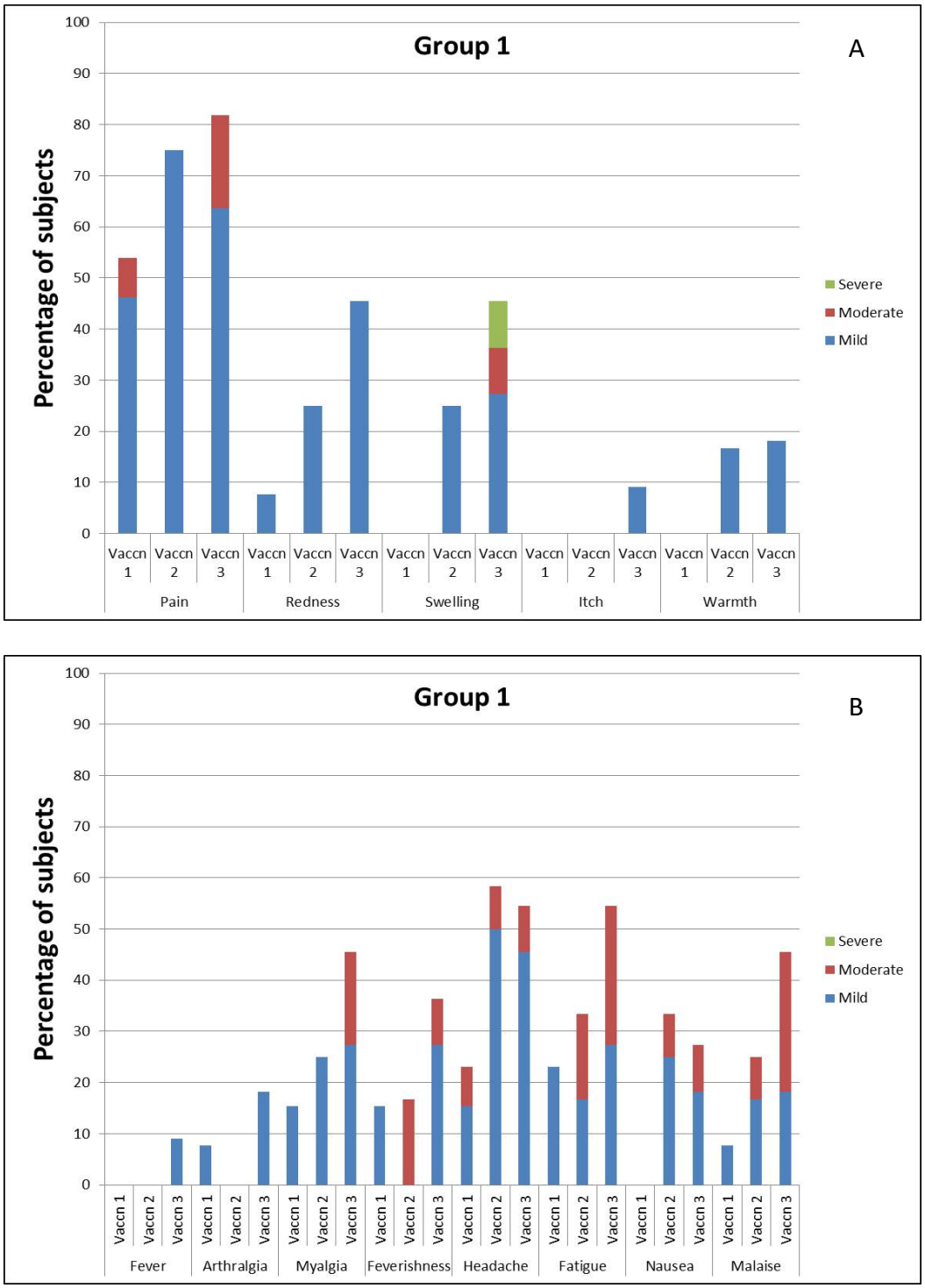
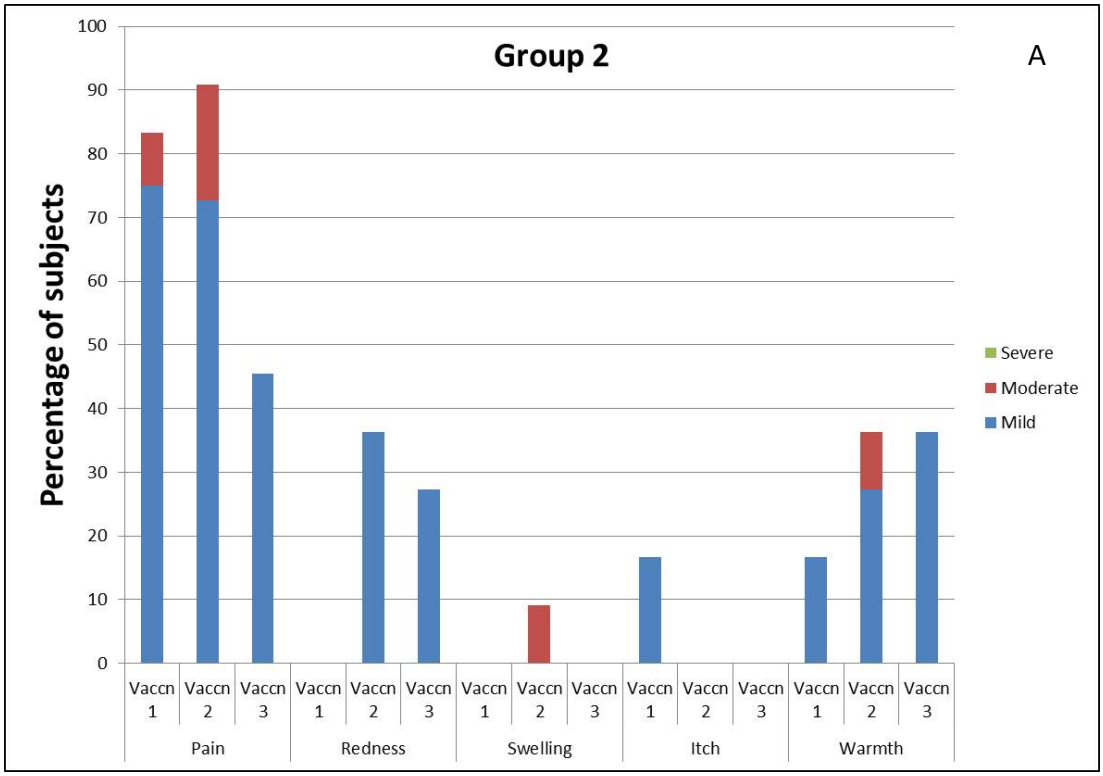


Figure 5-4. VAC065 - Local (A) and systemic (B) solicited adverse events (AE) reported by Group 1 volunteers during the first seven days after each vaccination. *Volunteers received three vaccinations of 10μg R21/MM, given 4 weeks apart. Only the highest intensity of each AE per subject is listed. Data are combined for all AEs for all volunteers receiving the same vaccine at the stated time point.*



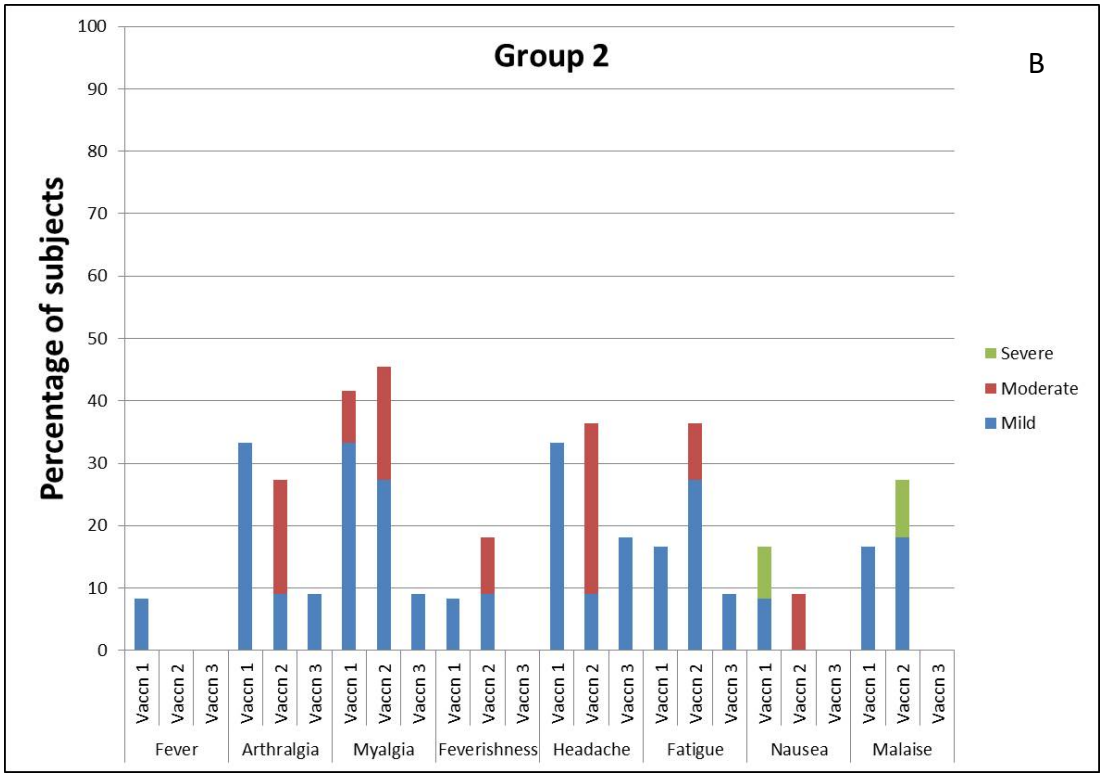


Figure 5-5. VAC065 - Local (A) and systemic (B) solicited adverse events (AE) reported by Group 2 volunteers during the first seven days after each vaccination. *Volunteers received two vaccinations of 50μg R21/MM, given 4 weeks apart followed by a third vaccination of 10μg R21/MM given at 8 weeks. Only the highest intensity of each AE per subject is listed. Data are combined for all AEs for all volunteers receiving the same vaccine at the stated time point.*

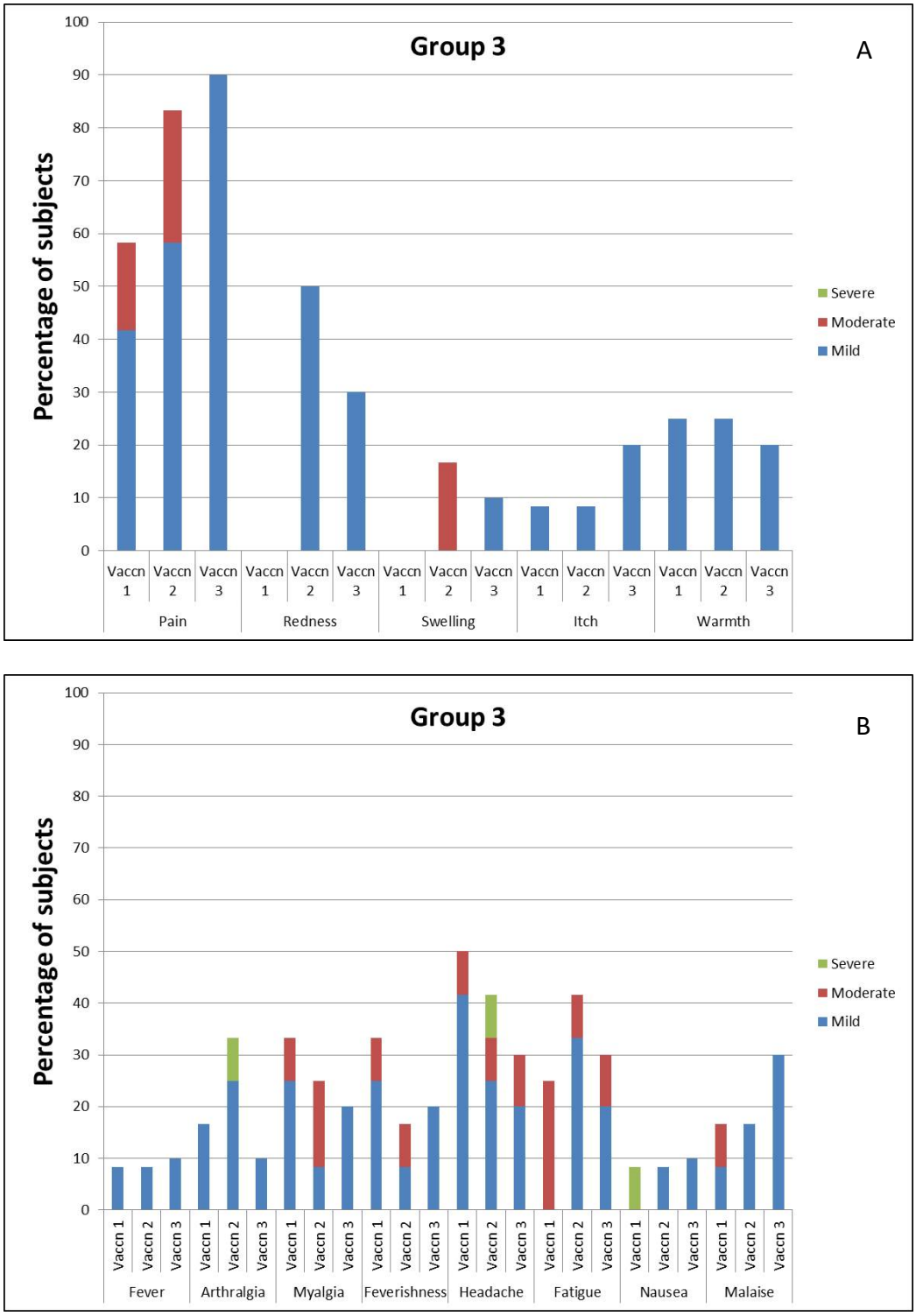


Figure 5-6. VAC065 - Local (A) and systemic (B) solicited adverse events (AE) reported by Group 3 volunteers during the first seven days after each vaccination. *Volunteers received three vaccinations of 10μg R21/MM, given 4 weeks apart. They had also received vaccinations with ChAd63 ME-TRAP and MVA ME-TRAP at weeks 1 and 9, respectively. The adverse event profile of these vaccinations is shown in Figure 5-7. Only the highest intensity of each AE per subject is listed. Data are combined for all AEs for all volunteers receiving the same vaccine at the stated time point.*

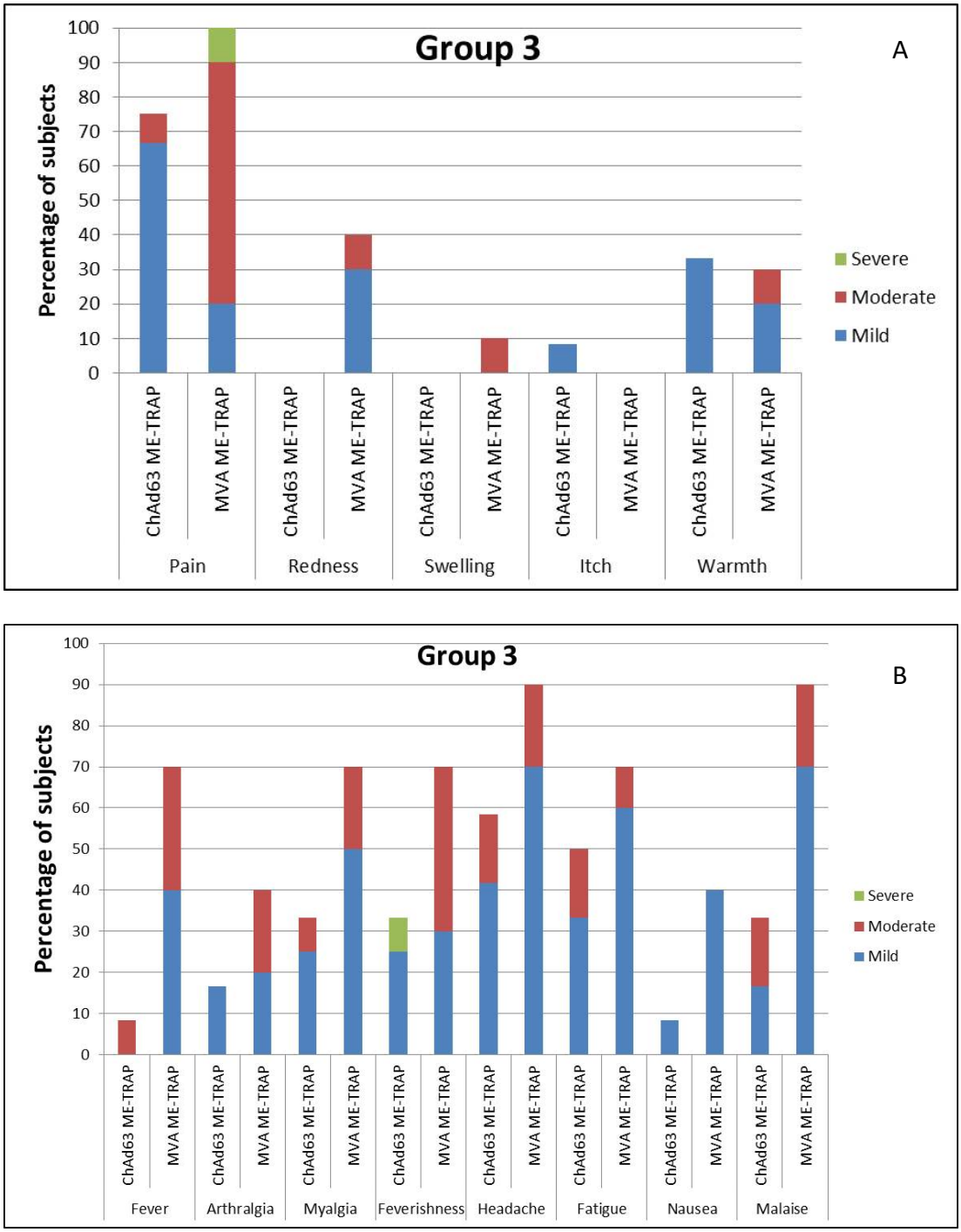


Figure 5-7. VAC065 - Local (A) and systemic (B) solicited adverse events (AE) reported by Group 3 volunteers during the first seven days after vaccination with ChAd63-MVA ME-TRAP. *They had also received three vaccinations of 10μg R21/MM, given 4 weeks apart. The adverse event profile of these vaccinations is shown in Figure 5-6. Vaccinations with ChAd63 ME-TRAP and MVA ME-TRAP were administered at weeks 1 and 9, respectively. Only the highest intensity of each AE per subject is listed. Data are combined for all AEs for all volunteers receiving the same vaccine at the stated time point.*

Symptom	MedDRA code	Intensity	Day of onset	Last day	R21 Vaccination no.	Causality
10/10/10µg R21/Matrix-M						
Dry skin	10013786	Mild	2	3	1	Possible
Fatigue	10016256	Mild	7	8	1	Possible
Light headedness	10024492	Moderate	1	1	3	Possible
Light headedness	10024492	Mild	0	0	2	Probable
Painful arm	10033503	Mild	18	18	2	Possible
Persistent dry cough	10034707	Moderate	0	12	2	Possible
Rash	10037844	Mild	2	5	1	Probable
Runny nose	10039296	Mild	1	1	2	Possible
Runny nose	10039296	Mild	0	1	2	Possible
Shortness of breath	10040604	Mild	1	4	3	Possible
Irritation lips	10049342	Mild	1	1	2	Possible
Phlegm	10059004	Mild	1	1	2	Possible
Dysaesthesia pharynx	10062665	Mild	0	0	2	Possible
Vaccination site scab	10069618	Mild	10	12	2	Probable
50/50/10µg R21/Matrix-M						
Arthralgia	10003239	Mild	7	7	2	Probable
Chest discomfort	10008469	Mild	4	4	3	Possible
Coryzal symptoms	10011216	Mild	4	4	3	Possible
Dry cough	10013773	Mild	0	14	2	Possible
Fatigue	10016256	Mild	7	9	2	Probable
Light headedness	10024492	Mild	3	3	3	Possible
Malaise	10025482	Mild	7	7	2	Probable
Migraine	10027599	Moderate	0	0	2	Possible
Sore throat	10041367	Mild	3	7	2	Possible
Sweating	10042661	Mild	2	2	2	Probable
Vaccination site erythema	10059079	Mild	8	8	1	Probable
Vaccination site pain	10068879	Mild	8	9	1	Probable
Vaccination site pruritus	10068881	Mild	10	10	3	Probable
Vaccination site pruritus	10068881	Mild	8	8	1	Probable

Symptom	MedDRA code	Intensity	Day of onset	Last day	R21 Vaccination no.	Causality
Vaccination site swelling	10069620	Severe	8	9	1	Probable
Vaccination site warmth	10069624	Mild	7	9	1	Probable
Vivid dreams	10047666	Mild	0	0	1	Probable
10/10/10μg R21/Matrix-M + ChAd63-MVA ME-TRAP						
Axillary pain	10048750	Mild	2	4	1	Probable
Cough	10011224	Mild	2	2	3	Possible
Dizziness	10013573	Mild	0	3	1	Possible
Feeling cold	10016326	Mild	1	2	3	Possible
Feeling cold	10016326	Moderate	0	1	MVA ME-TRAP	Possible
Feeling cold	10016326	Mild	1	1	ChAd63 ME-TRAP	Possible
Head cold	10019192	Mild	0	2	3	Possible
Head cold	10019192	Mild	0	4	3	Possible
Headache	10019211	Mild	8	9	ChAd63 ME-TRAP	Possible
Heart rate increased	10019303	Mild	1	1	MVA ME-TRAP	Probable
Hyperaesthesia	10020568	Mild	0	3	2	Possible
Migraine	10027599	Mild	0	7	3	Possible
Migraine	10027599	Mild	0	0	MVA ME-TRAP	Possible
Muscle spasms	10028334	Moderate	1	1	ChAd63 ME-TRAP	Possible
Nasal stuffiness	10028771	Mild	0	0	2	Possible
Pain in arm	10033421	Mild	8	8	2	Possible
Rash	10037844	Mild	1	1	2	Possible
Runny nose	10039296	Mild	0	2	3	Possible
Runny nose	10039296	Mild	0	1	1	Possible
Shakiness	10040527	Mild	2	3	3	Possible
Shivering	10040558	Mild	1	1	ChAd63 ME-TRAP	Probable
Sore throat	10041367	Mild	1	2	1	Possible
Sore throat	10041367	Mild	0	3	2	Possible
Sore throat	10041367	Mild	7	14	ChAd63 ME-TRAP	Possible
Sore throat	10041367	Mild	7	7	ChAd63 ME-TRAP	Possible
Stomach pain	10042112	Mild	1	5	2	Possible
Vaccination site erythema	10059079	Mild	7	7	1	Probable
Vaccination site lump	10078374	Moderate	0	9	MVA ME-TRAP	Probable

Symptom	MedDRA code	Intensity	Day of onset	Last day	R21 Vaccination no.	Causality
Vomiting	10047700	Mild	7	7	3	Possible
Vomiting	10047700	Mild	2	2	2	Possible

Table 5-6. VAC065 – Unsolicited AEs deemed definitely, probably and possibly related to vaccination reported in the 28 days following vaccination. *The maximum severity for each AE is shown. AEs have been classified according to the Medical Dictionary for Regulatory Activities (MedDRA) at the Lower Level (LLT) Term level.*

Lab AE and intensity	Number (% of volunteers)		
10/10/10μg R21/MM	**Vaccination 01 (n=13)**	**Vaccination 02 (n=12)**	**Vaccination 03 (n=11)**
Eosinophillia Grade 1	1 (7.7)	1(8.3)	0
Hyponatraemia Grade 1	0	0	1 (9.1)
Elevated Urea Grade 2	0	1 (8.3)	0
Elevated ALT Grade 1	0	0	1 (9.1)
Elevated ALP Grade 1	0	1 (8.3)	0
50/50/10μg R21/MM	**Vaccination 01 (n=12)**	**Vaccination 02 (n=11)**	**Vaccination 03 (n=11)**
Anaemia Grade 1	0	1 (9.1)	0
Elevated WBC Grade 1	1 (8.3)	0	0
Lymphopaenia Grade 1	0	1 (9.1)	0
Hypokalaemia Grade 1	0	0	2 (18.2)
Elevated ALT Grade 1	0	0	1 (9.1)

Table 5-7. VAC065 - Frequency of all laboratory adverse events following vaccination with R21 adjuvanted with Matrix-M. *Adverse events are defined as a deviation from the baseline result collected pre-vaccination on Day 0.*

Lab AE and intensity	Number (% of volunteers)

	R21 Vacc 01 (n=12)	ChAd63 ME-TRAP (n=12)	R21 Vacc 02 (n=12)	R21 Vacc 03 (n=10)	MVA ME-TRAP (n=10)
Anaemia					
Grade 1	0	2 (16.7)	1 (8.3)	0	0
Grade 2	0	0	0	1 (10)	0
Low WBC					
Grade 1	1 (8.3)	0	0	0	0
Eleveated WBC					
Grade 1	0	0	0	1 (10)	0
Thrombocytopaenia					
Grade 1	0	0	1 (8.3)	0	0
Grade 2	0	0	0	0	1 (10)
Neutropaenia					
Grade 1	0	0	1 (8.3)	0	1 (10)
Lymphopaenia					
Grade 2	0	0	0	1 (10)	0
Eosinophillia					
Grade 1	0	1 (8.3)	0	0	0
Hyperbilirubinaemia					
Grade 1	0	1 (8.3)	0	0	0

Table 5-8. VAC065 - Frequency of all laboratory adverse events following vaccination with R21 adjuvanted with Matrix-M administered with ChAd63-MVA ME-TRAP. *Adverse events are defined as a deviation from the baseline result collected pre-vaccination on Day 0.*

LOCAL AE's ALL VACCINATION

	10 μg R21/MM	50 μg RTS,S/AS01
None	**237**	**145**
Mild	**93**	**83**
Moderate	**4**	**17**
Severe	**1**	**5**
SYSTEMIC AE's ALL VACCINATION		
	10 μg R21/MM	**50 μg RTS,S/AS01**
None	**418**	**233**
Mild	**90**	**83**
Moderate	**28**	**66**
Severe	**0**	**18**
ALL AE's ALL VACCINATION		
	10 μg R21/MM	**50 μg RTS,S/AS01**
None	655	378
Mild	183	166
Moderate	32	83
Severe	1	23
Total possible AEs	**871**	**650**
% mild	**21.0**	**25.5**
% moderate	**3.7**	**12.8**
% severe	**0.1**	**3.5**
Total %	**24.8**	**41.8**

Table 5-9. Comparison of the local and systemic adverse event profile between 10μg R21/MM and 50μg RTS,S/AS01B. *The total number of local and systemic adverse events reported by all volunteers following all vaccinations of each vaccine regime is shown.*

5.4.4 CHMI safety

There were no serious adverse events or withdrawals from the trial due to CHMI-related adverse events. Adverse events related to CHMI in volunteers diagnosed with malaria are shown in Figure 5-8. None of the AEs occurring after CHMI were considered possibly, probably or definitely related to vaccination. The AE profile was significantly worse in the control volunteers who were diagnosed with malaria in comparison to the vaccinated volunteers who were diagnosed with malaria ($p<0.0001$). The control volunteers reported a higher proportion of severe AEs in comparison to the vaccinees (Table 5-10).

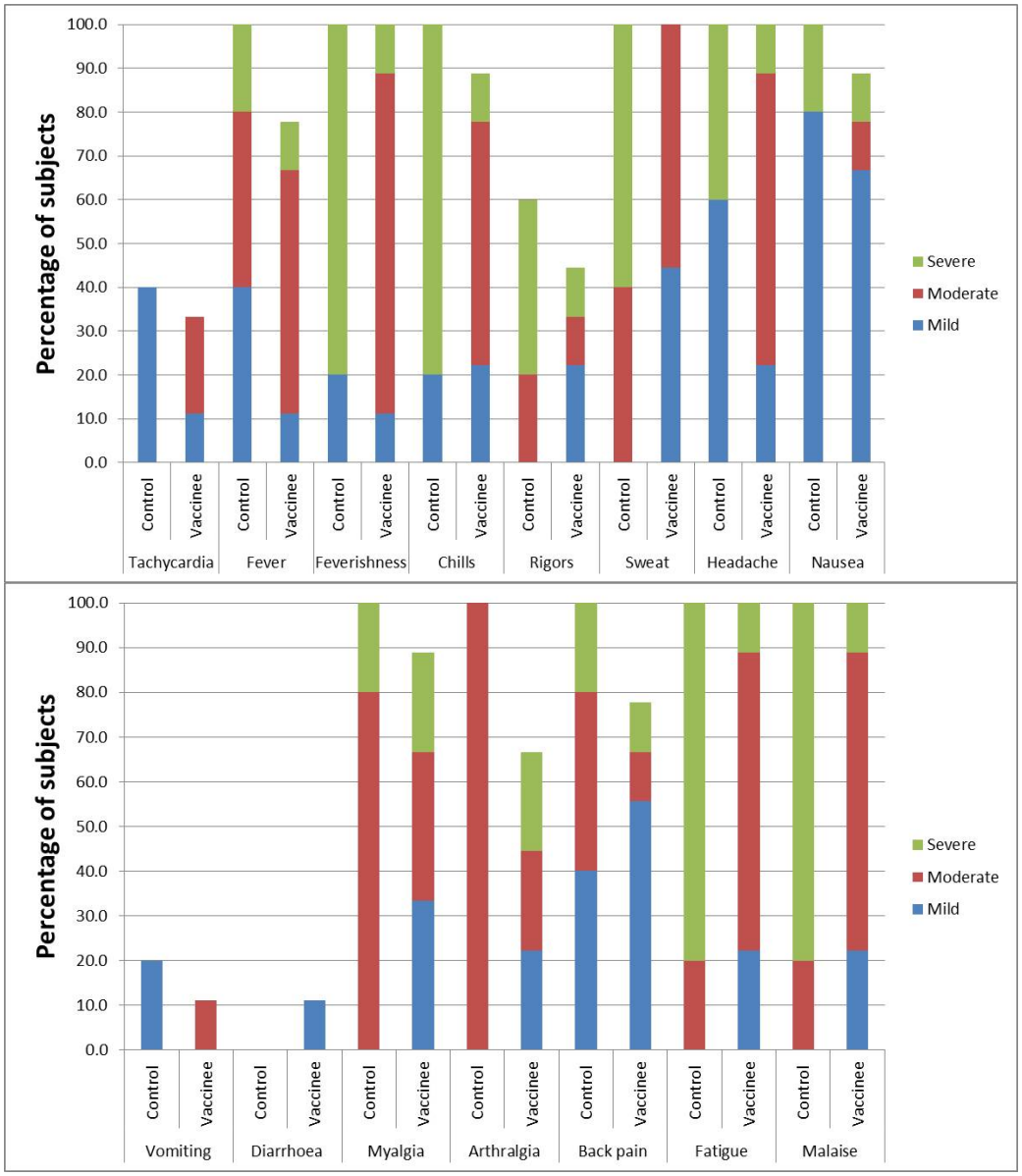


Figure 5-8: VAC065 - Adverse events reported by volunteers diagnosed with malaria after CHMI. *Percentage of control (n=5) and vaccinated (n=9) volunteers who developed CHMI-related AEs during the course of their follow-up. Only the highest intensity of each AE per subject is listed.*

	Control (n=5)	Vaccinee (n=9)
None	14	37
Mild	16	34

Moderate	**18**	**51**
Severe	**27**	**13**
%mild	21.3	25.2
%moderate	24.0	37.8
%severe	36.0	9.6

Table 5-10. Comparison of the adverse event profile between control volunteers (n=5) and vaccinated volunteers (n=9) who were diagnosed with malaria after CHMI. *Only the highest intensity of each AE is taken into account and the number of mild, moderate and severe AEs reported by volunteers is shown.*

5.4.5 Immunogenicity

The dynamics of the humoral response to the NANP region was similar across all three groups (Figure 5-9). The peak response was observed at day 70 in Group 2 [median (IQR): 1267 (989.5-1928.5 ELISA units)] and Group 3 [median (IQR): 1798.5 (1075.8-2300.5 ELISA units)]. The peak response in Group 1 volunteers was observed at day 42 [median (IQR): 1798 (978.5-3188 ELISA units)]. There were no significant differences in the antibody titres measured on the day before CHMI (C-1) (Figure 5-10). Furthermore, there was no correlation with the antibody titres and sterile protection (Figure 5-11). I have also made a comparison with C-1 antibody titres from a previous trial conducted in our centre (VAC055) where volunteers received either RTS,S/AS01 alone or RTS,S/AS01 with ChAd63-MVA ME-TRAP. Though the C-1 antibody titres were significantly higher in both VAC055 cohorts (R21/MM vs RTS,S/AS01 – $p<0.0001$, R21/MM + ChAd63-MVA ME-TRAP vs RTS,S/AS01 + ChAd63-MVA ME-TRAP – $p=0.01$) compared to VAC065, similar high level efficacy was observed across both trials (Figure 5-12).

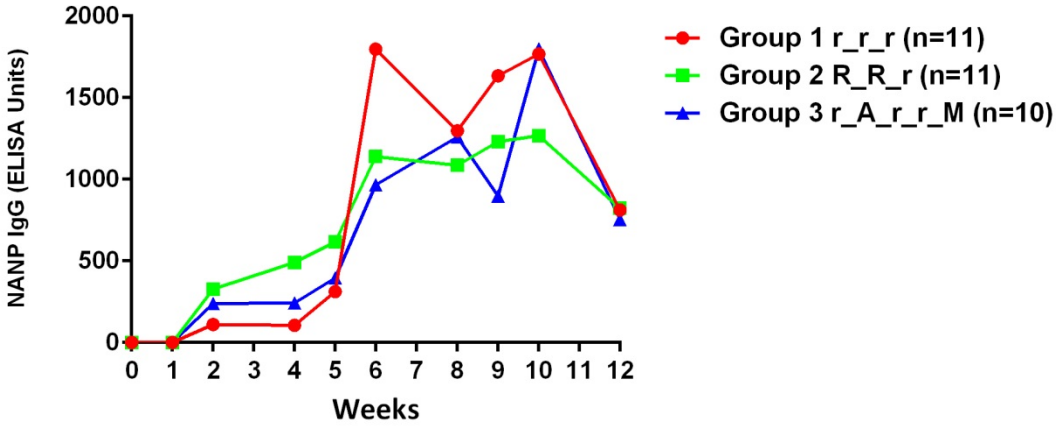


Figure 5-9. VAC065 - Median NANP IgG time courses in response to vaccination. *Volunteers in Groups 1 and 2 received three doses given four weeks apart. Group 1 (n=11) received 10/10/10μg R21/MM and Group 2 (n=11) received 50/50/10μg R21/MM. Group 3 (n=10) received three doses of 10μg R21/MM given four weeks apart with ChAd63 ME-TRAP and MVA ME-TRAP given at weeks 1 and 9, respectively.*

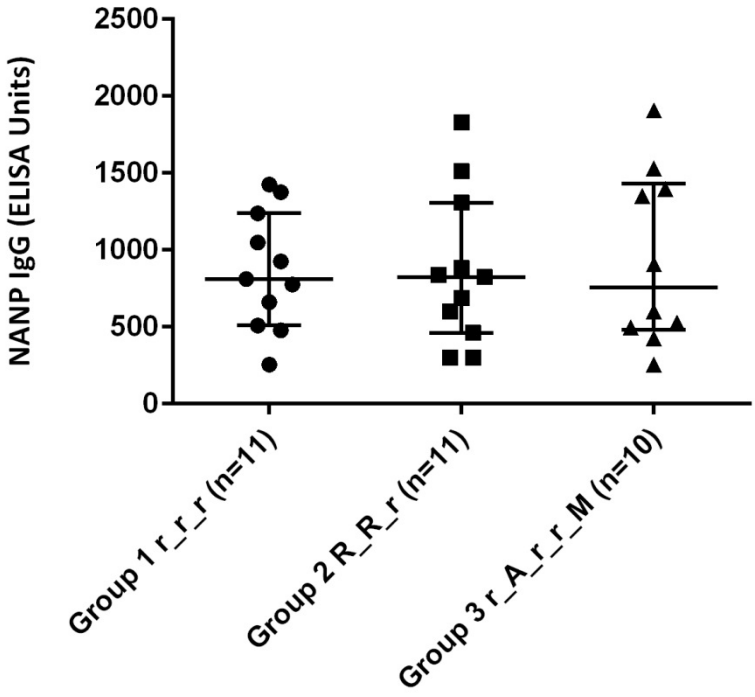


Figure 5-10. VAC065 - Individual NANP IgG responses to vaccination. *Abbreviations: r, 10μg R21/Matrix-M; R, 50μg R21/Matrix-M; A, ChAd63 ME-TRAP; M, MVA ME-TRAP. Median and interquartile ranges are shown.*

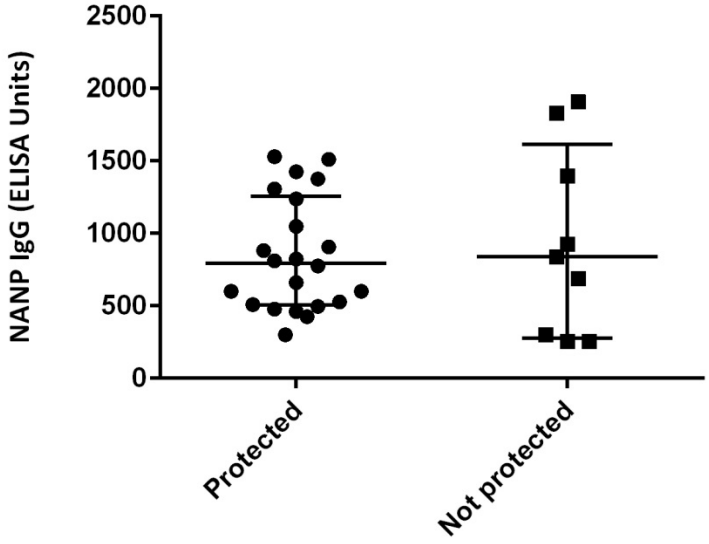


Figure 5-11. VAC065 - Comparison of individual NANP IgG responses in vaccinated volunteers who were protected and not protected from clinical malaria after CHMI. *Median and interquartile ranges are shown.*

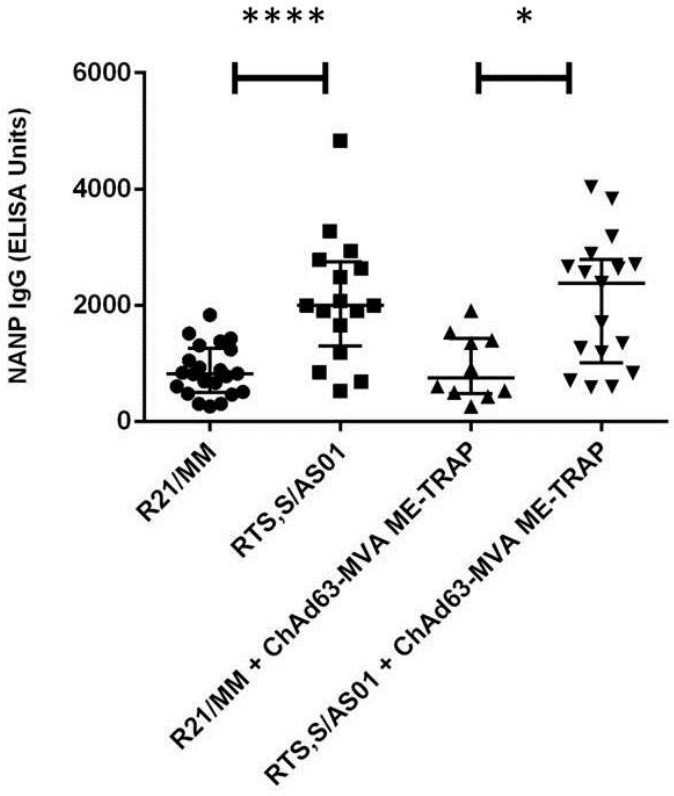


Figure 5-12. Comparison of individual NANP IgG responses in vaccinated volunteers on the day before CHMI between R21/Matrix-M and RTS,S/AS01 given alone and in combination with ChAd63-MVA ME-TRAP. *Median and interquartile ranges are shown. *p = 0.01, ****p < 0.0001.*

The additional immunoassay of interest in Group 3 volunteers is the IFN-γ ELISpot response to ME-TRAP as they received ChAd63-MVA ME-TRAP in addition to three doses of 10μg R21/MM. In keeping with VAC048, the peak response [median (IQR): 1468 (775-2676.7) SFC/10^6 PBMC)] was observed at 7 days post-MVA ME-TRAP vaccination (Figure 5-13). There were no significant differences in the peak ELISpot response in comparison to volunteers in VAC055 who received a similar regime where RTS,S/AS01 was given in a combination with ChAd63-MVA ME-TRAP (Figure 5-14). Antibody responses to TRAP were measured on the day before malaria challenge and were detected in all volunteers (Figure 5-15).

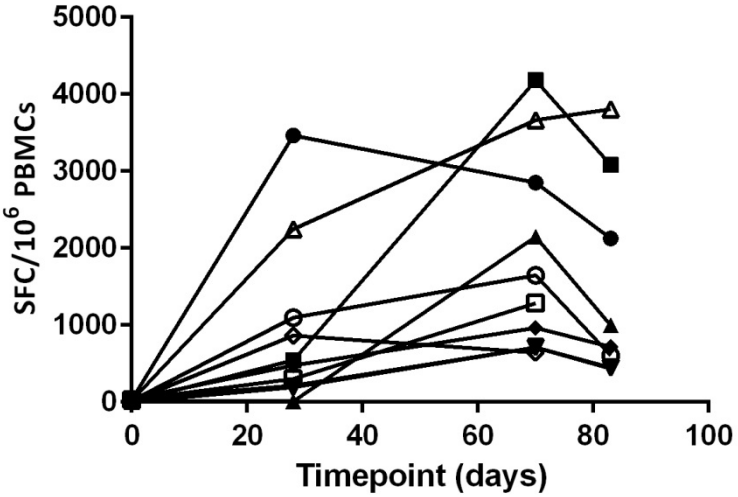


Figure 5-13. VAC065 - Individual IFN-γ ELISpot responses to ME-TRAP in Group 3 volunteers (n=10). *All volunteers received three doses of 10μg R21/MM at 0, 4 and 8 weeks and ChAd63 ME-TRAP and MVA ME-TRAP at weeks 1 and 9, respectively.*

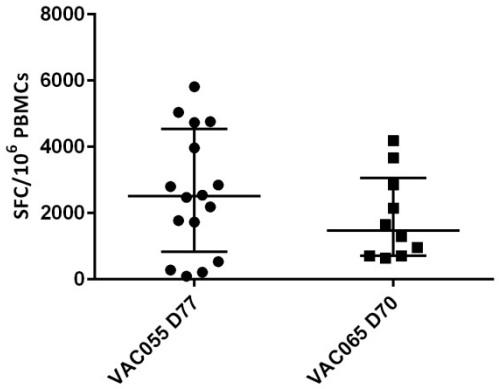


Figure 5-14. Comparison of peak IFN-γ ELISpot responses to ME-TRAP between RTS,S/AS01 and R21/MM administered with ChAd63-MVA ME-TRAP. *Comparison of the peak responses between Group 3 volunteers in VAC065 and volunteers in VAC055 who received three doses of 50μg RTS,S/AS01 at 0, 4 and 8 weeks and ChAd63 ME-TRAP and MVA ME-TRAP at weeks 2 and10, respectively.*

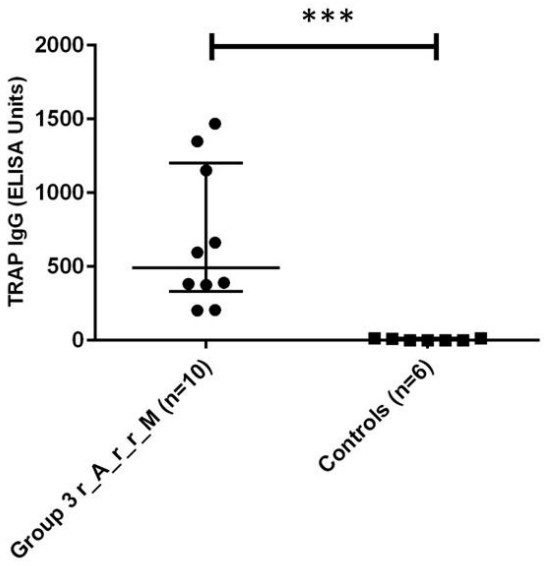


Figure 5-15. VAC065 - Individual TRAP IgG responses to vaccination. *Comparison of responses between Group 3 volunteers in VAC065 who received three doses of 10μg R21/MM at 0, 4 and 8 weeks and ChAd63 ME-TRAP and MVA ME-TRAP at weeks 1 and 9, respectively and control volunteers who received no vaccinations. ***p=0.0001*

5.4.6 Inhibition of sporozoite invasion

There were no differences in the functional ability of induced antibodies to inhibit sporozoite invasion between protected and non-protected volunteers (Figure 5-16).

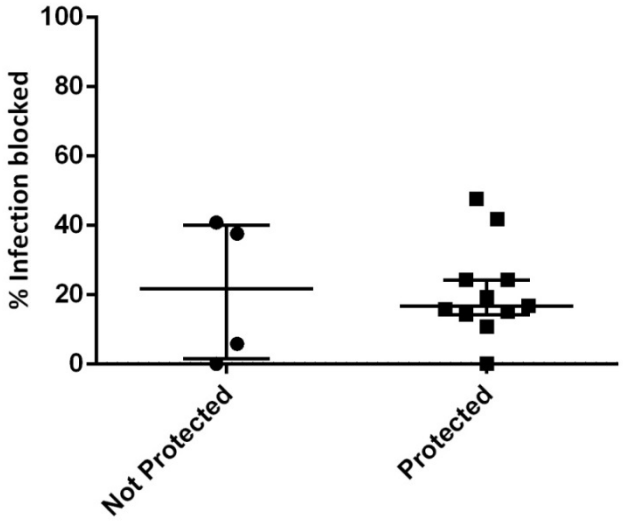


Figure 5-16. VAC065 - Comparison of the inhibition of sporozoite invasion between non-protected (n=4) and protected (n=11) volunteers.

5.5 Discussion

I report here for the first time, high level efficacy of a novel malaria vaccine candidate, R21 adjuvanted with Matrix-M at one-fifth of the dose of the most advanced malaria vaccine candidate, RTS,S/AS01. This will have significant dose-sparing and cost-saving implications for large scale production of the vaccine if it is proven to be efficacious in subjects living in malaria endemic regions. This is amongst the highest levels of sterile protection reported in previously published CHMI trials. A list of the previously published CHMI trials assessing pre-erythrocytic malaria vaccines of varying dosing schedules and intervals is shown in Table 5-11; the studies demonstrating high level efficacy (> 80%) are highlighted in yellow.

100% efficacy has only been reported using whole sporozoite vaccines. Though Seder et al. initially reported 100% efficacy, this was a small group of 6 volunteers and the intravenous regime required 5 immunisations over 19 weeks [140]. A more recent

study showed that efficacy of 86.7% can be achieved with a three-dose schedule of the same intravenous vaccine, *Pf*SPZ [142] but using many more sporozoites per dose. Delivery of non-irradiated intravenous whole sporozoites in combination with chemoprophylaxis has also demonstrated 100% efficacy in a group of 9 volunteers [148]. However, this may prove to be a logistically challenging regimen with a significant safety issue for deployment in malaria-endemic regions.

A delayed fractional third dose has demonstrated > 80% efficacy with RTS,S/AS [72, 246]. In view of this, we had reduced the dose of the third dose in Group 2 from 50μg to 10μg. In contrast to previous studies, we observed inferior efficacy in Group 2 compared to Group 1, though this was not statistically significant and this small efficacy study was not powered to compare between the different dose groups. In addition, we had not reduced the dose of the adjuvant as was the case in the studies using RTS,S/AS and our vaccines were given at 0, 1, and 2 months rather than 0, 1 and 7 months. As mentioned in Chapter 4, it may be of value for there to be to a delay between the 2nd and 3rd dose in the regime and this approach warrants further investigation.

We had previously shown that a combination regime of RTS,S/AS01 and ChAd63-MVA ME-TRAP resulted in > 80% efficacy. However, there was no synergistic effect of combining these two vaccine approaches in VAC065. Again, this regime would involve five different immunisation time points and we have shown that when RTS,S/AS01 and the viral-vectored vaccines are given concomitantly in the same arm to reduce the dose schedule to three vaccination time points, there is immunological

interference and reduced humoral responses associated with reduced efficacy (Rampling et al., manuscript submitted, 2017).

We did observe a significant delay in patency in 100% of vaccinated volunteers in comparison to the control volunteers. This was evident in both the mean time to diagnosis and parasite density dynamics measured by qPCR. A delay in patency is thought to reflect a reduction in liver-to-blood inoculum [28]. Given that there is a 10-fold multiplication in the blood stage parasite burden every 48 hours, a delay in patency of ~4 days would reflect a ~100-fold reduction in the number of injected sporozoites invading the hepatocytes in all volunteers. The parasite density dynamics in VAC065 also demonstrates a delay in parasites emerging from the liver in vaccinated volunteers compared to controls. The exact reasons for this are unclear and it is possible that the schizonts are damaged, but not killed by vaccine effects, resulting in a delay in schizonts releasing merozoites. Alternatively, this may simply reflect a sensitivity issue: detecting 100-time fewer parasites emerging from the liver will be more difficult and this would appear as a delay in emergence. In comparison, RTS,S only results in a delay in patency of ~48 hours, which would reflect a 10-fold reduction in parasite burden [136].

Whether this delay to patency would provide any additional public health benefit in enhancing field efficacy is unclear. Even if there is a reduction in parasite burden, if there are inadequate functional antibodies to facilitate destruction of all circulating sporozoites at this brief window of infection prior to invasion of hepatocytes, clinical malaria will still ensue even if one hepatocyte is infected. However, there may be some theoretical reasons that it could be beneficial but are yet to be proven in the

field. The malaria sporozoite challenge is undertaken by the bite of five infected mosquitoes, which would potentially be greater than the number of sporozoites injected in the field. Furthermore, if there is additional natural blood stage immunity, the reduced liver-to-blood inoculum may help protect from patency and clinical or severe disease. Finally, a reduced number of merozoites could result in reductions in circulating gametocytes and therefore likelihood of transmission.

Author ; year of publication	Vaccine	Dosing schedule (wks)	Protected	Efficacy
Gordon; 1995 [57]	RTS,S/ alum/MPL	50/50/50 (0, 8, 24)	2/8	25%
Stoute; 1997 [72]	RTS,S/AS02	50/50/50 (0, 4, 28)	6/7	85.7%
Kester; 2001 [247]	RTS,S/AS02 RTS,S/AS02 RTS,S/AS02 RTS,S/AS02	50/50 (0, 4) 50/50/50 (0, 4, 36) 25/25/25 (0, 4, 36) 10/10/10 (0, 4, 36)	7/14 3/6 4/7 1/4	50% 50% 57.1% 25%
Dunachie; 2006 [252]	RTS,S/AS02 + MVA-CS	RRM or MRR (0, 4, 12)	4/12	33.3%
Kester; 2007 [253]	RTS,S/AS02	RTS,S/AS02 (0, 4)	8/19	42.1%
Kester; 2008 [254]	RTS,S/AS02 RTS,S/AS02	50/50/50 (0, 4, 12) 50/50/50 (0, 1, 4)	9/20 7/18	45% 38.9%
Kester; 2009 [86]	RTS,S/AS01 RTS,S/AS02	50/50/50 (0, 4, 8) 50/50/50 (0, 4, 8)	18/36 14/44	50% 31.8%
Ewer; 2013 [129] (Hetorologous CHMI)	ChAd63-MVA ME-TRAP	N/A	3/14	21.4%
Seder; 2013 [140]	*Pf*SpZ (intravenous)	$5 \times 1.35 \times 10^5$ (0, 4, 8, 12, 19)	6/6	100%
Ockenhouse; 2015 [255]	RTS,S/AS01 Ad35.CS + RTS,S/AS01	50/50/50 (0, 4, 8) A/R/R (0, 4, 8)	11/21 11/25	52.4% 44%
Rampling; 2016 [136]	RTS,S/AS01 RTS,S/AS01 + ChAd63-MVA ME-TRAP	50/50/50 (0, 4, 8) 50/50/50 (0, 4, 8) + (2, 10)	12/16 14/17	75% 82.4%
Regules; 2016 [246]	RTS,S/AS01 RTS,S/AS01	50/50/50 (0, 4, 8) 50/50/10 (0, 4, 28)	10/16 26/30	62.5% 86.7%

Author ; year of publication	Vaccine	Dosing schedule (wks)	Protected	Efficacy
Epstein; 2017 [142] (Homologous CHMI)	*Pf*SpZ (intravenous)	5 x 2.7 x 10^5 (0, 4, 8, 12, 20)	12/13	92.3%
(Homologous CHMI)		3 x 4.5 x 10^5 (0, 8, 16)	13/15	86.7%
(Heterologous CHMI)		5 x 2.7 x 10^5 (0, 4, 8, 12, 20)	4/5	80%
Mordmuller; 2017 [148]	*Pf*SpZ-CVac (intravenous *Pf*SpZ challenge with chloroquine chemoprophylaxis)	5.12 x 10^4 (0, 4, 8)	9/9	100%

Table 5-11. Previously published CHMI trials assessing efficacy of pre-erythrocytic malaria vaccines. *Sterile efficacy > 80% is highlighted in yellow.*

I have described the efficacy assessed by CHMI of numerous pre-erythrocytic malaria vaccine approaches, including R21/MM. The key question that now remains to be answered is whether this high-level efficacy in malaria-naïve volunteers can translate to significant effectiveness in the target population of those living in malaria-endemic regions. There are numerous reasons that the high level efficacy observed in this trial may not be replicated in endemic populations including genetic differences in susceptibility to disease and responses to vaccines, prior exposure to malaria and malarial immunosuppression, co-existing medical conditions, accessibility, acceptability and local political will for deployment. The predominant number of deaths due to *Pf* malaria occur in the under 5's in Sub-Saharan Africa. This efficacy result in healthy malaria-naïve adults would require Phase I trials in adults in malaria-endemic populations before age de-escalation to children and infants to assess efficacy.

RTS,S/AS01 has demonstrated a pooled efficacy of ~60% in malaria sporozoite challenge trials. Initial assessment in a field efficacy trial in Kenyan adults showed efficacy of only ~30% [87]; however, comparable efficacy against clinical malaria of ~50% was observed in African children aged 5-17 months [91, 92]. As described previously, RTS,S/AS01 is the only malaria vaccine to have completed Phase III testing and a vaccine efficacy of ~50% is observed in 5-17 month African children during the first 12 months of follow-up [93]. This suggests that comparable efficacy to that detected in CHMI studies can be achieved in this population. However, the efficacy was much lower in African infants aged 6-12 weeks [94]. The other approach that has demonstrated high-level efficacy in CHMI studies is intravenous, irradiated, whole sporozoite vaccine, *Pf*SpZ. Assessment of the the intravenous *Pf*SpZ vaccine in the field was published recently where a five dose regime $2{\cdot}7 \times 10^5$ *Pf*SPZ given at days 0, 28, 56, 84, and 140 resulted in a 48% reduction in infection in high transmission settings, which is much lower than observed in previous CHMI studies [144].

Most of the CHMI studies detailed in Table 5-11 assess efficacy using sporozoite challenge 2-4 weeks after the vaccination schedule. Though this gives an indication of the likely efficacy of a vaccination regime, invariably the efficacy wanes when protected volunteers are re-challenged at intervals up to 6-12 months after their initial challenge. Ishizuka et al. reported efficacy of 55% at 59 weeks after the vaccination schedule with intravenous *Pf*SPZ in volunteers who had undergone a prior CHMI [141]. To my knowledge, this is the lengthiest vaccination-CHMI interval and I have invited Group 1 volunteers from VAC053 who received 10/10/10μg R21/MM 15-18 months ago to undergo sporozoite challenge in addition to Group 1

VAC065 volunteers who underwent the CHMI described in this chapter ~7 months ago. This study is currently ongoing and will give us a good indication of the likely durability of the high-level efficacy observed.

To date, no vaccine has demonstrated durable efficacy and it is well recognized that the field efficacy of RTS,S/AS01 wanes rapidly after the first year and there are also concerns of a rebound phenomenon after vaccine efficacy wanes, in high exposure populations, which is likely to reflect interference of acquisition of natural blood stage immunity [21]. R21, which is a biosimilar of RTS,S also predominantly induces antibodies to the CSP and it is likely that a combination with a blood stage vaccine will be required to circumvent this issue.

The safety data presented in this chapter also reaffirms that R21/MM is safe and well tolerated in healthy UK volunteers, even when administered with ChAd63-MVA ME-TRAP, with adverse events being predominantly mild in nature. It is also very reassuring to know the 10/10/10μg R21/MM offers a distinctly improved reactogenicity profile compared to RTS,S/AS01, which would be of value in improving acceptability for deployment.

The humoral response to the vaccine did not vary between Groups 1-3 and the addition of the viral-vectored vaccines to the regime in Group 3 in a staggered fashion did not result in reduced immunogenicity. The magnitude of the response did not predict efficacy, which suggests that the quality of the antibody response also contributes to efficacy. We have also previously shown that using a fractional third dose in a 0, 4 and 8 week regime with RTS,S/AS01 resulted in improved efficacy (non-statistically significant) and functional assessment of inhibition of sporozoite invasion

was significantly improved in this group (Rampling et al., manuscript submitted, 2017). However, there was no difference in the functional ability of the antibodies to inhibit sporozoite invasion between protected and non-protected volunteers in VAC065 although power in this analysis was very limited.

In keeping with VAC053, we did not observe any significant boosting of the humoral response after the 3rd dose in comparison to the 2nd dose in the schedule and the peak response in Group 1 was seen at 14 days after the second vaccination. I previously discussed this in Chapter 4 and it could be that a two-dose schedule would be sufficient to protect a malaria-primed population in endemic regions and a booster could be administered at 6 months. In view of this, we are currently assessing a two dose-schedule using 10μg of R21/MM in a CHMI study in healthy UK volunteers. If this is proven to have similar high level efficacy as a three-dose schedule, this would have significant cost-saving benefits and would be potentially easier to deploy in co-ordination with the EPI program.

One of the control volunteers did not develop clinical malaria, which is very unusual and has never occurred previously in CHMI trials in Oxford. However, the failure of one of the controls to develop malaria has been reported in a number of other CHMI trials undertaken by Sanaria Inc. to test the intravenous whole sporozoite vaccine [140, 141]. Kester et al. also reported that one of 23 control volunteers who underwent sporozoite challenge using the same model as used in VAC065 did not develop malaria [247]. The mean time to patency in the control group of 11.3 days indicates that this was not an unusually weak challenge, and the vaccination and CHMI methodology used in this trial are largely comparable to other CHMI studies

[136]. Furthermore, this volunteer had a very high sporozoite score (19/20) when the salivary glands of the infected mosquitoes were examined after they had taken a blood meal. There are no features in her history and examination that could explain this failure and antimalarial drug level assays did not reveal therapeutic concentrations of any of the antimalarials tested. To my knowledge, there are no other known factors that can explain why this volunteer did not develop malaria and it is likely to be a chance finding.

In conclusion, this Phase II malaria sporozoite challenge study demonstrated high level efficacy with a novel low-cost malaria vaccine, R21/MM. This study provides the first evidence that this vaccine regime should be tested in target populations in malaria endemic regions.

CHAPTER SIX:

Conclusion and future work

6.1 Conclusions and future work

Plasmodium falciparum is a highly immunoevasive pathogen and it is very likely that development of an efficacious vaccine will require a combination of different approaches acting on multiple antigens involved in different stages of its complex life cycle. The pre-erythrocytic stage of infection presents an attractive target for an efficacious human vaccine, as sufficient reduction in the number of viable merozoites reaching the blood from the liver will prevent parasitisation of red blood cells and initiation of the blood stage of infection. Prior to the work I have described in this thesis, R21, a novel malaria vaccine candidate had never been administered to humans. Similar to the most advanced malaria vaccine (RTS,S/AS01), R21 targets the circumsporozoite protein, which is expressed on the sporozoite surface and to a lesser degree on hepatic schizonts. Anti-CSP antibodies can target sporozoites, facilitating destruction of sporozoites prior to hepatocyte invasion. However, as sporozoites travel from the skin to the liver within hours, it may be difficult for a vaccine to confer high level protection based solely on antibodies to sporozoites. The liver stage of infection provides a longer window of opportunity for cell mediated immunity to recognize and destroy infected hepatocytes. The use of viral-vectored vaccines is a promising alternate approach that induces potent T-cell responses in addition to antibody responses. These Ag-specific T cells recognise and destroy parasitised liver cells and would complement CSP Ab-mediated prevention of hepatocyte invasion.

In Chapter 3, I reported for the first time that Matrix-M, a novel saponin-based adjuvant could be used safely with the heterologous prime-boost viral-vectored

regime ChAd63-MVA ME-TRAP without any evidence of immunological interference. This is an important finding as most subunit protein-particle based vaccines, including R21 require an adjuvant to be immunogenic. ChAd63-MVA ME-TRAP demonstrated 21% sterile efficacy in malaria-naïve UK volunteers [129] and a 67% reduction in infection in Kenyan adults [256]. However, though it was shown to be safe and potently immunogenic in African children and infants [132, 133], there was no significant efficacy observed in 5-17 month Burkinabe children in a double-blind randomised controlled study, against clinical malaria or severe malaria (Tiono A et al., manuscript in preparation). This suggests that significant improvements need to be made to this vaccine regime for it to be considered for deployment in this age group, although immunogenicity is far greater in young infants. Furthermore, I showed that there was no synergistic effect of adding ChAd63-MVA ME-TRAP to a sporozoite-based vaccine in VAC065, although power to detect an improvement in this trial was limited. We have also shown previously that administering RTS,S/AS01 concomitantly with ChAd63-MVA ME-TRAP resulted in reduced humoral immunogenicity and efficacy against CHMI (Rampling et al., manuscript submitted, 2017).

Attempts to improve on ChAd63-MVA ME-TRAP within the Jenner Institute have explored the use of intravenous administration in addition to the usual intramuscular route. This aims to increase the number of tissue-resident memory (TRM) T-cells localized in the liver rather than just the total number of circulating antigen-specific T-cells. In pre-clinical studies, a “prime-target” immunisation regimen involving an initial dose administered by the standard IM route followed by IV administration of

ChAd63 ME-TRAP generated substantially higher numbers of Ag-specific $CD8^+$ T-cells in the liver. This regime also conferred 100% efficacy against sporozoite challenge in mice, which correlated with the number of antigen-specific T-cells, with the characteristics of TRMs, found in the liver, rather than T-cells in the blood. The first IV vaccinations with ChAd63 ME-TRAP took place earlier this year in a dose-escalation study, which demonstrated that this approach was safe and well tolerated and elicited cellular immune responses at a dose of 5×10^{10} vp, which is the same as the optimal intramuscular dose (ClinicalTrials.gov NCT03084289; Gola et al, manuscript submitted) Though intravenous administration might not be ideal for acceptability in malaria-endemic populations, there is growing evidence that intravenous vaccinations with *Pf*SPZ can be safely and successfully administered. In addition, the prime-target regime only involves one intravenous immunisation in comparison to three to five intravenous immunisations required with *Pf*SPZ.

More recently, the ChAd-MVA platform has also been used to explore whether incorporating two hepatic antigens – Liver Stage Antigen 1 (LSA1) and Liver stage Associated Protein 2 (LSAP2) in the same vaccine would improve immunogenicity and efficacy. LSA1 is abundantly expressed on developing merozoites in the parasitophorous vacuole and is likely to be important in late liver stage differentiation prior to transitioning to a blood stage infection [257]. LSAP2 is increasingly expressed at the liver stage of infection and the antigen localises as part of the parasitophorous vacuole membrane (PVM) during liver stage development [258]. LS2, a fusion construct of LSA-1 and LSAP2 delivered using the ChAd-MVA platform conferred high level efficacy in pre-clinical studies. Preliminary immunogenicity in humans is very

encouraging and unprecedented T-cell responses (~6000 SFC/10^6PBMC) were seen at the peak time point. We are currently assessing the efficacy of this heterologous prime-boost regime against CHMI to see whether this improved immunogenicity translates to enhanced efficacy (ClinicalTrials.gov NCT03203421).

In Chapter 4, I reported on the safety and immunogenicity of the first-in-human administration of R21 administered with two different vaccine adjuvants. R21 is similar in composition to the most advanced malaria vaccine candidate RTS,S and comprises a portion of the CSP fused to HBsAg. The main difference is the ratio between the RTS,S:S, which is 1;4; whereas in R21 the proportion of CSP to HBsAg is 1:1 [226]. It was postulated that this would result in increased amount of CSP exposed on the virus-like particle and therefore result in a more robust response towards the CSP. I showed that R21 can be administered safely at doses of 2-50μg with either adjuvant. Though the addition of MM increased reactogenicity, this was markedly reduced when the dose of the antigen was reduced to 2μg. It was very reassuring that a dose of 2μg R21 administered with MM was also immunogenic after a three dose schedule. It is a very promising result that R21 administered with either adjuvant elicited comparable humoral responses to RTS,S even at one-fifth of the dose. Furthermore, this dose of 10/10/10μg R21/MM resulted in minimal reactogenicity in Burkinabe adults. Matrix-M resulted in reduced reactogenicity compared to $AS01_B$ and was therefore chosen as the adjuvant to test efficacy in a sporozoite challenge study. These results provided the basis for VAC065, which I detail in Chapter 5.

I report that 10/10/10μg R21/MM conferred high-level sterile efficacy against CHMI.

This is amongst the highest levels of efficacy reported in published CHMI trials. This provides strong evidence that this vaccine regime should be tested in adults, children and infants in malaria endemic regions. We have also previously reported high level efficacy with regimes involving RTS,S/AS01 in CHMI trials in our centre [136]. RTS,S has completed numerous Phase II and III field efficacy trials over the last 30 years, which culminated in a large multicenter randomised controlled trial in seven African nations. This trial required significant investment from stakeholders and would have cost hundreds of millions of dollars. It remains unclear whether the low-level short-lived efficacy rendered by RTS,S/AS01 would be adequate for licensure.

Mathematical modelling can assist in predicting the package of interventions that is most likely to be the optimally cost-effective approach towards reaching the ultimate goal of elimination. The local transmission intensity, seasonality profiles, local infrastructure and accessibility to health care, and population coverage are important predictors of the cost-effectiveness and public health impact of an intervention, such as a vaccine. For example, there was a higher coverage of ITNs in the trial cohort of the Phase III trial assessing RTS,S and the overall mortality was lower compared to the general population due to receipt of better medical care. A recent systematic comparison of predictions from four different mathematical models that used trial data assessing follow-up over at least 32 months in the 5-17 month age group predicted a positive public health impact over a 15-year timescale in moderate to high transmission settings. However, they did not observe the same benefit in low-transmission settings where cases typically occur in older children and adults. They considered two different dose schedules: three doses administered at 6, 7.5 and 9

months of age and a four dose schedule with a booster dose at 27 months. Overall, it was thought that there would be a public health benefit of the fourth dose assuming 90% coverage of the three-dose schedule and 80% of these individuals receiving the fourth dose (72% coverage) [259].

In this regard, the WHO is planning to undertake pilot deployment trials in three African nations to help further elucidate whether this would be a suitable vaccine for widespread deployment. This would also provide an opportunity to address safety concerns with the vaccine and compare it to the cost-effectiveness of other measures, like the usage of ITNs. In view of this, it is important to carefully examine the lessons learnt from the RTS,S trials to delineate the best approach to progressing the testing of R21 in endemic populations. R21 is a biosimilar of RTS,S and whether it will improve on field efficacy observed with RTS,S remains to be seen. We have observed efficacy of 75-80% with both vaccines in CHMI trials in our centre. It is also worth considering that R21 will face the same problems as RTS,S with a 'rebound phenomenon' as it is also a sporozoite-based vaccine, which would interfere with the acquisition of natural blood stage immunity in vaccinated volunteers. In this regard, recent advances in the Jenner Institute developing the Rh5 blood stage antigen are very promising and a trial testing efficacy against blood stage challenge is currently ongoing (ClinicalTrials.gov NCT02927145).

I have shown that R21/MM has a number of distinct advantages in comparison to RTS,S/AS01 – high-level efficacy is observed at one-fifth of the dose of RTS,S, which will significantly reduce the cost of production per dose. This lower dose also has the more durable humoral immunogenicity at 6 months. Assessment of efficacy against

CHMI at 7-18 months in volunteers who received 10/10/10μg R21/MM is currently ongoing. It may well be that a dose of 2μg would be sufficient in children and infants as it has already been shown to be immunogenic in adults. The use of MM instead of AS01 would also further reduce the cost of production and I have also shown that it results in a favourable reactogenicity profile. Overall, the reduced cost of production per dose would be advantageous in improving the cost-effectiveness of R21 in comparison with RTS,S/AS01.

R21 was initially manufactured on a small-scale at the CBF, University of Oxford with a high cost per dose. At present, there are very few doses of the vaccine that remain and there are plans for it to be manufactured on a large-scale at the beginning of next year, which will significantly reduce the cost of production per dose. Subsequently, there are plans to initiate Phase IIb trials in adults in malaria endemic regions to assess field efficacy. In addition, there are plans to initiate age de-escalation efficacy trials in African infants. The exact dosing schedule and interval remains to be defined and would ideally harmonise with the delivery of EPI vaccines. As mentioned in Chapter 5, we are currently testing the efficacy against CHMI of a two-dose 10/10μg R21/MM schedule and this would be a significant improvement on RTS,S/AS01, if high-level efficacy is again observed. This would also provide further merit to administering a delayed booster dose at 6 months which may prove beneficial for affinity maturation and antibody persistence.

There still remain a number of unanswered questions regarding the underlying immunological mechanisms that render immunity to a complex parasitic disease, both in natural settings and in response to vaccines. Antibody responses to the CSP

are the most widely studied and have been shown to be associated with protection [86]; however, a clear threshold level indicative of protection has long been sought after to no avail [260]. It is known that all NANP-binding antibodies don't have the same capacity for sporozoite neutralization and this may explain why though there is a broad correlation between antibody titres and protection, there is no very clear threshold for protection [261]. In Chapter 5, I report that there were no differences between the antibody titers between the three groups despite somewhat varying efficacy and there was a broad range of antibody responses that rendered efficacy. Furthermore, there were no differences in the functional ability of the induced antibodies to inhibit sporozoite invasion between protected and non-protected volunteers. We have previously reported much higher levels of anti-CSP antibodies in a CHMI trial using RTS,S/AS01; however, this did not result in improved efficacy in comparison to VAC065. In the same trial, we also assessed efficacy in sterilely protected volunteers at 6 months after the initial challenge; though antibody responses had waned significantly, 12/14 (86%) of volunteers remained protected [136]. This suggests that the quality of the initially induced humoral response was relevant to efficacy in addition to the magnitude of the response. In VAC053, though the antibody responses had waned at 6 months after the final dose, the more durable response was seen in volunteers who had been administered the lower dose (10μg). It would be of great interest to see whether the antibody responses seen at 6 months have persisted 15-18 months later and whether any of these volunteers are protected against CHMI. This study is currently ongoing.

RTS,S also induces very low level CD4 responses to the CSP and stronger CD4

responses are seen in protected individuals compared to susceptible individuals; however again there is no clear threshold that determines efficacy [86]. Similarly, in VAC065, minimal IFN-γ ELISpot responses to the CSP were observed. It is likely that T-dependent B cell responses play a role in immunity against malaria and it was recently shown in mice that CSP-specific B cells underwent somatic hypermutation and affinity maturation indicative of a T-dependent response [261]. I have also previously mentioned that a delayed fractional third dose of RTS,S/AS01 resulted in improved efficacy associated with enhanced somatic hypermutation [246]. Identification of a clear threshold of protection, as has been done with a number of other infectious diseases, would be a significant step forward in the development of malaria vaccines. This would circumvent the need to undertake large efficacy trials in malaria endemic populations to prove efficacy.

A protein particle subunit vaccine such as R21 requires an adjuvant for it to be immunogenic. There have been significant advances in the development of vaccine adjuvants over the last 20 years. In addition to aluminum salts, a number of novel adjuvants have been identified, some of which are components of recently licensed vaccines. Increased understanding of the underlying mechanisms of their action involving different arms of the immune system will prove invaluable. In addition to enhancing antigen presentation by prolonging deposition, they can also enhance the magnitude and influence the nature of the adaptive immune response (for eg. Th1 v Th2). The key focus of a number of the novel adjuvants are ligands for pattern recognition receptors (PRR) that are expressed by numerous cells of the innate immune system to identify two classes of molecules: pathogen-associated molecular

patterns (PAMPs) and damage-associated molecular patterns (DAMPs) that are released during host cell damage or death. PRRs can also orchestrate release of inflammatory cytokines and adaptive immune responses. The most extensively studied group of PRR-ligands that form constituents of vaccine adjuvants are ligands of toll-like receptors. AS01, which has been tested in VAC056 contains MPL, which is a TLR-4 agonist and stimulates the innate immune system by activating dendritic cells and facilitating antigen presentation. There are a number of other TLR-ligands that are being developed and have been tested in clinical studies. It would be of interest to assess the innate immune responses induced by R21/MM to see whether that could predict the quality and persistence of the antibody response as there was no correlation between the magnitude of the immune response and sterile efficacy.

Traditionally, efficacy of novel malaria vaccine candidates has been assessed using CHMI in malaria-naïve volunteers to prove proof-of-concept prior to testing field efficacy. One of the main disadvantages of this approach is that it does not take into account the impact of the interactions with natural immunity that may exist in populations living in malaria-endemic populations. Though there were three studies in the 1950s and 1960s where African volunteers were challenged with *Pf* malaria [262-265], there has been no further studies until recently. One of the main obstacles was that most recent CHMI studies in malaria-naïve volunteers have been conducted using the bites of *Pf*-infected *Anopheles stephensi* mosquitoes, which are not native to Africa. Furthermore, this method requires a CL3 insectary, which was unavailable in most African settings. Since 2012, there have been two studies conducted in centres in Tanzania [224] and Kenya [219] where *Pf*SPZ challenge (aseptic, purified,

cryopreserved sporozoites) has been administered intradermally and intramuscularly to adult volunteers. Both studies demonstrated that *Pf*SPZ challenge was safe, well tolerated and infective. Assessing efficacy of vaccines against malaria using this controlled model in the target population gives a better indication of the likely effectiveness of the vaccine and provides a unique opportunity to assess parasite multiplication rates in the target population, growth inhibition antibody activity, the mechanisms underlying field efficacy and interactions with host factors including naturally acquired immunity, innate immunity, and underlying haemoglobinopathies [219, 224]. There are plans to undertake a CHMI trial in Kilifi, Kenya to assess the efficacy of R21/MM in naturally immune adults.

The exact dosing schedule and the interval between vaccinations are important to consider when assessing R21/MM in malaria-endemic populations. I reported in Chapter 4 that a very-low dose of 2μg/MM is safe and immunogenic in adults and could potentially be a suitable dose for use in children and infants. I also reported that a lower dose of R21/MM (10μg) elicited the more durable response. As discussed in Chapter 4, using lower doses results in improved Th2-like Tfh responses associated with improved durability and memory B-cell responses. In this regard, a three-dose schedule of 2/2/10μg or 2/2/2μg R21/MM given at 3, 4 and 9 months in co-ordination with EPI vaccines could be an optimal regime that warrants testing in African children and infants.

6.2 Concluding Remarks

Though *Pf* malaria is one of the leading infectious causes of morbidity and mortality

worldwide, we have seen significant reductions over the last 15 years in the morbidity and mortality caused by this disease despite there not being a licensed vaccine. This is mainly due to increased uptake of ITNs, the widespread use of ACT and a significant increase in funding. However, resistance to artemisinins is well documented in the Mekong region and insecticide resistance is also widespread. The global scientific community has endeavoured to develop a durable, efficacious vaccine for over 50 years. Numerous attempts targeting different antigens in the various stages of its complex life cycle including antibody-based and T cell-mediated vaccines have yet to yield a licensed vaccine. In view of the fact that *Pf* is a complex, immunoevasive pathogen, it is unclear whether R21/MM administered alone would be adequate to progress towards the ultimate goal of elimination. However, it would be an invaluable complementary tool in a package of concurrent measures that are implemented in countries progressing towards elimination. In an attempt to curtail artemisinin resistance, there is a significant renewed interest in implementation of mass drug administration campaigns to interrupt malaria transmission in the Mekong region. This may provide a critical opportunity to deploy a vaccine even if protection is short-lived (6-12 months), especially in low-endemic populations nearing elimination. The safety, immunogenicity and efficacy data presented in this thesis make R21/MM a promising candidate malaria vaccine, with distinct advantages over RTS,S/AS01, that could be used in a malaria elimination package. I conclude on a somber note; the history of humanity's struggle with this disease is a gentle reminder of the tortuous path that lies ahead towards the ultimate goal of malaria elimination.

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APPENDICES

Appendix 1: Volunteer information sheets

VAC048 Volunteer information sheet

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REC REF: 12/SC/0036

VOLUNTEER INFORMATION SHEET: VAC048

Safety and Immunogenicity of ChAd63 ME-TRAP / MVA ME-TRAP heterologous prime boost malaria vaccination adjuvanted with Matrix M™: A Phase I Controlled Clinical Trial

We would like to invite you to take part in a clinical trial. Before you decide, it is important for you to understand why the research is being done and what it would involve. Please take time to read the following information carefully and discuss it with friends, relatives and your General Practitioner (GP) if you wish.

- Part 1 tells you the purpose of the study and what will happen to you if you take part.
- Part 2 tells you more information about the conduct of the study.

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Part 1

What is the purpose of the study?

Malaria affects around 250 million people each year, about a million of whom die from the disease. It is a major problem for those who live in affected areas and for travellers. There is a great need for a safe, effective malaria vaccine. Researchers around the world, including members of Professor Hill's group at the University of Oxford, have been investigating malaria for over 15 years. For over 10 years we have been conducting clinical studies of new malaria vaccines.

The **ChAd63 ME-TRAP vaccine** is based on a weakened version of an adenovirus (a common cold virus). The adenovirus has been inactivated so that it can not multiply within the body. A piece of DNA based on part of the malaria parasite has been added to the adenovirus to make the vaccine.

The **MVA ME-TRAP vaccine** is based on the Modified Vaccinia Virus Ankara (MVA). MVA was used for smallpox vaccination, and is unable to multiply within the body. A piece of DNA based on part of the malaria parasite has been added to the MVA virus to make the vaccine.

Matrix M™ is a product, known as a vaccine adjuvant, which is used in vaccine research to improve the immune responses to vaccines.

The purpose of this study is to assess the safety of the **ChAd63 ME-TRAP vaccine mixed with Matrix M™**, and the safety of the **MVA ME-TRAP vaccine mixed with Matrix M™.** We also want to look at what effect **Matrix M™** has on the immune response to the vaccines.

Some volunteers will be vaccinated with **ChAd63 ME-TRAP vaccine mixed with Matrix M™**, followed by a second vaccination with the **MVA ME-TRAP vaccine mixed with Matrix M™.** For comparison, other volunteers will receive the **ChAd63 ME-TRAP** vaccine, followed by a second vaccination with the **MVA ME-TRAP** vaccine**.**

We will do blood tests to assess the response of the immune system to the vaccines.

We hope to recruit 22 volunteers to be vaccinated.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. You are free to withdraw at any time and without giving a reason but you may be asked to return to the clinic for follow up for safety reasons.

What will happen if I decide to take part?

This study involves having two vaccinations, at an interval of two months, and being followed up with blood tests for a further four months.

Length of research

If you decide to take part in this study, you will be involved in the trial for 6 months.

Am I eligible to be involved in the trial?

In order to be involved in the study you must:

- be a healthy adult aged between 18 and 50 years.
- be able and willing (in the investigators' opinion) to comply with all study requirements.
- allow the investigators to discuss your medical history with your GP.
- if you are a woman, practice continuous effective contraception for the duration of the study
- if you are a man, use barrier contraception for 3 months from the day of any administration of Matrix M™
- refrain from blood donation during the course of the study and for 6 months after the end of your involvement in the study

You cannot participate in this study if:

- You have had malaria before.
- You have travelled to a malaria endemic region in the six months preceding your involvement in the study or are intending to travel to a malaria endemic region during the study.
- You have participated in another research study in the 30 days preceding involvement in this study.
- You are planning to participate in another study at the same time as participating in this study.
- You have previously received an investigational malaria vaccine
- You have had immunoglobulins and/or any blood products in the three months preceding your involvement this trial.
- You have problems with your immune system.
- You are pregnant, breast feeding or intend to become pregnant during the study.
- You have a history of a severe allergic reaction to a vaccination or an allergy to eggs.
- You have a history of cancer.
- You have a history of a serious psychiatric condition that may affect participation in the study.
- You have any other serious chronic illnesses requiring hospital follow-up.
- You drink on average more than 42 units of alcohol a week (a pint of beer is two units, a small glass of wine 1 unit and a shot of spirits one unit)
- You have injected drugs at any time in the last 5 years.
- You have hepatitis B, hepatitis C or HIV infection.
- You have a history of autoimmune disease.

Mild conditions, such as childhood asthma which is well controlled would not automatically exclude you from participating. If you are unclear whether you are eligible to be involved in the study you can contact the study team who will be able to advise you.

Considerations before taking part in this study

Screening Visit: This takes place up to 3 months before the study starts and lasts up to one and a half hours. The purpose of the screening visit is for you to discuss the trial with us and decide if you still wish to enter the study. If you decide to participate, you will be asked to sign a consent form. We then need to check that you are eligible to participate. You will be asked some medical questions and a doctor will examine you. Some blood tests will be taken to check your red and white cells, your liver and your kidney function. Your blood will also be tested for infection with hepatitis B, hepatitis C or HIV. These viruses are transmitted by infected blood and can affect the immune response to infection. Your blood will also be tested for anti-nuclear antibodies. If present, these antibodies can indicate the presence of, or pre-disposition to autoimmune disease. If there are any abnormal blood test results, we will inform you of the result and offer referral for medical review as required, with your permission. All participants are asked for urine samples at screening to check for glucose (to exclude diabetes), protein and blood (which can indicate kidney disease). For women, a urine pregnancy test will also be performed.

To avoid repeated testing, if you are not enrolled into this study and apply to enter another study conducted by the Jenner Clinical Trials Group based at the Centre for Clinical Vaccinology & Tropical Medicine (CCVTM) the screening blood results may be used in that study, where appropriate.

Blood Donation: Under current UK regulations, volunteers will not be able to donate blood during the study or for 6 months after the end of the trial.

Private Medical Insurance: If you have private medical insurance you are advised to contact your insurance company before participating in this trial.

Malaria Prophylaxis: You should note that the vaccines being tested in this trial are experimental. If you travel to a malaria endemic region in the future you should not assume that the vaccines you received in this study have given you any protection against malaria. Make sure you visit your GP before travelling to a malaria endemic region and use prophylactic anti-malarial medications, bed nets and insect repellent during your trip as directed by your GP.

Contraception: The vaccines being tested in this study are at an early stage of development and it is currently unknown as to whether they are safe in pregnancy. For this reason, it is important that all women use adequate contraception for the duration of the trial. Men must use barrier contraception from day of any vaccination with Matrix M™ for 3 months.

Vaccinations

What are the vaccinations that are being tested?

We are testing four vaccinations:

- ChAd63 ME-TRAP
- MVA ME-TRAP
- ChAd63 ME-TRAP mixed with Matrix M™
- MVA ME-TRAP mixed with Matrix M™.

Vaccinations will be given into the muscle of the arm.

ChAd63 ME-TRAP is based on a virus that infects chimpanzees, called chimpanzee adenovirus 63. We have genetically disabled the virus so that it is impossible for it to grow in humans. We added a section of DNA that encodes a protein of the malaria parasite. We want to try and make the body develop an immune response to this malaria protein. Over 180 people have been vaccinated with ChAd63 ME-TRAP with no serious side effects related to the vaccine. It can cause transient short-lived side effects, and appears to be safe and well tolerated.

MVA ME-TRAP is based on an attenuated virus called modified vaccinia virus Ankara (MVA) and also contains the added section of DNA. Over 700 people have received MVA ME-TRAP with no serious side effects related to the vaccine. It can cause transient short lived side effects, and appears to be safe and well tolerated.

Matrix M™ is a vaccine adjuvant. These are compounds that are mixed with vaccines to improve the immune response. Matrix M™ has been given to 45 people, mixed with an influenza vaccine. There have been no serious side effects related to it. Side effects have been transient and short lived, and it appears to be safe and well tolerated. A similar vaccine adjuvant, called ISCOMATRIX™, has been given with various vaccines to over 500 people, and appears to be safe and well tolerated.

What Vaccinations will I receive?

You will be allocated to one of three groups. Each group will receive a different course of vaccinations. All volunteers will receive two vaccinations. The vaccinations are summarised in the table below.

Group	Number of Volunteers	First vaccination	Second Vaccination eight weeks later
1	6	ChAd63 ME-TRAP	MVA ME-TRAP
2	8	ChAd63 ME-TRAP mixed with Low Dose Matrix M™	MVA ME-TRAP mixed with Low Dose Matrix M™
3	8	ChAd63 ME-TRAP mixed with Standard Dose Matrix M™	MVA ME-TRAP mixed with Standard Dose Matrix M™

Side effects of Vaccination
It is likely that you will experience some local or general side effects from vaccination. It is important to remember these vaccinations are in the early stage of development. Therefore the amount of safety data available is limited. For this reason there is a chance you could experience a side effect that is more severe than that described below, or that has not been seen before with these vaccines.

Local Reactions
You will probably experience some discomfort at the site as the injection is given. This is expected to get better in 5 minutes. Later, you might experience pain, and this might result in some difficulty moving your arm fully. Redness, swelling, or warmth at the injection site may also occur. The reactions should resolve completely, generally within 3 days.

General reactions
You will probably experience flu-like symptoms, for example, fever, chills, headache, nausea, aching in the joints and muscles, or tiredness after vaccination. The reactions should resolve completely, generally within 3 days. You are encouraged to take over the counter medications such as paracetamol or ibuprofen as soon as you experience these symptoms as this is likely to reduce the intensity of any symptoms you have.

Severe Reactions
With any vaccination there is a risk of rare serious adverse events, such as an allergic reaction, which may be related to the nervous system or the immune system. Severe allergic reactions to vaccines (anaphylaxis) are also rare, but can be fatal. Reactions in the nervous system are also extremely rare following vaccination and can cause an illness called Guillain-Barré syndrome. Guillain-Barré syndrome is an illness in which people can develop severe weakness and can also be fatal. These adverse events have not previously been seen with MVA ME-TRAP or AdCh63 ME-TRAP or Matrix M™.

If you experience unexpected events, or become in any way concerned you should contact one of the Investigators (who are available 24 hours a day) using the contact details at the end of Part 2.

Number, timing and purpose of visits

There are 13 study visits, with an extra two study visits for the first three volunteers in the Matrix M™ groups. All visits will take place at the Centre for Clinical Vaccinology & Tropical Medicine (CCVTM), on the Churchill Hospital site in Oxford (OX3 7LJ).

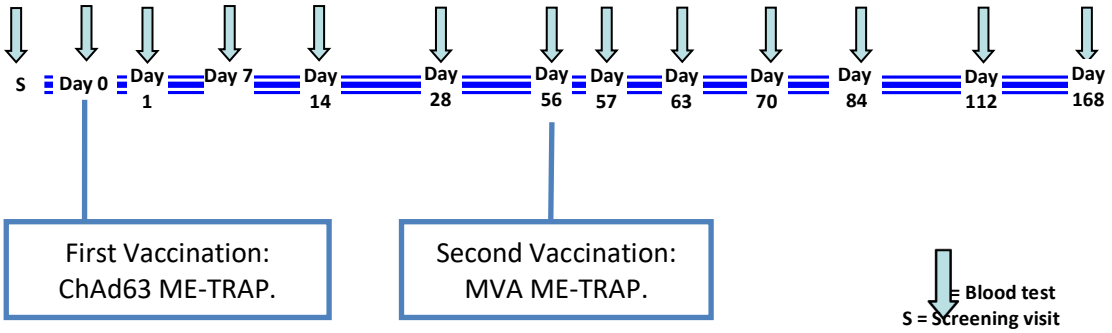
There are two visits where a vaccination will be given. We will ask you to wait for 45 minutes after each vaccination to make sure there are no immediate problems. You will be assessed again before leaving and given a diary card, thermometer and tape measure to take away. We will ask you to record your symptoms and the size of any redness or swelling every day for 7 days after vaccination.

Follow up of previous vaccinations will be done at all of the visits (except the first visit). Visits include a medical assessment, temperature, pulse and blood pressure readings, examination by a doctor if needed, and blood tests.

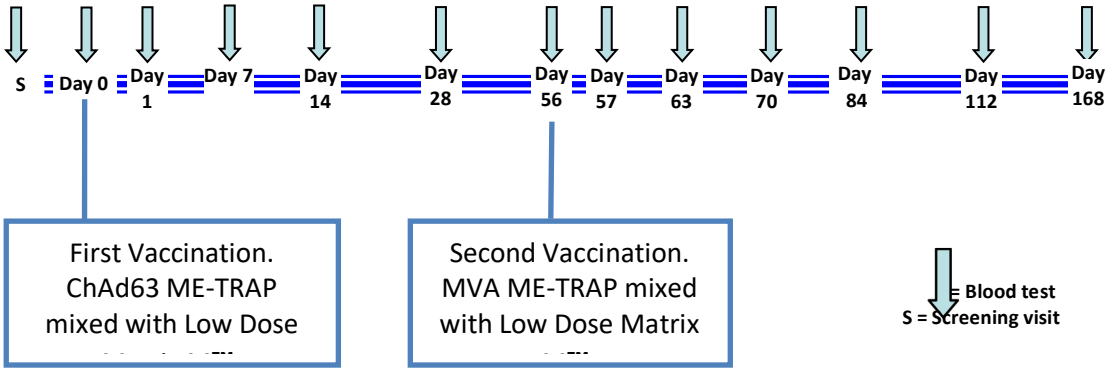
We may ask to photograph your vaccination site and you can choose whether or not to agree to this when you sign the consent form. You will not be identifiable in these photographs, as only the vaccination site and your unique trial number will be visible. These photographs may be shown to other professional staff, used for educational purposes or included in a scientific publication.

Timeline of visits

<u>Group 1</u>

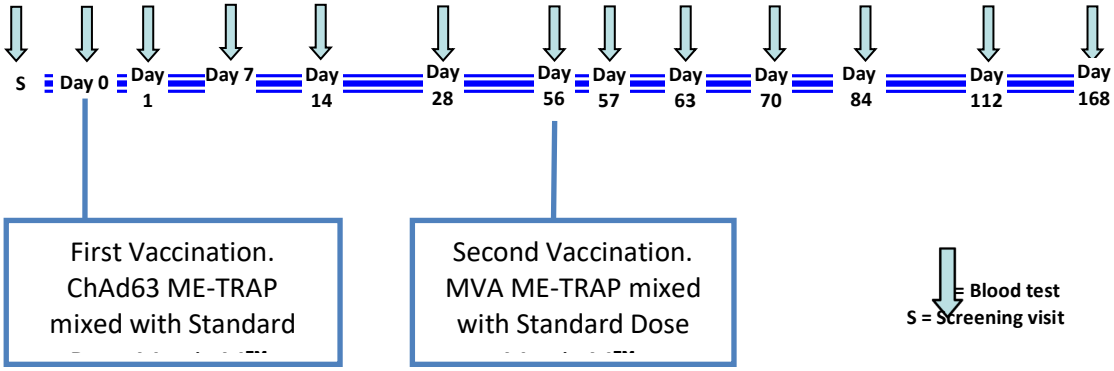


<u>Group 2</u>



The first three volunteers in Group 2 will have an additional visit three days after each vaccination.

Group 3



The first three volunteers in Group 3 will have an additional visit three days after each vaccination.

Other Information

Blood Tests

We take blood tests as part of the screening visit to help us to assess your general health. Blood tests are also taken at the study visits in order for us to assess your immune response to the vaccine and to monitor for side effects. If you would like them, we will give you the results of the blood tests. We only send the results to your GP if you wish us to and will not report them to anyone without your permission. The total volume of blood taken during the study is approximately 639ml (43 tablespoons) over six months.

To summarise the blood tests,

- Tests for Hepatitis B, Hepatitis C and HIV are done at the screening visit.
- HLA typing, a test of a component of the body's immune system, is done at the first vaccination visit.
- Tests of red and white blood cells and tests of liver and kidney function are done at the screening visit and most of the the other visits when you come for vaccination.

- The presence of anti-nuclear antibodies will be tested at screening. They will be tested again at two months after each vaccination.
- Tests of the immune responses are done at most visits.
- At some of the visits we also test to see what genes are being used to generate your immune response. We may also do DNA sequencing, that is, determining parts of the genetic code that may affect how people respond to the vaccine- you can opt out of DNA testing if you wish, without any effect on the rest of the study.
- Every time you have a blood test, the volume of blood taken ranges from 10ml to 90 ml

Urine Tests

- A urine sample will be tested at screening to check for glucose, protein and blood. For volunteers receiving Matrix M™, this will also be done a further five times during the study.
- All women will have urinary pregnancy testing at screening and before each vaccination.

Abnormal Results

If abnormal results or undiagnosed conditions are found in the course of the study these will be discussed with you and, if you agree, your GP will be informed. For example, a new diagnosis of high blood pressure might be made. Any newly diagnosed conditions will be looked after by your GP within the NHS.

Expenses and Payments

You will be compensated for:

o Travel expenses:	£10 per visit
o Time required for visit:	£20 per hour
o Inconvenience of blood tests:	£10 per blood donation

The total compensation will be £600 (£660 for the first three volunteers in the Matrix M™ groups, who will have an additional two visits).

If you choose to leave the study early or are withdrawn from the study, you will be compensated according to the length of your participation based on these figures. You should note that compensation payments received in this trial may have an impact on your entitlement to benefits.

What do I have to do?

- You **must** provide a name and 24 hour phone number for someone who lives near to you and who will know where you are for the duration of the study.
- You **must** attend all the visits that are outlined above.
- You should record in the study diary all the things you notice about injection sites, any other change in your health or the way you feel after each injection.
- Women **must** use an effective method of contraception for the duration of the study.

- Men **must** use barrier contraception from day of vaccination with Matrix M™ for 3 months.
- You **must not** donate blood during the study or for 6 months after the end of the trial (current National Blood Service guidelines).

What alternatives are present?
At present, there is no malaria vaccine licensed anywhere in the world. There are other malaria vaccines in various stages of development. This study may help develop an effective malaria vaccine.

What are the possible benefits of taking part?
This study will not benefit you, but the information gained from the study might help to prevent malaria infection and disease in those who live in areas where malaria is common and in travellers.

What if there is a problem?
Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2.

What happens when the research study stops?
If you have any queries or concerns once the study is over please do not hesitate to get in touch with us.

Will my taking part in the study be kept confidential?
Yes. All the information about your participation in this study will be kept confidential. The details are included in Part 2.

This completes Part 1 of the Information Sheet. If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2

What if relevant new information becomes available?
Sometimes during the course of a research project, new information becomes available about the vaccine that is being studied, for example if unexpected side effects occur. If this happens, we will tell you about it and discuss whether you want to or should continue in the study. If you decide to continue in the study you will be asked to sign an updated consent form. On receiving new information, we may consider it to be in your best interests to withdraw you from the study.

What will happen if I don't want to carry on with the study?
If, at anytime after agreeing to participate you change your mind about being involved with this study, you are free to withdraw without giving a reason**.** Your compensation would be paid as a proportion of the total compensation according to the length of your participation.

What if there is a problem?

The University has arrangements in place to provide for harm arising from participation in the study for which the University is the Research Sponsor.

If you wish to complain about any aspect of the way in which you have been approached or treated during the course of this study, you should contact Dr Nicholas Anagnostou, navin.venkatraman@ndm.ox.ac.uk, 01865 857135, or you may contact the University of Oxford Clinical Trials and Research Governance (CTRG) office on 01865 572224 or the head of CTRG, email heather.house@admin.ox.ac.uk

Will my taking part in this study be kept confidential?
All information that is collected about you during the course of the research will be coded with a study number and kept strictly confidential. The information is available to the study team, the safety monitors, the ethical review committee, the sponsors, government regulatory agencies and external monitors who can ask to audit or monitor the study. Any information about you that leaves the hospital or clinic will have your name and address removed so that you cannot be identified from it. Your information is stored on a secure server and any paper notes will be kept in a locked filing cabinet. Samples will be stored in anonymised form.

Involvement of the General Practitioner/Family doctor (GP)
In order to enrol into this study, you will be required to sign a form, documenting that you consent for us to contact your GP. This is to inform him / her that you are interested in being involved in the study and to ensure there are no medical reasons that they are aware of why this would not be safe. The final decision about your eligibility will be the responsibility of the study investigators.

Prevention of 'Over Volunteering'

Volunteers participating in this study must not be concurrently involved in another study. In order to check this, you will be asked to provide your National Insurance or Passport number (if you are not entitled to a NI number). This will be entered on to a national database which helps prevent volunteers from taking part in too many clinical trials. More information can be found at *www.tops.org.uk*. Your national insurance or passport number is also required to allow processing of compensation payments.

What will happen to any samples I give?

If you consent, some of your leftover blood samples will be stored and may be used for further studies of the human body's immune response to malaria vaccination. No studies concerning diseases other than malaria will be done. Any such tests will have an appropriate ethical review. Tests that may be performed include measurements of antibody levels, white cell activity and the ability of blood to inhibit the growth of malaria parasites in the laboratory. Samples may also be used to assess what genes are expressed by cells following vaccination. Samples will be stored for up to fifteen years. Upon your request at any time, your remaining blood samples will be destroyed. Your participation in this study will not be affected by your decision to allow or not allow storage and future use of your leftover blood samples. The blood tests mentioned in part 1 will be analysed in the hospital laboratory, and Oxford University research laboratories.

Will any genetic tests be done?

Yes. Some blood will be used to look at the pattern of your genes that can affect the immune system (including the Human Leukocyte Antigen or HLA genes). The immune response to vaccines is in part genetically controlled, so knowing your pattern of genes that regulate immune responses (HLA type) may help us to understand the responses to vaccination. We will also look at the expression of certain genes which relate specifically to the immune response to the vaccines and may also perform DNA sequencing on your blood, so we can understand how people respond to the vaccines - you can opt out of DNA testing if you wish, without any effect on your participation in the trial.

What will happen to the results of the research study?

The results of this research study may be published in a scientific medical journal. This may not happen until 1 or 2 years after the study is completed. If you contact the researchers in the future you can obtain a copy of the results. You will not be identified in any report or publication.

Who is organising and funding the research?

The study will be funded primarily by a grant from the National Institute of Health Research through the Oxford Biomedical Research Centre Vaccines Theme. Neither your GP, nor the researchers are paid for recruiting you into this study.

Who has reviewed the study?

This study has been reviewed by NRES Committee South Central - Oxford A and has been given a favourable ethical opinion. The Medicines and Healthcare products Regulatory Agency (MHRA) which regulates the use of all medicines in the UK has reviewed the study design and has granted permission to use these unlicensed vaccines in this clinical study.

Thank you for reading this information sheet. If you are interested in being involved in the study please contact the study team at your local trial site below to arrange a screening appointment.

Contact Details for Further Information

Study Co-ordinator
E-mail:vaccinetrials@well.ox.ac.uk
Telephone: 01865 857401
Centre of Clinical Vaccinology and Tropical Medicine,
Old Road, Headington, Oxford, OX3 7LJ.

Out of hours mobile number for emergencies: 07917882967

VAC053 Volunteer Information Sheet

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NRES Committee South Central Berkshire B number: 15/SC/0386

PARTICIPANT INFORMATION SHEET: VAC053

A study to assess new malaria vaccine R21 with Matrix M

A Phase Ia clinical trial to assess the safety and immunogenicity of a protein particle malaria vaccine candidate, R21, administered with and without Matrix-M1

We would like to invite you to take part in a research study. Before you make a decision, it is important you take the time to understand why the research is being done and what it would involve. Please read the following information carefully and discuss it with friends, relatives and your General Practitioner (GP) if you wish. Please ask us if there is anything that is not clear or if you would like more information.

- Part 1 tells you the purpose of the study and what will happen to you if you take part.
- Part 2 tells you more information about the conduct of the study.

Part 1

What is the purpose of the study?

Malaria is a major global problem, affecting around 207 million people each year and causing around 430,000 deaths. There is a great need for a safe, effective malaria vaccine as the range of effective medicines for treating malaria is limited and resistance to commonly used medicines is increasing. Currently there is no approved vaccine available for malaria.

The purpose of this study is to assess the new malaria vaccine candidate, **R21**, at different doses either alone or in combination with the adjuvant Matrix M. The study will enable us to assess the safety of the vaccines and the extent of the immune response in healthy volunteers. We will do this by giving volunteers three vaccinations over 8 weeks, doing blood tests and collecting information about any symptoms that occur after vaccination. This is the first trial to use this vaccine in humans. We plan to recruit a total of 34 volunteers to be vaccinated.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep (or sent it electronically) and be asked to sign a consent form. You are free to withdraw at any time and without giving a reason, but you may be asked to return to the clinic for follow up for safety reasons.

What will happen if I decide to take part?

You will be allocated to a study group once you are deemed eligible following screening and on the basis of your availability. This study involves having three vaccinations and then being followed up with blood tests. You will be asked to complete a diary, recording any symptoms you experience after the vaccination.

Length of research

If you decide to take part in this study, you will be involved in the trial for approximately 8 months.

Am I eligible to be involved in the trial?

In order to be involved in the study you **must**:

- Be a healthy adult aged between 18 and 50 years.
- Be able and willing (in the Investigator's opinion) to comply with all study requirements.
- Allow the Investigators to discuss your medical history with your GP.
- Practice continuous effective contraception for the duration of the study **(women only)**.
- Refrain from blood donation during the course of the study.

You cannot participate in this study if:

- You have had malaria before.
- You have travelled to a malaria endemic region in the last 6 months or are intending to travel to a malaria endemic region during the time you would be involved in the study.
- You have participated in another research study in the last 30 days.
- You are planning to participate in another study at the same time as this study.
- You have previously received an investigational vaccine likely to impact on interpretation of the trial data.
- You have had immunoglobulins and/or any blood products (such as a blood transfusion) in the 3 months preceding your involvement this trial.
- You have problems with your immune system.
- You are pregnant, breast feeding or intend to become pregnant during the study.
- You have a history of a severe allergic reaction to a vaccination.
- You have a history of cancer.

- You have a history of a serious psychiatric condition that may affect participation in the study.
- You have any other serious long-term illnesses requiring hospital follow-up.
- You drink on average more than 42 units of alcohol a week (a pint of beer is 2 - 3 units, a small glass of wine (125mL) 1 unit and a shot of spirits (25mL) one unit).
- You have injected drugs at any time in the last 5 years.
- You have hepatitis B, hepatitis C or HIV infection.

Mild conditions, such as childhood asthma, which are well-controlled, would not automatically exclude you from participating. If you are unclear whether you are eligible to be involved in the study you can contact the study team who will be able to advise you.

CONSIDERATIONS BEFORE TAKING PART IN THIS STUDY

Screening Visit: This takes place at your local study site and will last approximately an hour. The purpose of the screening visit is for you to discuss the trial with us and decide if you wish to enter the study. If you decide to participate, you will be asked to sign a consent form and we will check that you are eligible to participate.
During the screening visit:

- You will be asked some medical questions
- A doctor will examine you
- Blood samples and a urine sample will be taken. These tests will need to be normal for you to be enrolled in the study
- All women will have a urinary pregnancy test

Please note – The screening blood tests will look at your blood counts (e.g. to check if you are anaemic), your liver function and your kidney function. We will also test your blood to see if you are infected with hepatitis B, hepatitis C or HIV, as these conditions can affect your body's response to the vaccines we are assessing. If you test positive to any of these, we will let you know and arrange for a repeat test. We may also report positive results to your GP and offer to refer you for treatment.

Blood Donation: Under current UK regulations, volunteers will not be able to donate blood during the course of the study.

Private Medical Insurance: If you have private medical insurance you are advised to contact your insurance company before participating in this trial, as involvement may affect the cover provided.

Malaria Prophylaxis: If in future you travel to an area where malaria is common, you should not assume that the experimental vaccines you received in this study will give you any protection against malaria. Make sure you visit your GP or a travel clinic before travelling to a malaria endemic region and follow their advice on prevention measures.

Contraception: It is currently unknown whether the vaccines being tested are safe during pregnancy. For this reason, it is important that all women use adequate contraception throughout the trial. If you were to become pregnant during the trial you must tell us immediately and you will be withdrawn from the study, although we will ask to follow you up for safety reasons.

VACCINATIONS

What are the vaccines that are being tested?

We are testing four different vaccine schedules; R21, at different doses either alone or in combination with the adjuvant Matrix M. These vaccines will be injected into the muscle of your upper arm(s).

R21

R21 is a protein particle, which is produced by combining a protein from Hepatitis B with the circumsporozoite protein (CSP) of the malaria parasite. This is not a live vaccine and so there is no chance of catching malaria. CSP is a protein required by the malaria parasite to get into liver cells. We are hoping to make the body develop an immune response to this protein, in order to stop the malaria parasite from getting into liver cells. Side effects from this vaccine would be expected to be comparable to another similar vaccine RTS,S, which has already been tested in more than 10,000 babies and children. It was found to be safe and well tolerated, but can cause temporary side effects as detailed below.

Matrix M

Matrix M is a vaccine adjuvant used to boost the immune response, which will be given in combination with R21 for volunteers in Group 1, Group 3 and Group 4. It has been shown to boost the immune response when given in combination with an influenza vaccination. It has also been tested clinically in combination with a different malaria vaccine. Overall, Matrix M has been given to over 1000 healthy volunteers

and has been safe and well tolerated. Volunteers may experience temporary side effects as mentioned below.

What are the expected side effects from these vaccines?

Once the vaccinations have been given they cannot be undone, so it is important you understand the potential risks of the vaccines before you agree to be involved in the study.
This trial is the first use of R21 in humans. To date, it has been safely administered to 45 healthy human volunteers. We do not expect the side effects to be significantly different from previous trials with a similar malaria vaccine RTS,S. You may experience any of the following short-lived side effects:

- Injection site pain (most likely mild; however there is a chance this could be moderate or severe in intensity).
- Redness, swelling, itching and warmth at the vaccine site (symptoms are likely to be mild if present; however, there is a chance this could be moderate or severe in intensity).
- A 'flu-like illness within 24 hours of vaccination which usually resolves within 48 hours. (This can include headache, muscle aches, joint aches, feverishness, tiredness and feeling generally unwell). The majority of general symptoms are likely to be mild but there is a possibility of moderate or severe symptoms occurring.

It is important to remember this vaccine is in the early stage of development; therefore the amount of safety data available is limited. The malaria protein R21 has only been given to 45 humans before, so there is a chance you could experience a side effect different in nature, or more severe than those described.

Severe Reactions

With any vaccination there is a low risk of serious reactions, which may be related to the nervous system or the immune system. Severe allergic reactions to vaccines (anaphylaxis) are very rare but can be fatal. Doctors qualified in the management of anaphylaxis and appropriate equipment and medication will be present at each vaccination. Reactions in the nervous system are also extremely rare following vaccination, but can cause an illness called Guillain-Barré syndrome. Guillain-Barré syndrome is an illness in which people can develop severe weakness and may be fatal. However, these reactions have not previously been seen with the types of vaccines used in this study. If you experience unexpected symptoms, or become in any way concerned you should contact one of the Investigators (who are available 24 hours a day) using the emergency contact details that you will be given once you have been vaccinated.

Vaccination days

All women will have a urinary pregnancy test before each vaccination.

We will ask you to wait for 1 hour after each vaccination to check there are no immediate problems. You will be assessed again before leaving and we will ask you to record your symptoms and measure any redness every day for 7 days after each vaccination. After these 7 days we will ask you to record if you feel unwell or take any medications over the next 3 weeks.

We may ask to photograph your vaccination site. You will not be identifiable in these photographs and you can choose whether or not to agree to this when you sign the consent form. Photographs may be shown to other professional staff, used for educational purposes or included in a scientific or academic publication.

Number, timing and purpose of visits

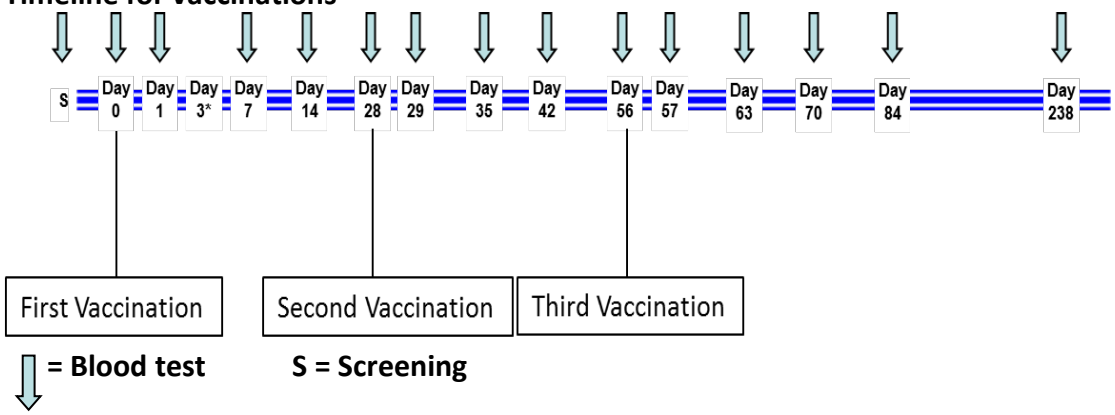
You will receive 3 vaccinations and attend up to 16 visits in total. Visits may include a medical assessment, temperature, pulse and blood pressure readings, examination by a doctor if needed and blood tests. All visits will take place at your local trial site. During the course of the trial you may be asked to attend for an extra visit, for example, if a blood test needs to be repeated. You will be compensated for the time and inconvenience of any extra visits.

You will be allocated to a study group once you are deemed eligible following screening and on the basis of your availability.

The vaccination groups are summarised in the table below – we have chosen these doses based on the adult dose of a very similar vaccine, RTS,S, which has been used at this dose in a number of trials.

	Day 0	Day 28	Day 56
Group 1 (n=10)	10μgR21/50μg Matrix M	10μgR21/50μg Matrix M	10μgR21/50μg Matrix M
Group 2 (n=4)	50μg R21	50μg R21	50μg R21
Group 3 (n=10)	50μgR21/50μg Matrix M	50μgR21/50μg Matrix M	50μgR21/50μg Matrix M
Group 4 (n=10)	2μgR21/50μg Matrix M	2μgR21/50μg Matrix M	2μgR21/50μg Matrix M

Timeline for vaccinations



= Blood test **S = Screening**

Day 3* = Additional safety visit for first three volunteers in each group only

As this is the first trial to test this vaccine in humans, the first three volunteers in Groups 1-3 will be required to attend an additional visit at Day 3 post-vaccination to assess for local and systemic adverse events.

OTHER INFORMATION

Blood Tests

We take blood tests as part of the screening visit and at the study visits in order for us to assess your general health, your immune response to the vaccine and to assess the safety of the vaccines. The volume of blood taken at each visit ranges from 3 to 62 ml. If you would like them, we can give you the results of your blood tests. Anonymised blood samples will be stored after testing, and may be used in future malaria research. You will be asked to consent specifically for blood to be stored and shared with other researchers.

To avoid repeated testing, if you are not enrolled into this study and apply to enter another study conducted by the Jenner Clinical Trials Group based at the CCVTM in Oxford, the screening blood results may be used in that study, where appropriate.

Abnormal Results

If abnormal results or undiagnosed conditions are found in the course of the study these will be discussed with you and, if you agree, your GP (or a hospital specialist, if more appropriate) will be informed. Any newly diagnosed conditions will be looked after within the NHS.

Expenses and Payments

You will be compensated for travel, time and the inconvenience of blood tests for each visit during the trial, and for attending a screening visit.

The approximate total compensation for taking part in this study will range from £675 to £705 depending on whether or not you are one of the first three volunteers in the group. There is a fixed payment of £25 for attending the screening visit. If additional pre-enrolment visits are required you will receive a fixed payment of £10 per visit. During the trial you will be compensated as follows:

- Travel expenses: £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).
- Time required for visit: £20 per hour
- Inconvenience of blood tests: £10 per blood donation

Payment will be made via BACS within six weeks of completing the study.
In addition, if during the trial you have to attend extra visits due to an abnormal result, you will also be compensated as per the above figures. If you choose to leave

the study early or are withdrawn from the study, you will be compensated according to the length of your participation based on these figures. Please note that if you do leave the study early it can take several weeks for your final payment to be made. You should note that compensation payments received in this trial may have an impact on your entitlement to benefits.

What alternatives are present?

At present, there is no malaria vaccine approved anywhere in the world. There are other malaria vaccines in various stages of development. This study may help develop an effective malaria vaccine.

What are the possible benefits of taking part?

This study will not benefit you, but the information gained from the study might help to prevent malaria infection and disease in those living in areas where malaria is common and in travellers to those areas.

Will my taking part in the study be kept confidential?

Yes. All the information about your participation in this study will be kept confidential. The details are included in Part 2.

This completes Part 1 of the Information Sheet. If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2

What if relevant new information becomes available?

Sometimes during the course of a trial, new information becomes available about the vaccine being studied. If this happens, we will tell you about it and discuss whether you want to or should continue in the study. If you decide to continue to take part you will be asked to sign an updated consent form. On receiving new information, we may consider it to be in your best interests to withdraw you from the study.

What will happen if I don't want to carry on with the study?

If, at any time after agreeing to participate you change your mind about being involved with this study, you are free to withdraw without giving a reason. Unless you state otherwise, any blood taken whilst you have been in the study will continue to be stored and used for research as detailed above. You are free to request that your blood samples are destroyed at any time during or after the study.

What if there is a problem?

The University of Oxford, as Sponsor, has appropriate insurance in place to provide no fault compensation in the unlikely event that you suffer any harm as a direct consequence of your participation in this trial.

The investigators recognise the important contribution that volunteers make to medical research, and make every effort to ensure your safety and well-being. In the unlikely event of harm being suffered, while the University will cooperate with any claim, you may wish to seek independent legal advice to ensure that you are properly represented in pursuing any complaint. At any time during the study you will be entirely free to change your mind and withdraw from the study. This will not affect your subsequent medical care in any way.

Complaints statement

If you wish to complain about any aspect of the way in which you have been approached or treated during the course of this study, you should contact your local trial team (contact details at the end of this document) or you may contact the University of Oxford Clinical Trials and Research Governance (CTRG) office on 01865 572224 or the head of CTRG, email ctrg@admin.ox.ac.uk.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the research will be coded with a study number and kept confidential. The data we collect about you will be entered onto an electronic database stored on a secure server. Research data is stored for 20 years because this is a new vaccine for which there is currently a lack of long term safety and immunogenicity data. Some of your leftover blood samples will

be stored indefinitely, but you can request for these to be destroyed at any time. The information is available to the study team, safety monitors, ethical review committee, Sponsors, government regulatory agencies and external monitors who can ask to audit or monitor the study. Any information about you that leaves the hospital or clinic will have your name and address removed so that you cannot be identified from it. Your information is stored electronically on a secure server and any paper notes are kept in a locked filing cabinet.

Involvement of the General Practitioner/Family doctor (GP)

In order to enrol into this study, you will be required to sign a form, documenting that you consent for us to contact your GP. This is to inform them that you are interested in being involved in the study and to check there are no medical reasons that they are aware of why your participation would be unadvisable. The researchers will not enrol you in the trial if they have any concerns about your eligibility or safety. We will write to your GP to let them know whether or not you are finally enrolled in the study, and whether or not you completed the study, so they can update your medical records accordingly.

Prevention of 'Over Volunteering'

Volunteers participating in this study must not be involved in another study at the same time. In order to check this, you will be asked to provide your National Insurance or Passport number. This will be entered on to a national database which helps prevent volunteers from taking part in too many clinical trials. More information can be found at *www.tops.org.uk*. Your national insurance or passport number is also required to allow processing of compensation payments.

What will happen to any samples I give?

If you consent, some of your leftover blood samples will be stored and may be used for further studies of the human body's immune response. Any such tests will have an appropriate ethical review. Upon your request at any time, your remaining blood samples will be destroyed. Your participation in this study will not be affected by your decision whether to allow storage and future use of your leftover samples.
Your study visit blood tests will be analysed in the hospital laboratories and Oxford University research laboratories. Other blood tests to look at the response of your body to the vaccine may be done with collaborating laboratories in other countries. Any samples or data sent to them would be anonymous.

Will any genetic tests be done?

Yes. Some blood may be used to look at the pattern of your genes that can affect the immune system (for example 'human leukocyte antigen [HLA] type). The immune response to vaccines is in part genetically controlled, so knowing your pattern of genes that regulate immune responses may help us to understand the responses to vaccination. You can opt out of 'genetic tests' if you wish, without any effect on your

participation in the trial. You will not receive the results of any genetic tests performed.

What will happen to the results of the research study?

The results of this research study may be presented at scientific meetings or conferences and published in a scientific medical journal. This may not happen until 1 or 2 years after the study is completed. A summary of published reports will be sent to all trial participants for information purposes. You will not be identified in any report or publication.

The anonymised data from this study will be shared with the collaborating partners who are organising and funding this research work, including the pharmaceutical company Novavax. Data from this study may be used to file patents, licence vaccines in the future or make profits in other ways. You will not be paid for any part of this. Data from this study may be used as part of a student post-graduate degree, for example a MD or DPhil.

Who is sponsoring, organising and funding the research?

The study is sponsored by the University of Oxford and funded by European Commission funding.

The study is organised by a research team at The Jenner Institute at the University of Oxford, headed by Prof Adrian Hill. Neither your GP, nor the researchers are paid for recruiting you into this study.

Who has reviewed the study?

This study has been reviewed by South Central Berkshire Research Ethics Committee B and has been given a favourable ethical opinion. The Medicines and Healthcare products Regulatory Agency (MHRA) which regulates the use of all medicines in the UK has reviewed the study design and has granted permission to use these unlicensed vaccines in this clinical study.

Thank you for reading this information sheet. If you are interested in taking part in the study please contact the study team at your local study site to arrange a screening appointment.
Contact details for further information:
INSERT LOCAL CONTACT DETAILS HERE

VAC056 Volunteer Information Sheet

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NRES Committee London
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PARTICIPANT INFORMATION SHEET: VAC056

A study to assess new malaria vaccine R21 with AS01$_B$

A Phase Ia clinical trial to assess the safety and immunogenicity of a protein particle malaria vaccine candidate, R21, administered with AS01$_B$ in healthy UK volunteers

We would like to invite you to take part in a research study. Before you make a decision, it is important you take the time to understand why the research is being done and what it would involve. Please read the following information carefully and discuss it with friends, relatives and your General Practitioner (GP) if you wish. Please ask us if there is anything that is not clear or if you would like more information.

- Part 1 tells you the purpose of the study and what will happen to you if you take part.
- Part 2 tells you more information about the conduct of the study.

Part 1

What is the purpose of the study?

Malaria is a major global problem, affecting around 207 million people each year and causing around 627,000 deaths. There is a great need for a safe, effective malaria vaccine as the range of effective medicines for treating malaria is limited and resistance to commonly used medicines is increasing. Currently there is no approved vaccine available for malaria.

The purpose of this study is to assess the new malaria vaccine candidate, **R21**, at different doses in combination with the adjuvant AS01$_B$. The study will enable us to assess the safety of the vaccines and the extent of the immune response in healthy volunteers. We will do this by giving volunteers three vaccinations over 8 weeks, doing blood tests and collecting information about any symptoms that occur after vaccination. This is the first trial to test this combination of R21 with the adjuvant AS01$_B$. We plan to recruit a total of 20 volunteers to be vaccinated.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep (or sent it electronically) and be asked to sign a consent form. You are free to withdraw at any time and without giving a reason, but you may be asked to return to the clinic for follow up for safety reasons.

What will happen if I decide to take part?
This study involves having three vaccinations and then being followed up with blood tests. You will be asked to complete a diary, recording any symptoms you experience after the vaccination. You will be able to choose which group you are enrolled in, although as groups fill up there will be less choice.

Length of research
If you decide to take part in this study, you will be involved in the trial for approximately 8 months.

Am I eligible to be involved in the trial?

In order to be involved in the study you **must**:

- Be a healthy adult aged between 18 and 50 years.
- Be able and willing (in the Investigator's opinion) to comply with all study requirements.
- Allow the Investigators to discuss your medical history with your GP.
- Practice continuous effective contraception for the duration of the study **(women only)**.
- Refrain from blood donation during the course of the study.
-

You cannot participate in this study if:

- You have had malaria before.
- You have travelled to a malaria endemic region in the last 6 months or are intending to travel to a malaria endemic region during the time you would be involved in the study.
- You have participated in another research study in the last 30 days.
- You are planning to participate in another study at the same time as this study.
- You have previously received an investigational vaccine likely to impact on interpretation of the trial data.
- You have had immunoglobulins and/or any blood products (such as a blood transfusion) in the 3 months preceding your involvement this trial.
- You have problems with your immune system.
- You are pregnant, breast feeding or intend to become pregnant during the study.
- You have a history of a severe allergic reaction to a vaccination.
- You have a history of allergic disease likely to be exacerbated by a component of the vaccine.
- You have a history of cancer.

- You have a history of a serious psychiatric condition that may affect participation in the study.
- You have any other serious long-term illnesses requiring hospital follow-up.
- You drink on average more than 42 units of alcohol a week (a pint of beer is 2 - 3 units, a small glass of wine (125mL) 1 unit and a shot of spirits (25mL) one unit).
- You have injected drugs at any time in the last 5 years.
- You have hepatitis B, hepatitis C or HIV infection.

Mild conditions, such as childhood asthma, which are well-controlled, would not automatically exclude you from participating. If you are unclear whether you are eligible to be involved in the study you can contact the study team who will be able to advise you.

CONSIDERATIONS BEFORE TAKING PART IN THIS STUDY

Screening Visit: This takes place at your local study site and will last approximately one and a half hours. The purpose of the screening visit is for you to discuss the trial with us and decide if you wish to enter the study. If you decide to participate, you will be asked to sign a consent form and we will check that you are eligible to participate.
During the screening visit:

- You will be asked some medical questions
- A doctor will examine you
- Blood samples and a urine sample will be taken. These tests will need to be normal for you to be enrolled in the study
- All women will have a urinary pregnancy test

Please note – The screening blood tests will look at your blood counts (e.g. to check if you are anaemic), your liver function and your kidney function. We will also test your blood to see if you are infected with hepatitis B, hepatitis C or HIV, as these conditions can affect your body's response to the vaccines we are assessing. If you test positive to any of these, we will let you know and offer to refer you for treatment. We may also report positive results to your GP.

Blood Donation: Under current UK regulations, volunteers will not be able to donate blood during the course of the study.

Private Medical Insurance: If you have private medical insurance you are advised to contact your insurance company before participating in this trial, as involvement may affect the cover provided.

Malaria Prophylaxis: If in future you travel to an area where malaria is common, you should not assume that the experimental vaccines you received in this study will give you any protection against malaria. Make sure you visit your GP or a travel clinic before travelling to a malaria endemic region and follow their advice on prevention measures.

Contraception: It is currently unknown whether the vaccines being tested are safe during pregnancy. For this reason, it is important that all women use adequate contraception throughout the trial. If you were to become pregnant during the trial you must tell us immediately and you will be withdrawn from the study, although we will ask to follow you up for safety reasons. There is no known risk of transmission of this vaccine through semen of a man who has received this vaccination.

VACCINATIONS

What are the vaccines that are being tested?
We are testing two different vaccine schedules; R21, at two different doses in combination with the adjuvant $AS01_B$. These vaccines will be injected into the muscle of your upper arm(s).

R21
R21 is a protein particle, which is produced by combining a protein from Hepatitis B with the circumsporozoite protein (CSP) of the malaria parasite. CSP is a protein required by the malaria parasite to get into liver cells. We are hoping to make the body develop an immune response to this protein, in order to stop the malaria parasite from getting into liver cells. Side effects from this vaccine would be expected to be comparable to another similar vaccine RTS,S, which has already been tested in more than 10,000 babies and children. It was found to be safe and well tolerated, but can cause temporary side effects as detailed below.

$AS01_B$
$AS01_B$ is a vaccine adjuvant, which will be given in combination with R21 in order to boost the immune response. This family of adjuvant systems has been tested in healthy volunteers in candidate vaccines against a number of diseases including malaria, TB and HIV. It has been most extensively studied in combination with the leading malaria vaccine candidate RTS,S, which has already been tested in more than 10,000 babies and children. It was found to be safe and well tolerated, but can cause temporary side effects as detailed below.

What are the expected side effects from these vaccines?

Once the vaccinations have been given they cannot be undone, so it is important you are clear of the potential risks of the vaccines before you agree to be involved in the study.

R21 has not been used in combination with $AS01_B$ in humans before but we do not expect the side effects to be significantly different from previous trials with a similar malaria vaccine RTS,S/$AS01_B$. You may experience any of the following short-lived side effects:

- Injection site pain (most likely mild; however there is a chance this could be moderate or severe in intensity).
- Redness, swelling, itching and warmth at the vaccine site (symptoms are likely to be mild if present).
- A 'flu-like' illness within 24 hours of vaccination which usually resolves within 48 hours. (This can include headache, muscle aches, joint aches, feverishness, tiredness and feeling generally unwell). The majority of general symptoms are likely to be mild but there is a possibility of moderate or severe symptoms occurring.

It is important to remember this vaccine is in the early stage of development; therefore the amount of safety data available is limited. The malaria protein R21 in combination with adjuvant $AS01_B$ has not been given to humans before, so there is a chance you could experience a side effect more severe than or different to those described.

Severe Reactions

With any vaccination there is a rare risk of serious reactions, which may be related to the nervous system or the immune system. Severe allergic reactions to vaccines (anaphylaxis) are very rare but can be fatal. Doctors qualified in the management of anaphylaxis will be present at each vaccination. Reactions in the nervous system are also extremely rare following vaccination, but can cause an illness called Guillain-Barré syndrome. Guillain-Barré syndrome is an illness in which people can develop severe weakness and may be fatal. However, these reactions have not previously been seen with the types of vaccines used in this study. If you experience unexpected symptoms, or become in any way concerned you should contact one of the Investigators (who are available 24 hours a day) using the emergency contact details that you will be given once you have been vaccinated.

Vaccination days

All women will have a urinary pregnancy test before each vaccination. We will ask you to wait for 1 hour after each vaccination to check there are no immediate problems. You will be assessed again before leaving and we will ask you to record your symptoms and measure any redness every day for 7 days after each vaccination. After these 7 days we will ask you to record if you feel unwell or take any medications over the next 3 weeks.

We may ask to photograph your vaccination site. You will not be identifiable in these photographs and you can choose whether or not to agree to this when you sign the

consent form. Photographs may be shown to other professional staff, used for educational purposes or included in a scientific or academic publication.

Number, timing and purpose of visits

You will receive 3 vaccinations and attend up to 16 visits in total. Visits may include a medical assessment, temperature, pulse and blood pressure readings, examination by a doctor if needed and blood tests. All visits will take place at your local trial site. During the course of the trial you may be asked to attend for an extra visit, for example, if a blood test needs to be repeated. You will be compensated for the time and inconvenience of any extra visits.

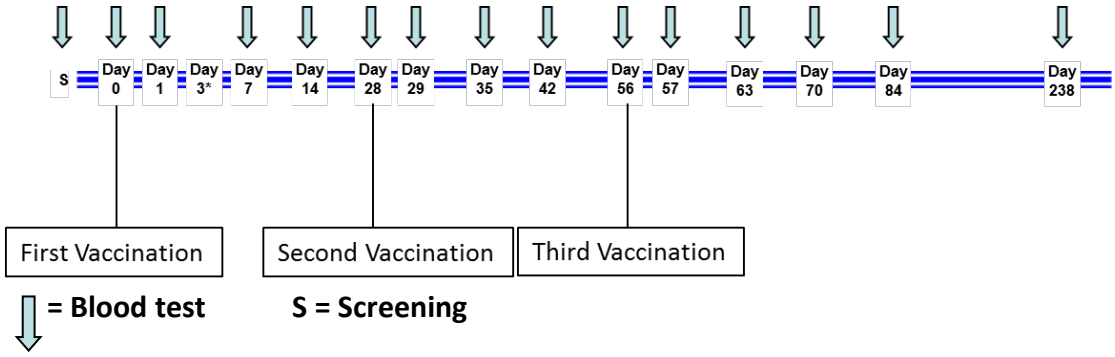
You will be able to choose which group you are enrolled in, although as groups fill up there will be less choice.

The vaccination groups are summarised in the following table:

Week	**0**	**4**	**8**
Group 1 (n=10)	**10 ug R21/ AS01$_B$**	**10 ug R21/ AS01$_B$**	**10 ug R21/ AS01$_B$**
Group 2 (n=10)	**50 ug R21/ AS01$_B$**	**50 ug R21/ AS01$_B$**	**50 ug R21/ AS01$_B$**

Table 1: *Study Groups*

Timeline for vaccinations



Day 3* = Additional safety visit for first three volunteers in each group only

OTHER INFORMATION

Blood Tests
We take blood tests as part of the screening visit and at the study visits in order for us to assess your general health, immune response to the vaccine and for safety reasons. The volume of blood taken at each visit ranges from 3 to 62 ml (about 1 to 13 teaspoons). If you would like them, we can give you the results of your blood tests. Anonymised blood samples will be stored after testing, and may be used in future malaria research. You will be asked to consent specifically for blood to be stored and shared with other researchers.

To avoid repeated testing, if you are not enrolled into this study and apply to enter another study conducted by the Jenner Clinical Trials Group based at the CCVTM in Oxford, the screening blood results may be used in that study, where appropriate.

Abnormal Results
If abnormal results or undiagnosed conditions are found in the course of the study these will be discussed with you and, if you agree, your GP (or a hospital specialist, if more appropriate) will be informed. Any newly diagnosed conditions will be looked after within the NHS.

Expenses and Payments
You will be compensated for travel, time and the inconvenience of blood tests for each visit during the trial, and for attending a screening visit.

The approximate total compensation for taking part in this study will range from £675 to £705 depending on whether or not you are one of the first three volunteers in the group. There is a fixed payment of £25 for attending the screening visit. If additional pre-enrolment visits are required you will receive a fixed payment of £10 per visit. During the trial you will be compensated as follows:

- Travel expenses: £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.
- Time required for visit: £20 per hour
- Inconvenience of blood tests: £10 per blood donation

Payment will be made via BACS within six weeks of completing the study.
In addition, if during the trial you have to attend extra visits due to an abnormal result, you will also be compensated as per the above figures. If you choose to leave the study early or are withdrawn from the study, you will be compensated according to the length of your participation based on these figures. Please note that if you do leave the study early it can take several weeks for your final payment to be made. You should note that compensation payments received in this trial must be reported for tax and benefit purposes.

What alternatives are present?
At present, there is no malaria vaccine approved anywhere in the world. There are other malaria vaccines in various stages of development. This study may help develop an effective malaria vaccine.

What are the possible benefits of taking part?
This study will not benefit you, but the information gained from the study might help to prevent malaria infection and disease in those living in areas where malaria is common and in travellers to those areas.

Will my taking part in the study be kept confidential?
Yes. All the information about your participation in this study will be kept confidential. The details are included in Part 2.

This completes Part 1 of the Information Sheet. If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2

What if relevant new information becomes available?
Sometimes during the course of a trial, new information becomes available about the vaccine being studied. If this happens, we will tell you about it and discuss whether you want to or should continue in the study. If you decide to continue to take part you will be asked to sign an updated consent form. On receiving new information, we may consider it to be in your best interests to withdraw you from the study.

What will happen if I don't want to carry on with the study?
If, at any time after agreeing to participate you change your mind about being involved with this study, you are free to withdraw without giving a reason. Unless you state otherwise, any blood taken whilst you have been in the study will continue to be stored and used for research as detailed above. You are free to request that your blood samples are destroyed at any time during or after the study.

What if there is a problem?
The University of Oxford, as Sponsor, has appropriate insurance in place in the unlikely event that you suffer any harm as a direct consequence of your participation in this trial.

The investigators recognise the important contribution that volunteers make to medical research, and make every effort to ensure your safety and well-being. In the unlikely event of harm being suffered, while the University will cooperate with any claim, you may wish to seek independent legal advice to ensure that you are properly represented in pursuing any complaint. At any time during the study you will be entirely free to change your mind and withdraw from the study. This will not affect your subsequent medical care in any way.

<u>Complaints statement</u>

If you wish to complain about any aspect of the way in which you have been approached or treated during the course of this study, you should contact your local trial team (contact details at the end of this document) or you may contact the University of Oxford Clinical Trials and Research Governance (CTRG) office on 01865 572224 or the head of CTRG, email ctrg@admin.ox.ac.uk.

Will my taking part in this study be kept confidential?
All information that is collected about you during the course of the research will be coded with a study number and kept confidential. The data we collect about you will be entered onto an electronic database stored on a secure server. Research data is stored for 20 years because this is a new vaccine for which there is currently a lack of long term safety and immunogenicity data. Some of your leftover blood samples will be stored indefinitely, but you can request for these to be destroyed at any time. The information is available to the study team, safety monitors, ethical review committee, Sponsors, government regulatory agencies and external monitors who can ask to audit or monitor the study. Any information about you that leaves the hospital or clinic will have your name and address removed so that you cannot be identified from it. Your information is stored electronically on a secure server and any paper notes are kept in a locked filing cabinet.

Involvement of the General Practitioner/Family doctor (GP)
In order to enrol into this study, you will be required to sign a form, documenting that you consent for us to contact your GP. This is to inform them that you are interested in being involved in the study and to check there are no medical reasons that they are aware of why your participation would be unadvisable. The researchers will not enrol you in the trial if they have any concerns about your eligibility or safety. We will write to your GP to let them know whether or not you are finally enrolled in the study, and whether or not you completed the study, so they can update your medical records accordingly.

Prevention of 'Over Volunteering'
Volunteers participating in this study must not be involved in another study at the same time. In order to check this, you will be asked to provide your National Insurance or Passport number. This will be entered on to a national database which helps prevent volunteers from taking part in too many clinical trials. More information can be found at *www.tops.org.uk*. Your national insurance or passport number is also required to allow processing of compensation payments.

What will happen to any samples I give?
If you consent, some of your leftover blood samples will be stored and may be used for further studies of the human body's immune response. Any such tests will have an appropriate ethical review. Upon your request at any time, your remaining blood samples will be destroyed. Your participation in this study will not be affected by your decision whether to allow storage and future use of your leftover samples.
Your study visit blood tests will be analysed in the hospital laboratories and Oxford University research laboratories. Other blood tests to look at the response of your

body to the vaccine may be done with collaborating laboratories in other countries. Any samples or data sent to them would be anonymous.

Will any genetic tests be done?
Yes. Some blood may be used to look at the pattern of your genes that can affect the immune system (for example 'human leukocyte antigen [HLA] type). The immune response to vaccines is in part genetically controlled, so knowing your pattern of genes that regulate immune responses may help us to understand the responses to vaccination. You can opt out of 'genetic tests' if you wish, without any effect on your participation in the trial.

What will happen to the results of the research study?
The results of this research study may be presented at scientific meetings or conferences and published in a scientific medical journal. This may not happen until 1 or 2 years after the study is completed. A summary of published reports will be sent to all trial participants for information purposes. You will not be identified in any report or publication.

The anonymised data from this study will be shared with the collaborating partners who are organising and funding this research work, including the pharmaceutical company GlaxoSmithKline. Data from this study may be used to file patents, licence vaccines in the future or make profits in other ways. You will not be paid for any part of this. Data from this study will be used as part of a student's PhD degree. Any data used for this degree will be completely anonymous. Nobody will know that you took part in this study.

Who is sponsoring, organising and funding the research?
The study is sponsored by the University of Oxford and funded by European Commission funding.

The study is organised by a research team at The Jenner Institute at the University of Oxford, headed by Prof Adrian Hill. Neither your GP, nor the researchers are paid for recruiting you into this study.

Who has reviewed the study?
This study has been reviewed by the London Westminster Research Ethics Committee and has been given a favourable ethical opinion. The Medicines and Healthcare products Regulatory Agency (MHRA) which regulates the use of all medicines in the UK has reviewed the study design and has granted permission to use these unlicensed vaccines in this clinical study.

Thank you for reading this information sheet. If you are interested in taking part in the study please contact the study team at your local study site to arrange a screening appointment.
Contact details for further information:
INSERT LOCAL CONTACT DETAILS HERE

VAC065 Volunteer Information Sheet

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NRES Committee South Central - Berkshire Number: 16/SC/0261

PARTICIPANT INFORMATION SHEET: VAC065

A study to assess the effectiveness of novel malaria vaccine candidates by infecting vaccinated volunteers with malaria parasites

A Phase I/IIa Sporozoite Challenge Study to Assess the Safety and Protective Efficacy of adjuvanted R21 at two different doses and the Combination Malaria Vaccine Candidate Regimen of adjuvanted R21 + ChAd63 and MVA encoding ME-TRAP

We would like to invite you to take part in a research study. Before you make a decision, it is important you take the time to understand why the research is being done and what it would involve. Please read the following information carefully and discuss it with friends, relatives and your General Practitioner (GP) if you wish. Please ask us if there is anything that is not clear, or if you would like more information.

- Part 1 tells you the purpose of the study and what will happen to you if you take part.
- Part 2 tells you more information about the conduct of the study.

Part 1

What is the purpose of the study?
Malaria is a major global health problem, with approximately 214 million cases and 438, 000 deaths each year. There is a great need for a safe, effective malaria vaccine as the range of malaria medicines is limited and drug resistance is increasing. Currently there is no approved malaria vaccine.

The purpose of this study is to assess two types of malaria vaccines in different combinations. The study will enable us to assess:

1. The ability of the vaccines to prevent malaria infection.
2. The safety of the vaccines in healthy participants.
3. The response of the human immune system to the vaccines.

We will do this by giving 36 participants either three or five vaccinations over 8 or 9 weeks, then exposing them to malaria infection by allowing malaria-infected mosquitoes to bite under carefully regulated conditions. We will follow participants closely to observe if and when they develop malaria. If the vaccines provide some protection against malaria, participants will take longer than usual to develop malaria, or will not develop malaria at all. We will also recruit 6 individuals to be control subjects – these participants won't receive any vaccinations, but will be challenged with malaria.

Vaccinated volunteers who do not develop malaria infection in the blood after being challenged with malaria the first time may be invited back to be infected with malaria again in a repeat challenge experiment. This would happen approximately 5-7 months after the first challenge. Follow-up after this second challenge will last approximately 3 months. Up to another 6 individuals will be recruited to be control subjects for the repeat challenge. The purpose of this second challenge is to see how long the protection of the investigational malaria vaccine lasts. You do not need to agree to take part in the second challenge in order to take part in this study.

Do I have to take part?

No. It is up to you to decide whether or not to take part. Your decision will not result in any penalty, or loss of benefits to which you are otherwise entitled. If you do decide to take part, you will be given this information sheet to keep (or be sent it electronically) and be asked to sign a consent form. You are free to withdraw at any time and without giving a reason, but you may be asked to return to the clinic for follow up for safety reasons.

The University of Oxford does not urge, influence, or encourage any employees/students of the institution to take part in this research study. Your decision to not participate in the study, or a decision on your part to withdraw from the study, will have no effect whatsoever on your employment/student status at the University.

What will happen if I decide to take part?

This study involves having either three or five vaccinations and then undergoing challenge infection with malaria (groups 1, 2 &3), or receiving no vaccinations then undergoing challenge infection with malaria (groups 4 & 5).

You will be able to choose whether you are vaccinated or not, but not be able to choose which specific group you are enrolled in.
There are 4 vaccines being assessed in this trial:
Vaccine 1a = R21 10μg with adjuvant Matrix M1
Vaccine 1b = R21 50μg with adjuvant Matrix M1

Vaccine 2 = ChAd63 ME-TRAP
Vaccine 3 = MVA ME-TRAP

The groups are summarised in the following table:

Week	Group 1	Group 2	Group 3	Group 4	Group 5
0	Vaccine 1a	Vaccine 1b	Vaccine 1a	No vaccine	No vaccine
1			Vaccine 2	No vaccine	No vaccine
4	Vaccine 1a	Vaccine 1b	Vaccine 1a	No vaccine	No vaccine
8	Vaccine 1a	Vaccine 1a	Vaccine 1a	No vaccine	No vaccine
9			Vaccine 3	No vaccine	No vaccine
12	Malaria challenge	Malaria challenge	Malaria challenge	Malaria challenge	
32 - 40	Repeat malaria challenge if protected**	Repeat malaria challenge if protected**	Repeat malaria challenge if protected**		Malaria challenge

**If you receive the vaccines and do not develop malaria infection after the first challenge, you will be offered the opportunity to participate in a second challenge 5-7 months after the first.

Length of research
If you decide to take part in this study, you will be involved in the trial for approximately 3 to 6 months, depending on which group you are in.
Participants in groups 1 – 3 who are protected from malaria following the first challenge, and who choose to take part in the second malaria challenge, will continue for up to 13 months.

Am I eligible to be involved in the trial?
In order to be involved in the study you **must be**:

- A healthy adult aged between 18 and 45 years.
- Able and willing (in the investigators' opinion) to comply with all study requirements.
- Willing to allow the investigators to discuss your medical history with your GP (General Practitioner)
- Practice continuous effective contraception for the duration of the study **(women only)**.
- Willing to refrain from blood donation during the study and for at least 3 years after the end of the trial.

You cannot participate in this study if:

- You have had malaria before.
- You have travelled to a malaria endemic region in the past 6 months, or are intending to travel to a malaria endemic region during the study period.

- You have received an investigational product in the 30 days preceding enrolment, or planned receipt during the study period.
- You have used antibiotics which could treat malaria in the 30 days prior to malaria challenge.
- You have previously received an investigational vaccine likely to impact on interpretation of the trial data.
- You have had immunoglobulins and/or any blood products in the three months preceding your involvement in this trial.
- You have problems with your immune system.
- You have sickle cell anaemia or thalassemia, or any other haematological condition that might affect susceptibility to malaria infection.
- You are pregnant, breast feeding or intend to become pregnant during the study.
- You have a history of allergic disease or reactions likely to be exacerbated by any component of the vaccines, by malaria infection or by the medications used to treat malaria infection.
- You have a history of cancer.
- You have a history of a serious psychiatric condition that may affect participation in the study.
- You have any other serious chronic illnesses requiring hospital follow-up.
- You drink on average more than 42 units of alcohol a week
- You have injected drugs at any time in the last 5 years.
- You have hepatitis B, hepatitis C or HIV infection.
- Close family members have developed heart disease when aged less than 50 years.

Mild conditions, such as childhood asthma, which are well-controlled would not automatically exclude you from participating. If you are unclear whether you are eligible for the study you can contact the study team who will be able to advise you.

CONSIDERATIONS BEFORE TAKING PART IN THIS STUDY

Medications: You should not take any drugs other than vitamin pills, contraceptive pills or those medications assessed by the doctor as appropriately safe during a malaria challenge. This also applies for drugs bought over the counter. If at any time you need any medication then you should take it, however it is very important that you let us know **before** you start any treatment, as some drugs might interfere with the malaria infection and/or anti-malaria treatment you would receive.

Blood Donation: Under current UK regulations, participants will not be able to donate blood during the study or for 3 years after the end of the trial. At this point, The National Blood Transfusion service can test your blood to see whether or not you are able to donate blood again. If you are positive for certain antibodies, you will not be able to donate blood again in the UK. It is not possible to determine in advance whether you will still have a positive antibody test as a result of the trial after 3 years.

Private Insurance: If you have private medical or travel insurance you are advised to contact your insurance company before participating in this trial, as involvement may affect the cover provided.

Contraception: It is currently unknown whether the vaccines being tested are safe during pregnancy. Also, malaria infection can be particularly dangerous during pregnancy to both the mother and the foetus. For this reason, it is important that all women use adequate contraception throughout the trial. If you were to become pregnant during the trial you must tell us immediately and you will be withdrawn from the study, although we will ask to follow you up for safety reasons.

Antimalarial Medication: Volunteers will take Riamet, an antimalarial medication, for three days to treat malaria infection. As Riamet may temporarily reduce the effectiveness of hormonal contraceptives, women taking hormonal contraceptives will need to use an additional form of contraception (e.g. condoms) while taking Riamet, and until the start of the next menstrual period after Riamet treatment. A urinary pregnancy test will be carried out at screening, just prior to each study vaccination, before malaria challenge, and again before anti-malarial treatment is started.

VACCINATIONS

What are the vaccines that are being tested?
We are testing three vaccines; R21 with the adjuvant Matrix M1, ChAd63 ME-TRAP and MVA ME-TRAP. These vaccines will be injected into the muscle of your upper arm.

R21
R21 is a protein particle, which is produced by combining a protein from Hepatitis B with the circumsporozoite protein (CSP) of the malaria parasite. This is not a live vaccine and so there is no chance of catching malaria. CSP is a protein required by the malaria parasite to get into liver cells. We are hoping to make the body develop an immune response to this protein, in order to stop the malaria parasite from getting into liver cells. There is limited experience with R21 administered to humans – it has been given to around 45 healthy volunteers in Phase I trials in the UK. Side effects from this vaccine are comparable to another similar vaccine RTS,S, which has already been tested in more than 10,000 babies and children. It was found to be safe and well tolerated, but can cause temporary side effects as detailed in the section titled "What are the risks of taking part in the study?".

Matrix M
Matrix M is a vaccine adjuvant used to boost the immune response, which will be given in combination with R21 for volunteers in Groups 1-3. It has been shown to boost the immune response when given in combination with an influenza vaccination. It has also been tested clinically in combination with a different malaria vaccine. Overall, Matrix M has been given to over 1000 healthy volunteers and has

been safe and well tolerated. Volunteers may experience temporary side effects as mentioned in the section titled “What are the risks of taking part in the study?”.

ChAd63 ME-TRAP
ChAd63 ME-TRAP consists of a virus (ChAd63), which is a weakened version of a chimpanzee adenovirus that has been genetically altered so that it is impossible for it to grow in humans. To this virus we have added genes that make proteins from the malaria parasite called ME-TRAP. By vaccinating with ChAd63 ME-TRAP, we are hoping to make the body recognise and develop an immune response to the ME-TRAP protein that will help stop the malaria parasite from progressing after initial infection with malaria. Vaccines made from the ChAd63 virus have been given to more than 950 people to date, and have been shown to be safe and well tolerated, although they can cause temporary side effects which are explained in the section titled “What are the risks of taking part in the study?”.

MVA ME-TRAP
MVA ME-TRAP is based on a different virus (MVA) but contains the same malaria genes as the ChAd63 ME-TRAP vaccine. Modified Vaccinia Ankara Virus (MVA) is a safer form of the vaccinia virus previously widely used for smallpox vaccination. Investigational vaccines made from MVA have been widely used and are currently in development as vaccines for many diseases. MVA ME-TRAP has been given to more than 1000 people. They have been shown to be safe and well tolerated, but can cause temporary side effects which are explained in the section titled “What are the risks of taking part in the study?”.

VISITS

Screening Visit: This takes place at your local study site (i.e. Oxford, London or Southampton) up to 6 months before the study starts and will last up to two hours. The purpose of the screening visit is for you to discuss the trial with us and decide if you wish to enter the study. If you decide to participate, you will be asked to complete a questionnaire assessing your understanding of the study in order for us to be confident that you fully understand what taking part will involve. You will then be asked to sign a consent form and we will check that you are eligible to participate.
During the screening visit:

- You will be asked some medical questions
- A doctor will examine you
- Blood samples and a urine sample will be taken. These tests will need to be normal for you to be enrolled in the study
- An electrocardiogram (ECG) will be done, to check the rhythm of the heart
- All women will have a urinary pregnancy test

The screening blood tests will look at your blood counts (e.g. to check if you are anaemic), your liver and kidney function, and your potential risk of heart disease (including checking your cholesterol levels). We will also test your blood to see if you are infected with hepatitis B, hepatitis C or HIV, as these conditions can affect your

body's response to the vaccines we are assessing. If you test positive to any of these, we will let you know and offer to refer you for treatment.

Vaccination days and follow-up visits post-vaccination
If you are in one of the vaccine groups (Groups 1-3), you will receive either 3 or 5 vaccinations and attend either 14 or 16 visits in total before the malaria challenge. Vaccination and follow up visits post-vaccination will take place at your local clinic site (i.e. Oxford, London or Southampton). All women will have a urinary pregnancy test before each vaccination. We will ask all volunteers to wait for 60 minutes after each vaccination to check there are no immediate problems. You will be given a diary card (electronic or paper), thermometer and tape measure to take away. We will ask you to use the diary card to record solicited symptoms every day for 7 days after each vaccination and any other symptoms experienced for 28 days post-vaccination.

We may ask to photograph your vaccination site(s) and you can choose whether or not to agree to this when you sign the consent form. You will not be identifiable in these photographs, as only the vaccination site and your unique trial number will be visible. These photographs may be shown to other professional staff, used for educational purposes, or included in a scientific publication.

Visits may include a medical assessment, temperature, pulse and blood pressure readings, examination by a doctor if needed and blood tests. During the course of the trial you may be asked to attend for an extra visit, for example, if a blood test needs to be repeated. You will be compensated for the time and inconvenience of any extra visits.

Clinic visit before challenge: all groups
All participants will be asked to attend clinic for a review, blood test and, for female participants, a urinary pregnancy test, a day or two before the malaria challenge to ensure you are completely healthy and can be infected with malaria. This visit will take place at your local clinic site (i.e. Oxford, London or Southampton).

THE MALARIA CHALLENGE

What happens during the Malaria Challenge?
The best way of assessing how well new malaria vaccines work is to test whether they protect against malaria - as we plan to do in this trial. The challenge involves spending the whole day at **Imperial College in West London**. Transportation to and from Imperial College will be provided. You will be bitten by up to five malaria-infected mosquitoes contained in paper cups with a gauze screen over the top. The cup is placed on the skin of the forearm, as most participants prefer this site. However, if you prefer another site then we can try putting the cup there. It will need to stay in place for between five and fifteen minutes to allow the mosquitoes to feed. Once they have fed, the cup will be removed and the mosquitoes examined for signs of blood and parasites. If this examination reveals that you have not received the required number of infected bites, then additional mosquitoes will be allowed to bite until you have received 5 infected bites. This process can take up to 2 hours.

The mosquitoes may not bite well if you have used perfume, aftershave, or a perfumed soap or cream on the skin where they are going to bite. We ask that you don't use any of these items, nor should you shower or bathe on the morning of the challenge.

What happens at follow up after the Malaria Challenge?

After an infected bite you will not develop malaria for at least 7 days. For this reason, you do not need to attend clinic on Days 1-5 post challenge, however one of the study team will call you each day to ensure that you are well and contactable during this time. Volunteers in Group 4 will attend an extra follow-up visit on Day 5 for blood tests.

Days 6-23 after Malaria Challenge

We will review you in clinic once on Day 6 following malaria challenge and then twice a day until Day 14 following challenge. From Day 15 until Day 21 after challenge, or until you have completed antimalarial treatment if you develop malaria, we will review you in clinic once a day. **All these clinic visits will take place at the Centre for Clinical Vaccinology & Tropical Medicine (CCVTM) at the Churchill Hospital in Oxford. You will be asked to remain in Oxford** for careful monitoring and regular review by the study team, until you have had two negative malaria films after starting antimalarial treatment, or completing 2 days of antimalarial treatment from Day 21 if you do not develop malaria. If you do not normally live in or close to Oxford, and if you joined the study at the London or Southampton sites, accommodation will be provided for this time, as well as transportation to and from Oxford. We will give you a medication diary card on which you will be asked to record all medications that you take. At each visit, which will last approximately 10 minutes, we will ask about symptoms of malaria, measure your pulse, blood pressure and temperature, and take a blood sample. This will be examined under the microscope for malaria parasites. This is called a thick film and is the standard test for diagnosing malaria infection. Your blood will also be tested for malaria parasite genetic material (DNA) using a technique called polymerase chain reaction (PCR). The total number of visits post challenge will vary depending on when and if you get malaria. It is important you are able to attend all the visits. **If you plan to travel outside of Oxford during Days 6 to 23 post challenge, then you should discuss your plans with one of the study physicians before participating in this study.**

If you are diagnosed with malaria you will be immediately started on a course of antimalarial tablets. Usually the blood test result is available after you have left clinic so we will contact you by telephone and ask you to return to the CCVTM as soon as possible to start treatment. It is therefore essential that we are able to contact you at all times on your telephone and that you are available to return to the CCVTM to start treatment at short notice any time between Day 6 and Day 21 post challenge. You **must** also provide a name and 24-hour phone number for someone who lives near to you and who will know where you are for the duration of the study. If you fail to attend for review during the 23 days after challenge and are un-contactable we will contact this person.

Our experience tells us that the malaria parasites should disappear from your blood within 2 or 3 days of starting the treatment. If you do develop malaria, then you will be seen each day during the treatment until there are no malaria parasites in your blood on two consecutive days. When you finish treatment we will give you a diary card on which to note when any ongoing symptoms of malaria stop. Once malaria has been diagnosed and treated, with 2 consecutive negative thick blood films confirmed after treatment, the twice daily / daily visits and blood tests after challenge will no longer be required.

If you become unwell with malaria then you may be admitted to the John Warin Ward (the Infectious Diseases and Tropical Medicine Unit at the Churchill Hospital in Oxford) as a precaution until you have recovered, but it is very unlikely that this will be necessary.

If the vaccines do not work, or you are in the control group (group 4 or 5), you are most likely to develop malaria between Day 7 and Day 14 following challenge. If the vaccines protect you against malaria you may develop malaria later than Day 14, or not at all. If we do not find malaria parasites in your blood by Day 21 post challenge then we will presume the vaccines have protected you against malaria, but will give you a course of antimalarial treatment anyway to kill any undetected parasites.

Days 35 and 90 after Malaria Challenge
You will be seen in clinic on Day 35 and Day 90 post challenge. At these visits a blood sample will be taken. The appointments will last about 10 minutes and will take place at your local clinic site (i.e. Oxford, London or Southampton).

REPEAT CHALLENGE
If you are a Group 1, 2 or 3 volunteer and do not develop malaria infection in the blood after the malaria challenge this means that the vaccine had some effect at preventing malaria infection. However, you should still assume that you are not protected from malaria infection, and take all the usual precautionary measures you would otherwise take if you travel to an area where there is malaria.
You may be invited to take part in a repeat challenge experiment. If you decide to take part in the second challenge you will be asked to attend a screening visit, which will take place up to 6 months before the repeat challenge. This screening visit will be similar to the first screening visit and you will be asked to sign a consent form for the repeat challenge. We will check that you are still eligible to participate and will conduct the same tests as at the first screening visit. Your participation in the repeat challenge is at the discretion of the researchers.
As before, a clinic visit will be conducted the day before the second challenge, and the challenge and follow up visits will be the same as for the first challenge (as described above) up to and including the visit 90 days after mosquito bite.

WHAT ARE THE RISKS OF TAKING PART IN THE STUDY?
The potential risks in the study can be divided into six categories;

1. **Blood Tests**

The total volume of blood taken during the study depends on the group. The amount taken at each visit will vary between 2.5ml (half a teaspoon) and 100ml (around 6 tablespoons). The volume of blood being taken over the course of the trial should not cause any problems in healthy people. There may be some temporary mild discomfort, such as bruising and tenderness, at the site from which blood is taken. You may experience faintness as a result of collecting blood. We will give you a copy of your blood tests if you request them, and will only send the results to your GP if you wish us to.
At different time points throughout the trial, we will take blood tests that include measures of:

- Red and white blood cells
- Liver and kidney function
- Blood borne viruses (HIV, hepatitis B & C)
- Cholesterol
- HLA type
- RNA tests
- Malaria parasites (for diagnosis after challenge)
- Immune responses to vaccines and to malaria infection
- Antibodies to malaria (only if necessary, for people who have travelled to malaria endemic regions before the trial)

2. Vaccination Side Effects (Groups 1, 2 and 3 only)

Side Effects of Vaccination
It is likely that you will experience some symptoms at the vaccination site as well as general symptoms due to vaccination. It is important to remember these are vaccines in the early stage of development and the amount of safety data available is limited. For this reason, there is a chance you could experience a side effect that is more severe than that described below, or that has not been seen before with these vaccines.

Although the combination of vaccines being given for group 3 is being tested for the first time, the individual vaccines in all of the schedules have previously been administered in other clinical trials. We can predict from past experience what the symptoms should be like with these schedules. We don't expect any new symptoms and we expect that symptoms will be mild in strength most of the time, although symptoms may also be moderate or severe. All symptoms should resolve completely within a few days.

Local Reactions
You may experience some discomfort at the injection site as the vaccination is given. This usually gets better in 5 minutes. Later, you might experience pain resulting in some difficulty moving your arm, but this should resolve within a few days. In addition to pain, you may experience redness, swelling, or warmth at the injection site.

General reactions

During the first 24 - 48 hours after vaccination you may experience flu-like symptoms such as muscle aches, joint aches, feverishness, chills, headache, nausea, tiredness and/or feeling generally unwell. These symptoms should usually resolve within a few days.
You are encouraged to take over the counter medications, such as paracetamol or ibuprofen, as soon as you experience these symptoms as they are likely to reduce the intensity of any symptoms you have.

Serious Reactions
With any vaccination there is a risk of rare serious adverse events, such as an allergic reaction, which may be related to the nervous system or the immune system. Severe allergic reactions to vaccines (anaphylaxis) are rare, but can be fatal. Reactions in the nervous system are also extremely rare, but can cause an illness called Guillain-Barré syndrome. This is a condition in which people can develop severe weakness, and can be fatal. These adverse events have not previously been seen following administration of the R21, MVA or ChAd63 based vaccines.
The investigators are contactable any time if you are concerned about any possible vaccine side effects.

3. Mosquito Bites

The mosquitoes that we are using have been laboratory-reared under very careful conditions by mosquito experts. They are infected with a strain of malaria (*Plasmodium falciparum*) that we know can be cured by antimalarial medications. There are other diseases that may be transmitted by the bite of a mosquito. However, the strains of mosquitoes that we are using have never shown any evidence of other mosquito-transmitted diseases and therefore the chance of contracting one of these diseases is very low.
After malaria challenge you will have mosquito bites on your arm which can become red and itchy for several days. Some people develop quite pronounced local reactions to mosquito bites with itching, swelling, redness and discomfort. We will give you antihistamine cream to apply to the bite sites as required to help relieve symptoms.

4. Malaria Infection

The risks of taking part in this study are very low provided that you return for follow-up as outlined above. **If untreated, the malaria infection that we propose to give you could result in death.** Worldwide over 1300 people have been deliberately infected with malaria and all have made a complete recovery. In Oxford more than 400 people have been infected with malaria.

The early symptoms of malaria include a flu-like illness, fever, chills, headache, muscle aches, diarrhoea and vomiting. If you develop any of these then you **must let one of the study physicians know immediately.** Study doctors can be contacted 24 hours a day. We hope to diagnose and treat your infection before the onset of symptoms, but in previous studies most participants did experience some of the above symptoms. About one-fifth of participants temporarily develop symptoms

graded as severe (i.e. symptoms that prevent daily activities). It is possible that you might need to take one or two days off work due to symptoms of malaria. We will prescribe pain-killers, such as paracetamol, and anti-sickness tablets, which you can take as required. Symptoms can start or persist after treatment has started, but usually last no more than 1 to 3 days. If malaria is not treated appropriately, possible complications include jaundice, kidney failure, fluid on the lung, low blood sugar and collapse. Seizures, altered consciousness, coma and even death may occur. It is for this reason **it is crucial that you attend all the scheduled follow-up visits and contact us immediately if you have any symptoms at all.**

In the unlikely event that it is necessary, you may be admitted to the Infectious Diseases Ward (The John Warin Ward) at the Churchill Hospital, Oxford for observation and treatment. In the last 10 years, only 4 participants out of more than 400 challenged with malaria in Oxford have required hospital admission. There have been no long term problems in participants challenged with malaria.

There have been two unexpected serious adverse events in persons infected in malaria challenge studies in the Netherlands. The first individual experienced an episode of chest pain diagnosed as acute coronary syndrome that occurred two days after completion of malaria treatment with a full recovery. It is uncertain whether this was a form of coronary artery spasm or blockage or cardiac inflammation. More recently, a second individual was found to have an abnormal blood test suggesting cardiac inflammation. They subsequently suffered a very short episode of chest pain. They were also found to be suffering with a viral upper respiratory tract infection (common cold virus) at the time. Again, this individual made a full recovery. It is unclear at this stage whether these findings were related to the malaria vaccine the participants received, the malaria infection, malaria treatment, or some other cause. As a result of these events we will exclude people at high risk of heart disease from involvement in this study. These individuals will be identified by medical history, family history, appropriate blood tests, and performing an ECG.

In 2010 in a malaria challenge study in Oxford, a participant failed to attend for a scheduled study visit after being infected with malaria. The police were immediately informed and began a nationwide search for the individual, which involved the national media. The participant was found 17 days following challenge when he had mild malaria symptoms. He was admitted to a local hospital where he received treatment for malaria and made a full recovery. The reason for the participant's disappearance was unrelated to the malaria vaccine he received or the malaria challenge.

It is important that you understand that if you fail to attend a clinic appointment after challenge, but before you have completed a full course of antimalarial therapy, the police may be notified and your name may be released to the national media in order to find you.

There are no known long term side effects of contracting or being treated for malaria. For 6 months after the challenge you should contact a doctor if you develop any of

the symptoms of malaria as detailed above. In this circumstance, please contact one of the study doctors or your General Practitioner and remind them that you have been involved in this study. If you have any queries or concerns once the study is over, please do not hesitate to get in touch with us.

5. Treatment of Malaria

The drug you will be treated with is called Riamet. It is a licensed drug in the UK for the treatment of acute uncomplicated malaria caused by *Plasmodium falciparum* (the type of malaria you will be infected with). 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. We will need to watch you take at least three of these doses. We will continue taking blood to look for parasites until 2 consecutive blood tests are negative for malaria parasites. Blood tests usually become negative for malaria parasites after 24 hours of treatment. Tablets should be taken with a meal or snack. We will provide a light snack with the doses of Riamet that we observe at the clinic. You should avoid taking grapefruit juice while taking Riamet.

Riamet is generally well tolerated, but may cause some side effects. Side effects can include headache, dizziness, abdominal pain and loss of appetite, sleeping problems, palpitations, nausea, vomiting, diarrhoea, skin rash, cough, muscle or joint pain, and fatigue. Side effects such as dizziness may impact on the performance of skilled tasks such as driving. Riamet can have an effect on the electrical conduction in the heart (increase in the QT interval) which could potentially increase the risk for a cardiac arrhythmia as an extremely rare side effect; as a precaution we will use a different malaria treatment if we find any reason that you would be at increased risk. Severe allergic reactions could potentially occur, but the exact frequency is unknown. Signs of severe allergic reactions include rash and itching, sudden wheezing, tightness of the chest or throat, difficulty breathing, and swollen eyelids, face, lips, tongue or other part of the body. If you experience any of these symptoms you should contact the trial doctor immediately on the emergency contact number you will be provided with, or telephone 999 and ask for an ambulance if you are having difficulty breathing.

Some other medicines cannot be taken at the same time as Riamet. If you cannot take Riamet, or need to stop taking Riamet during the study, then there are other antimalarial drugs that can be used effectively instead. If at screening the doctor thinks you may not be able to take Riamet they will discuss an alternative medication with you (Malarone or Chloroquine) and give you an information sheet from the manufacturer of this drug to take away.

6. Treatment of Symptoms Associated with Challenge

Provided there are no contraindications, all participants will be given some medications to help with symptoms associated with malaria challenge. These are licensed, commonly used, medications. If you wish you can see the sheets from the manufacturers, provided inside the packets of these medications, prior to taking part in the study. As with all medications, these drugs can cause a severe allergic reaction in a small number of people. If you develop any concerning symptoms you should contact the trial doctor immediately on the emergency contact number, you will be provided with.

Antihistamine Cream: Antihistamine cream can help reduce the itch and redness associated with mosquito bites. Antihistamine cream is generally well tolerated. However, there is a risk of local redness, swelling or itching in the area where the cream has been applied.

Cyclizine: This is a tablet that can be taken as and when needed to help reduce nausea and vomiting. Cyclizine is generally well tolerated. However, side effects include skin rashes or itching, drowsiness, headache, dry mouth, nose or throat, blurred vision, palpitations, difficulty passing water, constipation, anxiety, and difficulty sleeping. It should be noted that drowsiness may affect your performance of skilled tasks such as driving.

Paracetamol: This is a tablet that can be taken 6 hourly to reduce feverishness, muscle and joint pain, back ache and headache. Paracetamol is generally well tolerated.

There may be risks or side effects that are unknown at this time.

OTHER INFORMATION

Blood Tests
We take blood tests as part of the screening visit and at the study visits in order for us to assess your general health, immune response to the vaccine and for safety reasons. If you would like them, we can give you the results of your blood tests. Coded blood samples will be stored after testing, and, if you consent, may be used in future malaria research. You will be asked to consent specifically for blood to be stored and used in future research. To avoid repeated testing, if you are not enrolled into this study and apply to enter another study conducted by the Jenner Clinical Trials Group based at the CCVTM in Oxford, the screening blood results may be used in that study, where appropriate.

Abnormal Results
If abnormal results or undiagnosed conditions are found in the course of the study these will be discussed with you and, if you agree, your GP (or a hospital specialist, if more appropriate) will be informed. Any newly diagnosed conditions will be looked after within the NHS.

Expenses and Payments
You will be compensated for:

- Screening Visit: £25
- Travel expenses: £10 per visit
- Time required for visit: £20 per hour
- Inconvenience of blood tests: £10 per blood donation
- Illness compensation £480

Group No.	Time in Trial (approx.)	Maximum No. of Visits	Maximum Volume of Blood Taken (ml)	Compensation Amount
1 & 2	6 months	48	687	£2475
3	6 months	50	702	£2615
4	3 months	31	517	£1705
5	3 months	29	262	£1625

*The exact number of visits depends on when/if you are diagnosed with malaria following challenge.

**The exact amount of blood taken will depend on when/if you are diagnosed with malaria and also where you are recruited- blood volumes for volunteers outside of Oxford may be slightly higher than those for Oxford volunteers.

If you choose to leave the study early, or are withdrawn from the study, you will be compensated according to the length of your participation based on these figures. You should note that compensation payments received in this trial may have an impact on your entitlement to benefits.

If you are in Group 1-3 and undergo rechallenge 5-7 months after the initial challenge, you will attend additional visits, undergo additional blood tests, and receive additional compensation. These additional visits, blood tests and compensation will be roughly equivalent to those detailed above for the control volunteers in groups 4&5.

'Back-up' participants
In addition to the participants to be included in the study, we will also recruit a small number of back-up participants. Back-up control participants will complete the clinic visit day before challenge, but will not necessarily undergo challenge. These 'back-up' participants will be asked to be available to take part in the challenge part of the study at short notice if another participant is unable to take part at the last minute. We will recruit a total of 4 back-up control participants (two for each control group). If back-up participants do not undergo challenge, they will be compensated in addition to compensation for visits they have already attended (eg. Screening and day before challenge). Back-ups will receive £200.

What alternatives are present?
Your alternative is not to participate in this study.

What are the possible benefits of taking part?

This study will not benefit you, but the information gained from the study might help to develop an effective malaria vaccine and prevent malaria infection and disease in those living in areas where malaria is common and in travellers to those areas. At present, there is no malaria vaccine licensed anywhere in the world. There are other malaria vaccines in various stages of development.

Will my taking part in the study be kept confidential?
Yes. All the information about your participation in this study will be kept confidential. The details are included in Part 2.

This completes Part 1 of the Information Sheet. If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2

What if relevant new information becomes available?
Sometimes during the course of a trial, new information relevant to the trial becomes available. If this happens, we will tell you about it and discuss whether you want to, or should, continue in the study. If you decide to continue to take part, you will be asked to sign an updated consent form. On receiving new information, we may consider it to be in your best interests to withdraw you from the study. Your participation in this study may also be stopped at any time by the study doctor or the Sponsor for other reasons.

What will happen if I don't want to carry on with the study?
If, at any time after agreeing to participate, you change your mind about being involved with this study you are free to withdraw without giving a reason. Your decision will not result in any penalty, or loss of benefits to which you are otherwise entitled. However, **if you wish to leave after malaria challenge then you must take the treatment course of Riamet (or an agreed alternative) because of the potentially very serious consequences of untreated malaria infection.** Unless you state otherwise, any blood taken whilst you have been in the study will continue to be stored and used for research as detailed above. You are free to request that your blood samples are destroyed at any time during or after the study.

What if there is a problem?
If you are harmed as a result of taking part in this study, the study doctor can advise you of further action and refer you to a doctor within the NHS for treatment, if necessary. The University of Oxford, as Sponsor, has appropriate insurance in place in the unlikely event that you suffer any harm as a direct consequence of your participation in this trial. NHS indemnity operates in respect of the clinical treatment which may be provided if you needed to be admitted to hospital.

The investigators recognise the important contribution that volunteers make to medical research, and make every effort to ensure your safety and well-being. In the event of harm being suffered, while the University will cooperate with any claim, you may wish to seek independent legal advice to ensure that you are properly represented in pursuing any complaint. At any time during the study you will be entirely free to change your mind and withdraw from the study. This will not affect your subsequent medical care in any way.

Complaints statement
If you wish to complain about any aspect of the way in which you have been approached or treated during the course of this study, you should contact your local trial team (contact details at the end of this document) or you may contact the University of Oxford Clinical Trials and Research Governance (CTRG) office on 01865 572224 or the head of CTRG, email ctrg@admin.ox.ac.uk.

Will my taking part in this study be kept confidential?
All information that is collected about you during the course of the research will be coded with a study number and kept confidential. The information is available to the study team, safety monitors, ethical review committees, Sponsors, government regulatory agencies, authorised collaborators and external monitors who can ask to audit or monitor the study. Any information about you that leaves the hospital or clinic will have your name and address removed so that you cannot be identified from it. Your information is stored electronically on a secure server and any paper notes are kept in a locked filing cabinet. Records are stored for at least 15 years, and in accordance with the applicable regulations. Information generated from this study will be shared with GlaxoSmithKline (GSK), who are providing one of the vaccines for this trial. GSK is a company that studies and makes vaccines, medicines and other health products. GSK will have access to the study data collected, however, the link between your name and the code number will not be shared. Only the code number and coded information will be sent to GSK.

Involvement of the General Practitioner/Family doctor (GP)
In order to enrol into this study, you will be required to sign a form documenting that you consent for us to contact your GP. This is to inform them that you are interested in being involved in the study, and to check there are no medical reasons that they are aware of that would make your participation inadvisable. Your GP may be asked to share information about your medical history and give access to any other medical records as required. The researchers will not enrol you in the trial if your GP has relevant concerns about your eligibility or safety. We will write to your GP to let them know whether or not you are finally enrolled in the study, and whether or not you completed the study, so they can update your medical records accordingly.

Prevention of 'Over Volunteering'
Volunteers participating in this study must not be concurrently receiving investigational medications or vaccines in another study at the same time. In order to check this, you will be asked to provide your National Insurance or Passport number. This will be entered on to a national database which helps prevent volunteers from taking part in too many clinical trials. More information can be found at *www.tops.org.uk*. Your national insurance or passport number is also required to allow processing of compensation payments.

What will happen to any samples I give?
If you consent, some of your leftover blood samples will be stored and may be used for further studies of the human body's immune response and/or the vaccines used in this study, and/or your safety. Any such tests will have an appropriate ethical review. Upon your request at any time, your remaining blood samples will be destroyed. Your participation in this study will not be affected by your decision whether to allow storage and future use of your leftover samples. To avoid repeat testing, if you are not enrolled into this study and you apply to enter another study conducted by the Jenner Clinical Vaccine Trials Group based at the CCVTM, the results from your screening visit blood tests may be used to determine whether you are eligible for the trial you applied for.

Your study visit blood tests will be analysed in the hospital laboratories and Oxford University research laboratories. Other blood tests to look at the response of your body to the vaccine will be done with collaborating laboratories in the UK and in other countries. Any samples or data sent to them would be anonymous.

Will any genetic tests be done?
Yes. Some blood will be used to look at the pattern of your genes that can affect the immune system (including your 'human leukocyte antigen' [HLA] type). The immune response to vaccines is in part genetically controlled, so knowing your pattern of genes that regulate immune responses may help us to understand the responses to vaccination. We may also look at the expression of certain genes which relate specifically to the immune response to malaria. You can opt out of 'genetic tests' if you wish, without any effect on your participation in the trial.

What will happen to the results of the research study?
The results of this research study may be presented at scientific meetings or conferences and published in a scientific medical journal. This may not happen until 1 or 2 years after the study is completed. If you contact the researchers in the future, you can obtain a copy of the results. You will not be identified in any report or publication.

The anonymised data from this study will be shared with the collaborating partners who are organising and funding this research work, including the pharmaceutical company GlaxoSmithKline (GSK). Data from this study may be used to file patents, licence vaccines in the future or make profits in other ways. You will not be paid for any part of this. Data from this study may be used as part of a student post-graduate degree, for example a MD or PhD.

A description of this study will be available on www.clinicaltrials.gov, as required by US law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

Who is sponsoring, organising and funding the research?
The study is organised by the University of Oxford. The study is funded through financial support to Oxford University from several funders. Neither your GP nor the researchers are paid for recruiting you into this study.

Who has reviewed the study?
This study has been reviewed by the National Research Ethics Service (NRES) Committee South Central Berkshire Research Ethics Committee and has been given a favourable ethical opinion. The Medicines and Healthcare products Regulatory Agency (MHRA), which regulates the use of all medicines in the UK, has reviewed the study design and has granted permission to use these unlicensed vaccines in this clinical study.

Thank you for reading this information sheet. If you are interested in taking part in the study please contact the study team at your local study site to arrange a screening appointment.

Contact details for further information:

Volunteer Recruitment Co-ordinator

INSERT LOCAL CONTACT DETAILS HERE

Appendix 2: Schedule of attendances

VAC048 Schedule of attendances

Attendance Number	1	2	3	3a*	4	5	6	7	8	8a*	9	10	11	12	13
Timeline** (days) (weeks)	≥-90	0 0	1[@]	3	7 1	14 2	28 4	56 8	57[@]	59	63 9	70 10	84 12	112 16	168 24
Time window (days)			+1		±2	±2	±7	±7	+1		±2	±2	±7	±7	±14
Screening Visit	X														
Informed Consent	X														
Review contraindications, inclusion and exclusion criteria	X	X						X							
Vaccination		X						X							
Vital signs	X	X	X	X	X	X		X	X	X	X	X		X	X
Ascertainment of adverse events		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Diary cards provided		X						X							
Diary cards collected					X						X				
Medical History, Physical Examination	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Biochemistry, Haematology (ml)	6	6			6		6	6			6		6		6
Exploratory immunology (ml)		60				60	60	60			60		60	60	60
Gene expression profiling (ml)		10	10		10	10		10	10		10	10			
Anti-nuclear antibody	4							4[¥]						4[¥]	
Urinalysis	X	X[¥]					X[¥]	X[¥]					X[¥]	X[¥]	
Urinary β–HCG (women only)	X	X						X							
HLA typing (ml)		4													
HBsAg, HCV Ab, HIV serology (ml)	5														
Blood volume per visit	15	80	10	0	16	70	66	80	10	0	76	10	66	64	66
Cumulative blood volume	15	95	105	105	121	191	257	337	347	347	423	433	499	563	629

*The first three volunteers to receive each Matrix M™- adjuvanted vaccination were reviewed at 3 days post-vaccination

**Timeline is approximate only. Exact timings of visits relate to the previous visit, ie, each visit must fall at the interval indicated after the last visit ± time window.

[@] The visit will take place as close to 24 hours following vaccination as practicable

X: visit or procedure takes place

(X): procedure takes place if considered necessary, emphasising any acute complaints

[¥]Only for volunteers receiving Matrix M™

VAC053 & VAC056 Schedule of attendances

Attendance Number	1[S]	2	3	3a*	4	5	6	7	8	9	10	11	12	13	14	15
Timeline** (days) (weeks)	≤ 90	0 0	1	3	7 1	14 2	28 4	29	35 5	42 6	56 8	57	63 9	70 10	84 12	238 34
Time window (days)			+1	+1	±2	±3	±2	+1	±2	±3	±2	+1	±2	±3	±2	±14
Informed Consent	X															
Review contraindications, inclusion and exclusion criteria	X	X					X				X					
Vaccination		X					X				X					
Vital signs^	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	(X)
Ascertainment of adverse events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	(X)
Diary cards provided		X					X				X					
Diary cards collected							X				X				X	
Medical History, Physical Examination	X	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Biochemistry[$], Haematology (ml)	5	5			5		5		5		5		5		5	
Exploratory immunology[£] (ml)		50			50	50	50		50	50	50		50	50	50	50
Gene expression profiling (ml)		3	3		3			3	3			3	3			
Urinalysis	X															
Urinary β−HCG (women only)	X	X					X				X					
HLA typing (ml)		4														
HBsAg, HCV Ab, HIV serology (ml)	5															
Blood volume per visit	10	62	3	0	58	50	55	3	58	50	55	3	58	50	55	50
Cumulative blood volume	10	72	75	75	133	183	238	241	299	349	404	407	465	515	570	620[%]

[S] = screening visit; (X) = if considered necessary ^ = Vital signs included pulse, blood pressure and temperature; [$] = Biochemistry included Sodium, Potassium, Urea, Creatinine, Albumin and Liver function tests. [£] = Exploratory immunology included antibodies to CSP and NANP, antibodies to HepBsAg, ex vivo ELISpot responses to CSP and flow cytometry assays, functional antibody assays and B cell analyses.

**The first three volunteers in each group were reviewed at 3 days post-vaccination 1.*

***Timeline is approximate only. Exact timings of visits relate to the day on enrolment, ie, each visit must occur at indicated number of days after enrollment ± time window. [%]Cumulative blood volume for Oxford volunteers if blood taken as per schedule, and excluding any repeat safety blood test that may be necessary. Volunteers at other sites may have a slightly higher cumulative volume.*

VAC065 Schedule of attendances

Event	S	VAC1				VAC2				VAC3				C-1
Attendance number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Timeline (days)		0	1	7	14	28	29	35	42	56	57	63	70	83
Window (days)	-180		2	±3	±3	±7	2	±3	±3	±7	2	±3	±3	-1
Inclusion / Exclusion criteria	X	X				X				X				X
Informed Consent Questionnaire	X													
Informed consent	X													
Medical History	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)
Urinalysis	X													
Electrocardiogram	X													(X)
β-HCG urine (♀)	X	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
AEs reviewed		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
Biochemistry (mL)**	3	3		3		3		3		3		3		3
Anti- *P. Falciparum* serology (mL)$^{\$}$	2													
Immunology (mL)		50		30	30	50		30	60	30		30	30	90
RNA analysis (mL)		5	2.5				2.5				2.5			2.5
Blood Film/PCR (mL)														3
Blood volume per visit (mL)	12	64	2.5	35	30	55	2.5	35	60	35	2.5	35	30	100.5
Cumulative blood volume (mL)***	12	76	78.5	113.5	143.5	198.5	201	236	296	331	333.5	368.5	398.5	499

Schedule of clinical reviews pre CHMI for Groups 1&2 *(Windows refer to time since last vaccination apart from the window for the C-1 visit, which is in relation to the day of challenge); S = screening visit, (x) = If considered necessary, emphasising any acute complaints; ^Physical observations included blood pressure, pulse and temperature, height and weight, however height and weight was only measured at screening; ** Biochemistry included Sodium, Potassium, Urea, Creatinine, Albumin, Liver Function Tests, Magnesium & Cholesterol, however Magnesium and cholesterol were only measured at screening;$^{\$}$ Anti-P.falciparum serology may be tested at screening in certain individuals to help determine prior malaria exposure; *** The exact cumulative volume of blood taken will depend on the local site - blood volumes for volunteers outside Oxford may be slightly lower or higher than those for Oxford volunteers, due to use of different volume vacutainers for biochemistry, haematology and serology samples as per local Trust standard procedures.*

Event	S	VAC 1		VAC 2			VAC 3				VAC 4		VAC 5			C-1
Attendance	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Timeline (days)		0	1	7	8	14	28	29	35	42	56	57	63	64	70	83
Window (days)	-180		2	±3	2	±3	±7	2	±3	±3	±7	2	±3	2	±3	-1
Inclusion / Exclusion criteria	X	X		X			X				X		X			X
Informed Consent Questionnaire	X															
Informed consent	X															
Medical History	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)
Urinalysis	X															
Electrocardiogram	X															(X)
β-HCG urine (♀)	X	X		X			X				X		X			X
Review contraindications	X	X		X			X				X		X			X
Vaccination		X		X			X				X		X			
Physical Observations^	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
Diary card commenced		X		X			X				X		X			
Diary card completed				X			X				X		X			X
HLA typing (mL)		4														
HBV,HCV,HIV (mL)	5															
Haematology	2	2		2		2	2		2		2		2		2	2
Biochemistry (mL)**	3	3		3		3	3		3		3		3		3	3
Anti- *P. Falciparum* serology[$] (mL)	2															
Immunology (mL)		50		30		30	50		30	60	30		30		30	90
RNA analysis (mL)		5	2.5		2.5			2.5				2.5		2.5		2.5
Blood Film/PCR (mL)																3
Blood volume per visit (mL)	12	64	2.5	35	2.5	35	55	2.5	35	60	35	2.5	35	2.5	35	100.5
Cumulative blood volume (mL)***	12	76	78.5	113.5	116	151	206	208.5	243.5	303.5	338.5	341	376	378.5	413.5	514

Schedule of clinical reviews pre CHMI for Group 3 *(Windows refer to time since last vaccination apart from the window for the C-1 visit, which is in relation to the day of challenge); S = screening visit, (x) = If considered necessary, emphasising any acute complaints; ^Physical observations included blood pressure, pulse and temperature, height and weight, however height and weight was only measured at screening; ** Biochemistry included Sodium, Potassium, Urea, Creatinine, Albumin, Liver Function Tests, Magnesium & Cholesterol, however Magnesium and cholesterol were only measured at screening;[$] Anti-P.falciparum serology may be tested at screening in certain individuals to help determine prior malaria exposure; *** The exact cumulative volume of blood taken will depend on the local site - blood volumes for volunteers outside Oxford may be slightly lower or higher than those for Oxford volunteers, due to use of different volume vacutainers for biochemistry, haematology and serology samples as per local Trust standard procedures.*