UNIVERSITY OF OXFORD

Safety and immunogenicity of a protein particle malaria vaccine candidate, R21, administered with and without Matrix-M1 in healthy UK volunteers

A Phase I Clinical Trial

Study Reference: VAC 053

Protocol Version Number: 5.0

Date: 11th July 2016

Chief Investigator: Professor A.V.S Hill

Sponsor: University of Oxford

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Confidentiality Statement

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

Statement of Compliance

The trial will be conducted in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice Guideline E6 (R1) (ICH-GCP) and the applicable regulatory requirements.

Signatures

"I have read this protocol and agree to abide by all provisions set forth therein. I agree to comply with the principles of the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice."

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Modification history

Version	Date	Modifications	Author(s)
2.0	26/08/2015	Addition of the John Warin ward as an NHS site (pages 3 and 8). Correction of minor typographical errors (pages 3 and 4)	Emma Bakpa
2.1	09/11/2015	Typographical error corrected in section 6.4.	Emma Bakpa
3.0	24/11/2015	Clarification of previous receipt of an investigational vaccine in exclusion criteria (page 43, Section 7.3.2) Clarification that safety data is collected and stored through an electronic diary on a secure server; this data is uploaded to the study database at the end of the trial. (Page 58, Section 9.6)	Navin Venkatraman
4.0	08/06/2016	Addition of Group 4 – changes made throughout the document	Navin Venkatraman
5.0	11/07/2016	New concentration of Matrix-M1 added to be used for Group 4 volunteers	Navin Venkatraman

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1 SYNOPSIS

Trial Title	A Phase I study to assess the safety and immunogenicity of a protein particle malaria vaccine candidate, R21, administered with and without Matrix-M1 in healthy UK volunteers.
Trial Identifier	VAC053
Clinical phase	I
Active ingredients of vaccines/products	R21, a protein particle produced by using recombinant HBsAg particles expressing the central repeat and the C-terminus of the circumsporozoite protein (CSP), which will be administered either alone or adjuvanted with Matrix-M1.
Finished products and doses	1. R21 10μg mixed with Matrix-M1 50μg 2. R21 50μg without adjuvant 3. R21 50μg mixed with Matrix-M1 50μg 4. R21 2μg mixed with Matrix-M1 50μg
Form	Liquid (all finished products)
Route	Intramuscularly into the deltoid region of the arm
Principal Investigator	Adrian V. S. Hill
Trial Centres	Clinical Vaccinology & Tropical Medicine, University of Oxford, Churchill Hospital, Old Road, Headington, Oxford, OX3 7LE John Warin Ward, Oxford University Hospital NHS Trust, Churchill Hospital, Old Road, Headington, Oxford, OX3 7LE NIHR/Wellcome Trust Clinical Research Facility, Hammersmith Hospital, 150 Du Cane Road, London, W12 0HS
Planned Trial Period	Q3 2015 to Q1 2017

Study Duration	34 weeks per subject
Primary Objective	To assess the safety profile of the candidate vaccine R21 with and without the adjuvant Matrix-M1 in healthy adult volunteers
Secondary Objective	To assess the immunogenicity of the candidate vaccine R21 with and without the adjuvant Matrix-M1 in healthy adult volunteers
Population	Healthy UK adults aged 18 – 50 years
Planned Sample	34 volunteers

Vaccination Schedule

	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

Follow-up duration	26 weeks post final vaccine dose

Primary Evaluation Criteria

Actively and passively collected data on local and systemic adverse events

The following parameters will be 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

Volunteers will undergo clinical follow up for adverse events for a further 182 days following completion of the vaccination regimen.

Secondary Evaluation	Measures of immunogenicity of R21 formulations with and without adjuvant may include:

Criteria	• ELISA to quantify antibodies to CSP and NANP • ELISPOT to enumerate IFN-γ producing T cells • Ex vivo ELISPOT responses to NANP • Flow cytometry and intracellular cytokine staining to enumerate and functionally characterise immune cell populations such as; T cells (e.g. CD4+ and CD8+), B cells and dendritic cells • ELISPOT for enumeration of antibody-secreting cells (e.g. B cell ELISPOT responses to NANP) • Gene expression profiling including RNA analysis, DNA sequencing and other genotypic methods
Study Design	Open–labelled, non-randomised multicentre Phase I study

2 ABBREVIATIONS

AE	Adverse event
AS01	Adjuvant System 1 comprising the immunoenhancers QS-21 and MPL with proprietary liposomes. In the context of malaria vaccine development, AS01 has been tested as two formulations: $AS01_B$ and $AS01_E$
AR	Adverse reaction
CBF	Clinical Bio-Manufacturing Facility
CCVTM	Centre for Clinical Vaccinology and Tropical Medicine
CHMI	Controlled human malaria infection
CI	Confidence Interval
CI	Chief Investigator
CRF	Case report form or Clinical Research Facility
CSP/CS	Circumsporozoite Protein
CTL	Cytotoxic lymphocytes
CTRG	Clinical Trials Research Governance
Da	Dalton
DSUR	Development Safety Update Report
DTPwHepB/Hib	Diphtheria, tetanus, whole-cell pertussis, hepatitis B and Haemophilus Influenza type B
ELISA	Enzyme linked immunosorbent assay
ELISPOT	Enzyme linked immunospot assay
EPI	Expanded Programme of Immunisation
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HA	Haemagglutinin
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HI	Haemagglutination inhibition
HIV	Human Immunodeficiency virus
HLA	Human leukocyte antigen
ICH	International Conference on Harmonisation
IFN-γ	Interferon-gamma
IM	Intramuscular
IMP	Investigational Medicinal Product
LPS	Lipopolysaccharide

LSC	Local Safety Committee
LSM	Local Safety Monitor
MHRA	Medicines and Healthcare products Regulatory Agency
MM	Matrix-M1
MPL	Monophosphoryl lipid A
MRC	Medical Research Council
μg	microgram
nm	nanometer
PIS	Participant information sheet
ppm	parts per million
QP	Qualified Person
QS	*Quillaja saponaria*
R21	Protein particle malaria vaccine candidate
RTS,S	A hybrid polypeptide consisting of a portion of the CS 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
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
UOXF	University of Oxford
VE	Vaccine efficacy
WHO	World Health Organisation

3 BACKGROUND & RATIONALE

3.1 Impact of malaria and the need for a vaccine

Falciparum malaria remains one of the leading infectious causes of morbidity and mortality worldwide, predominantly affecting children and pregnant women in sub-Saharan Africa.[1] There are over 430,000 deaths annually and a huge socioeconomic burden associated with this parasitic disease.[1, 2] In high transmission countries, a child presents with 1.6 - 5.4 episodes of clinical malaria per year [1], with about 5% of malaria episodes turning to severe disease [3].

The advent of artemisinin-combination therapy and increased uptake of insecticide-treated nets has resulted in significant recent reductions in mortality in many places. However, emergence of resistance to artemisinins and insecticides may hinder progress made towards the ultimate goal of eradication.[4] In this regard, the development of a vaccine would be an invaluable tool in the fight against malaria. *Plasmodium falciparum* is a complex pathogen, which is highly immunoevasive and the development of an efficacious vaccine has remained elusive for many years.

3.1.1 Lifecycle of Plasmodium falciparum

The lifecycle of *P. falciparum* is complex with stages in both human and mosquito hosts (Figure 1). The bite of infected female Anopheles mosquitoes transmits malaria sporozoites to the human host where they travel via the bloodstream to the liver and invade hepatocytes. Here, during the liver stage, they mature into merozoites for 6 to 7 days. Malaria parasites are not detectable in the blood stream during the liver stage. The hepatocytes then rupture, releasing a large number of merozoites into the bloodstream (blood stage of infection). Merozoites invade erythrocytes where they multiply and after 2 days cause the erythrocyte to rupture, releasing progeny merozoites that in turn invade new erythrocytes. A small percentage of merozoites differentiate into gametocytes, which when ingested by a mosquito, unite with another gametocyte to create a zygote. The zygote matures and releases sporozoites which migrate to the mosquito's salivary glands and are injected into the human when the mosquito feeds.

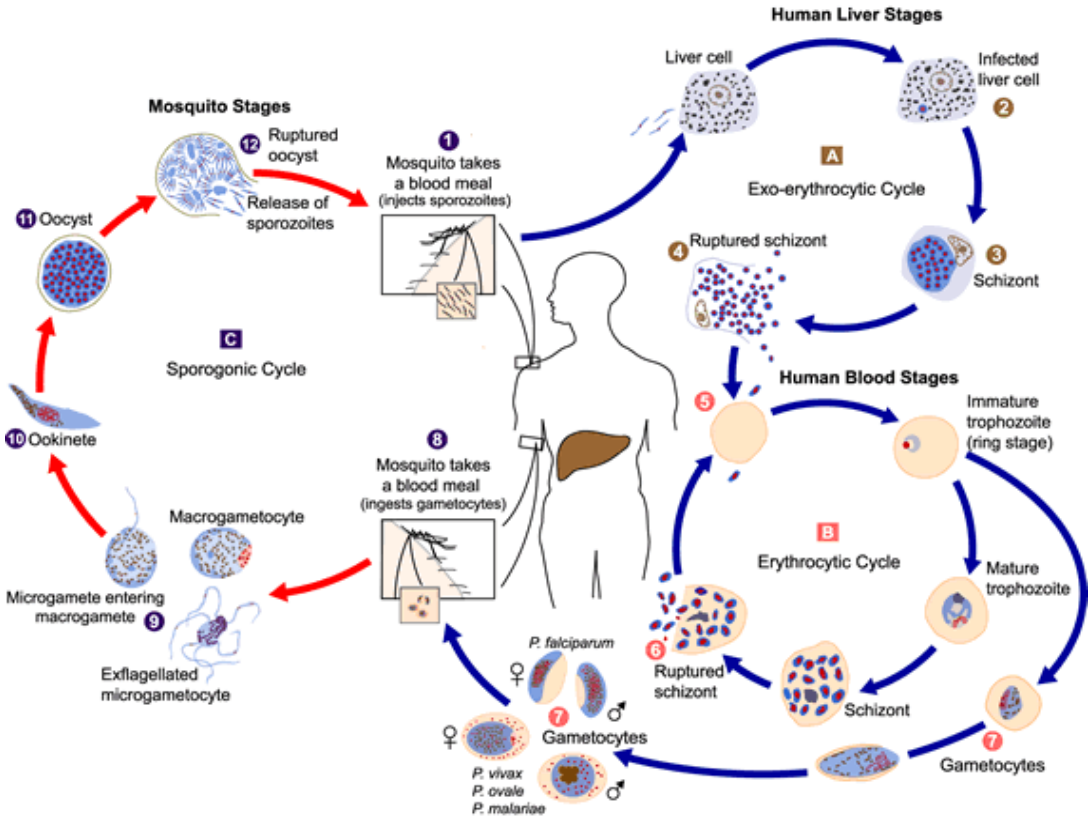


Figure 1. *Lifecycle of Plasmodium falciparum*

3.1.2 Progress towards a *P. falciparum* vaccine

Recently, there have been significant advances; the leading vaccine candidate RTS,S/AS01 has been tested in a Phase III study in African infants that completed very recently. RTS,S is based on the major malaria sporozoite surface protein the circumsporozoite protein, or CS protein.

The pivotal Malaria-055 Phase III efficacy study is a large double-blinded, randomized, controlled multicentre trial, which includes 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% (97.5% CI: 50.6, 60.4; p<0.001) reduction in the number of all malaria episodes during the first 12 months of follow-up, with 47.3% (95% CI: 22.4, 64.2; p<0.001) efficacy against severe, life-threatening malaria.[5] The vaccine did not perform as well in children vaccinated aged 6-14 weeks of age, in co-administration with other EPI (expanded programme of immunisation) 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.[5]

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% (95% CI 42% to 50%). This waned over time, but it persisted throughout the 18-month period. Efficacy against severe malaria was 36% (95% CI 15% to 51%) and reductions in both malaria hospitalizations (41%) and all-cause hospitalizations (19%) were noted over 18 months.[6] In the 6-14 week age group, an overall efficacy of 27% (95% CI 20% to 32%) was observed in the incidence of all episodes of clinical malaria.[6]

The final results of this Phase 3 trial were published in April 2015.[7] 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% (95% CI 31.8-40.5). However, achieving this efficacy would require four immunisations at time points not currently in the standard schedule of infant immunisations and therefore be likely very expensive and logistically difficult to achieve. 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% (95% CI 23-33). Vaccine efficacy against severe malaria was 32% (95% CI 44-47) in those that received RTS,S/AS01 at months 0, 1, 2 and 20, but only 1% (95% CI -23-21) in those administered the vaccine at 0, 1 and 2 months.

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% (95% CI 20-32) and 18% (95% CI 12-24) in those receiving immunisations at 0, 1 and 2 months. Efficacy against severe malaria was 17% (95% CI -9-38) with four doses and 10% (95% CI -18-32) with three doses. There was no vaccine efficacy against malaria mortality but power was low.

Though these results show significant low level efficacy against most endpoints, there remains an urgent need to improve efficacy to achieve World Health Organisation (WHO) goals - development of a suitable vaccine with at least 75% durable efficacy against clinical malaria by 2030. Potential explanations for the reduced immune response in 6-12 week old infants include: 1) the infant's immature immune system; 2) the co-administration of RTS,S with other childhood vaccines (DTPw-HepB/Hib and oral poliovirus vaccines); 3) the absence of priming with hepatitis B vaccine or with *P. falciparum* infection; and 4) maternal antibodies. It is also possible that the excess of Hepatitis B surface antigen present in the

formulation interferes with the induction of a potent immune response to circumsporozoite protein.

RTS,S targets the pre-erythrocytic circumsporozoite (CS) protein, which is the major functional protein that plays a key role in sporozoite development and hepatocyte invasion.[8] 80% of the molecules in each RTS,S particle are hepatitis B surface antigen, and only 20% are fusion proteins of the malaria circumsporozoite protein moiety fused to hepatitis B suface antigen. R21, to be tested in this trial, is a biosimilar protein particle that lacks the excess of HBsAg in RTS,S has been developed at the University of Oxford. Indeed R21 comprises only fusion protein moieties, i.e. as 100% of its molecules, in contrast to RTS,S which comprises 20% of these with the remaining 80% being HBsAg molecules (Figure 2).

3.2 Pre-erythrocytic stage as a vaccine target

The pre-erythrocytic stage of *P. falciparum* 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. Anti-CS antibodies can target sporozoites, facilitating destruction of sporozoites prior to hepatocyte invasion. As sporozoites travel from the skin to the liver within minutes, it may be difficult for a vaccine to achieve complete protection against *P. falciparum* 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. Research suggests that, in isolation the RTS,S vaccine targeting the pre-erythrocytic stage antigen, the CS protein, and vaccines targeting ME-TRAP, a liver-stage insert in Oxford's viral vectored vaccines, do not delay the initial emergence of parasites in to the blood, nor the rate of parasite multiplication in the blood, but rather reduce the size of this initial inoculum.[9] A delay to patent blood stage infection in persons receiving these vaccines reflects a reduced liver-to-blood inoculum. The efficacy of these pre-erythrocytic vaccine strategies can be assessed experimentally by subjecting volunteers to inoculation with *P. falciparum* sporozoites by the bite of infected mosquitoes. Complete protection against blood-stage infection, or a delay in the time to patent blood stage infection in vaccinees compared to controls, reflect vaccine efficacy.

There are a number of reasons that support the selection of circumsporozoite protein as a potential target for a malaria vaccine candidate. This protein is expressed on the sporozoite surface [10] and to a lesser degree on hepatic schizonts and plays a pivotal role in alignment and sporozoite invasion of hepatocytes.[8, 11] In vitro, antibodies directed against B cell epitopes derived from this protein can inhibit the infectivity of sporozoites to liver cells.[12] In murine models, passive transfer of antibodies to the immunodominant B

cell repeat epitope of the CS protein as well as active immunisation with constructs containing this epitope, confer protection against sporozoite challenge. [13, 14] Furthermore, it has been shown that adoptive transfer of CD8+ cytotoxic lymphocytes (CTL) or CD4+ T cell clones specific for epitopes on the CS protein can provide protection against a sporozoite challenge. [15, 16] Finally the leading malaria vaccine candidate, RTS,S, induces partial efficacy by inducing antibodies against the central repeat (NANP) of the circumsporozoite protein.

3.3 R21 vaccine development

Manufacture of clinical grade R21 particle was undertaken at the University of Oxford Clinical Bio-Manufacturing Facility (CBF) (www.cbf.ox.ac.uk), with financial support from the UK Medical Research Council (MRC) and the EC FP7 programme.

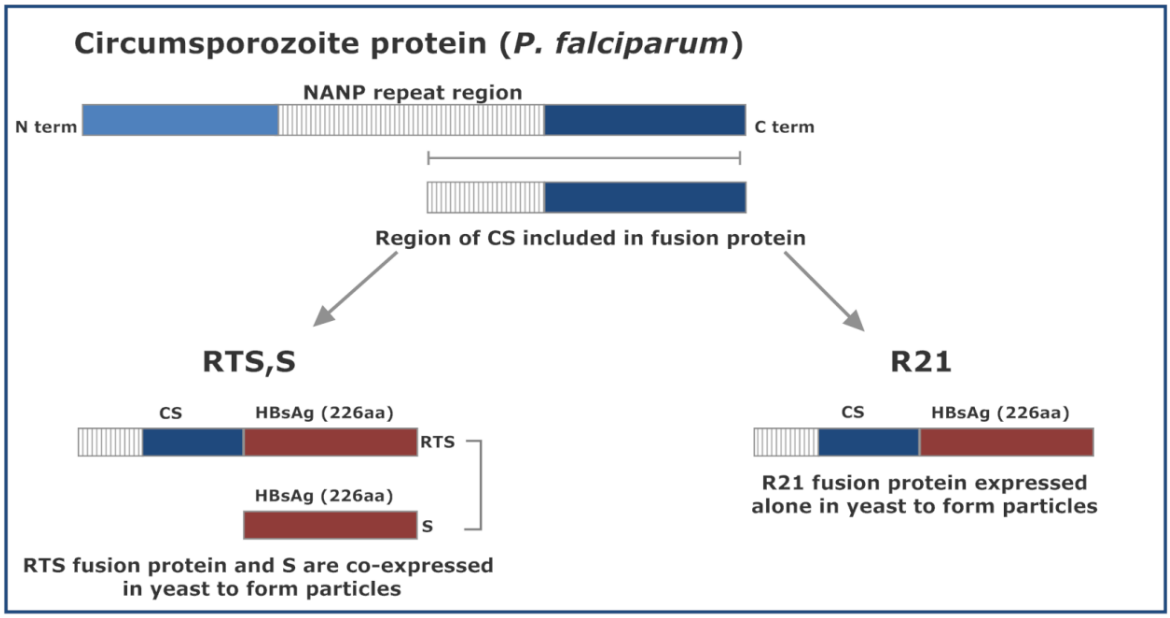


Figure 2: *Schematic diagram showing RTS,S and R21 fusion proteins. Both RTS,S and R21 include the fusion protein of hepatitis B surface antigen to the C-terminus and central repeats of the circumsporozoite (CS) protein. These repeats comprise many copies of the four amino acid sequence NANP. R21 is a virus like-particle that results for spontaneous assembly of the R21 molecules. RTS,S, expressed in a different yeast types required expression of an four fold excess of the unfused hepatitis B surface antigen to allow it to form hybrid particles. Generation of virus-like particles by both RTS,S and R21 has been shown to be important for allowing induction of high level antibody responses.*

RTS,S/AS01 vaccine, induces very strong antibody responses to the conserved central repeat of circumsporozoite protein (CSP), of the order of 100 - 600 micrograms per ml, very

weak mainly IL-2 containing CD4+ T cells and no CD8+ T cells to CSP.[17] The most reproducible correlate of protection in clinical studies is with antibody levels.[5, 17]

We propose here to test clinically a biosimilar of the RTS,S vaccine called R21 adjuvanted with Matrix-M1 (MM). As a biosimilar of the RTS,S vaccine, the R21 particle contains no *P. falciparum* sequences that are not present in RTS,S, which has been safely used in thousands of individuals. It is a hybrid protein of the majority of the CS protein of *P. falciparum* fused to the hepatitis B surface antigen (Figure 2). It spontaneously forms a particle just like RTS,S. However, we anticipate that R21 may be a more immunogenic particle than RTS,S in humans for two reasons. It induces predominantly malaria rather than hepatitis antibodies in pre-clinical studies probably because it has a higher proportion of malaria to hepatitis antigen in its composition than RTS,S. This is made possible by expressing R21 in the better expressing yeast *Pichia pastoris*, rather than in *Saccharomyces cerevisiae*. Secondly, in pre-clinical studies R21 has been found to be exceptionally immunogenic for induction of anti-NANP antibodies, likely the key protective immune mechanism of RTS,S, yielding titres of a mean of 800,000 ELISA (enzyme linked immunosorbent assay) units (K Collins and A Hill, unpublished data). 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.

3.4 R21 - pre-clinical studies

3.4.1 Immunogenicity

Initial pre-clinical assessment of immunogenicity was undertaken in BALB/c mice that were immunised intramuscularly with 0.5μg of R21 alone or in combination with an adjuvant (Alhydrogel, or “Abisco” which is almost identical to the Matrix M to be used in this trial and made by the same company). Immune responses including antibody levels to the central NANP repeat region and antigen–specific T cell responses were measured three weeks after a 3-dose immunisation schedule (Figures 3.1 & 3.3). R21 + Abisco-100, a potent saponin-based adjuvant resulted in the greatest humoral immune response at each time point in the vaccination schedule.

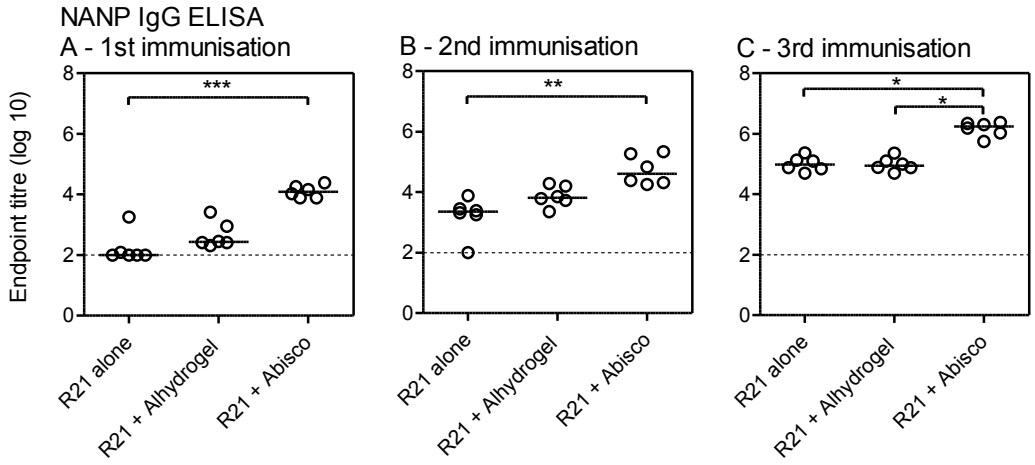


Figure 3.1*: Pre-clinical assessment of immunogenicity with 0.5μg of R21 alone or in combination with an adjuvant (Alhydrogel, Abisco).*

The responses in all groups were boosted by a third immunisation and R21 + Abisco-100 induced the highest titres of NANP specific IgG and the response for this group was significantly higher than both the R21 + Alhydrogel and R21 alone groups after the final immunisation (Figure 3.1).

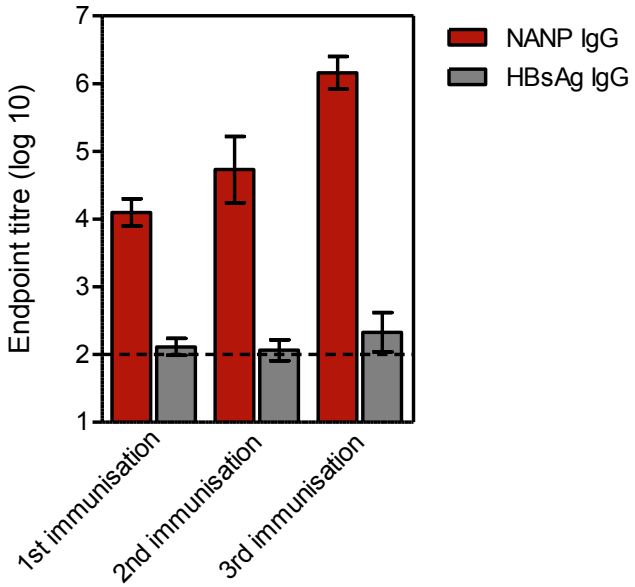


Figure 3.2: *Relative proportions of IgG to NANP and HBsAg after immunisation with R21 + Abisco-100 in BALB/c mice.*

Antibodies to hepatitis B surface antigen were measured in the same study. As expected from the composition of R21 and RTS,S antibodies to hepatitis B surface antigen were much lower with R21 than RTS,S (Figure 3.2) probably reflecting a structure in R21 where the CS sequences are found on the exposed surface of the virus-like particle whereas the surface of RTS,S comprises both hepatitis B and malaria epitopes.

CS-specific IFN-γ producing T cells measured after the third immunisation were only detected at a significant level in mice immunised with R21 + Abisco-100 (Figure 3.3). R21 alone was ineffective at inducing CS-specific T cell responses on its own. Further comparison to other adjuvants including a squalene-based oil-in-water emulsion (Addavax) and a polyionic carbomer (Carbopol) showed that Abisco-100 was the best adjuvant to induce high levels of humoral and cell-mediated immunity.

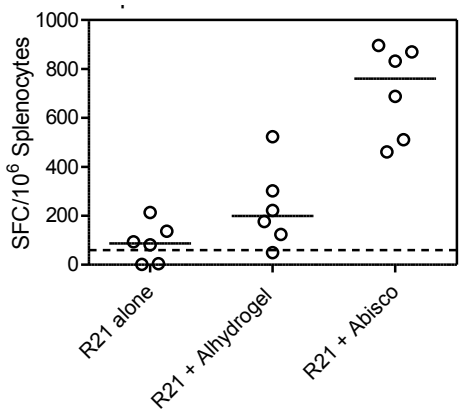


Figure 3.3: *CS-specific IFN-γ producing T cells measured after the third immunisation*

3.4.2 Efficacy

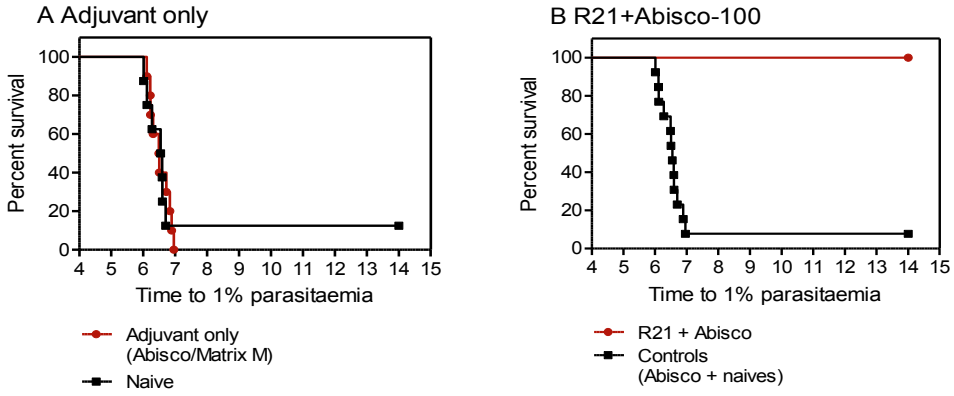
Sporozoite challenge (1000 sporozoites per mouse injected intravenously) using transgenic *P. berghei* parasite were performed in BALB/c mice (Figure 3.4). R21 + adjuvant were given twice, eight weeks apart and mice were challenged three weeks after the second dose. Thin blood films looking microscopically for parasitaemia were performed daily from day 5 post-challenge. Sterile protection was defined as remaining slide negative at day 14, and significant delay in development of 1% parasitaemia compared to non-immunised control mice was regarded as partial efficacy.

R21 + Abisco-100 steriley protected 100% of the challenged mice (p=< 0.0001) and R21 + Matrix M steriley protected 87.5% (p=0.0002) and this was confirmed in a second

independent challenge. (p = < 0.0001). There was no significant difference between the two adjuvants.

The durability of efficacy was assessed by undertaking sporozoite challenge in mice seven and fourteen weeks after immunisation. Efficacy was maintained well at seven weeks post immunisation with 75% of mice sterilely protected (6/8) and this was not significantly different when compared to efficacy at three weeks post immunisation (p=0.4468, by Log-rank (Mantel-Cox) Test). At 14 weeks post immunisation however, sterile efficacy was reduced to 50% (2/4) and this was 37% lower than the efficacy at three weeks. This was not significantly lower probably due to the small number in the group (p=0.0636).

Sterile efficacy 14 weeks after immunisation is 37% lower than efficacy three weeks after immunisation. This reduction in protective efficacy can however be boosted to 100% if mice are challenged once (three weeks post immunisation) within the 14 weeks. Therefore efficacy after vaccination and one sporozoite infection is very durable and 100% sterile efficacy is maintained for at least 14 weeks.



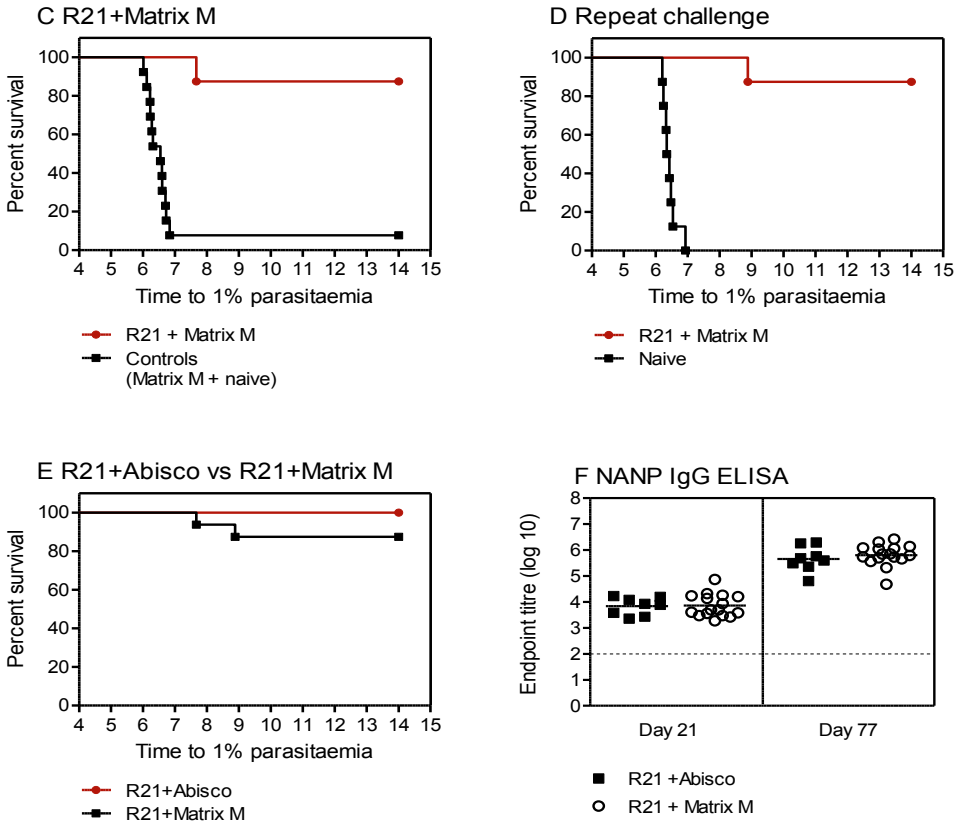


Figure 3.4 (A-F): *Protective efficacy elicited by saponin based ISCOM adjuvants with R21 in a transgenic sporozoite model. BALB/c mice were immunised i.m. with 0.5µg R21 + adjuvant (Abisco-100 or Matrix M), twice eight weeks apart (n=8/group). Mice were challenged three weeks after the final vaccination by i.v. injection of 1000 sporozoites (P. berghei transgenic for P. falciparum CSP) along with eight naïve mice. Two groups of adjuvant control mice (n=5/group) were also challenged three weeks after receiving two shots of adjuvant (Abisco-100 or Matrix M) i.m., eight weeks apart. Blood stage parasitemia was monitored from day 5 after challenge by thin-film blood smear, and time to 1% parasitemia was calculated using linear regression. The results are presented in the Kaplan-Meier survival graphs and survival curves were compared by Log-rank (Mantel-Cox) Test.* ***(A)*** *Adjuvant control = no significant difference,* ***(B)*** *R21 + Abisco-100 p<0.0001,* ***(C)*** *R21 + Matrix M p=0.0002,* ***(D)*** *R21 + Matrix M repeat p=<0.0001,* ***(E)*** *R21 + Abisco vs R21 + Matrix M = no significant difference. Blood was taken three weeks after each vaccination (Day 21 and Day 77) for immunology and NANP specific IgG was assayed by ELISA* ***(F)****, group mean responses shown and dotted line indicates the limit of detection.*

3.5 RTS,S/AS01

The RTS,S antigen consists of two proteins, RTS and S, that are expressed intracellularly in yeast and spontaneously assemble into mixed polymeric particulate structures. RTS,S is produced in *Saccharomyces cerevisiae*, genetically modified to contain the coding sequences of the RTS and S proteins. RTS is a hybrid polypeptide consisting of a portion of the CS antigen of the malaria parasite *P. falciparum* strain NF54, fused to the amino

terminal end of the Hepatitis B virus S protein. S corresponds to the surface antigen of Hepatitis B virus (HBsAg) (Figure 2).

The AS01 family of Adjuvant Systems contains 2 immunostimulants formulated with liposomes. The monophosphoryl lipid A (MPL) molecule consists of a chemically detoxified form of the parent lipopolysaccharide (LPS) from the Gram-negative bacterium *Salmonella minnesota*. QS21 is a natural saponin molecule purified from the bark of the South American tree, *Quillaja saponaria*.

The RTS,S/AS malaria vaccines have been assessed in 14 clinical studies conducted in malaria-naïve adults and in five adult field studies conducted in Africa. It was initially assessed in malaria-naïve adults to establish proof-of-concept. These studies demonstrated efficacy, immunogenicity and an acceptable safety profile of the RTS,S/AS02 vaccine formulation.[18, 19] Subsequently, studies demonstrated protection against natural infection in semi-immune adult Gambian men.[20] The AS01 adjuvant system was preferred for further evaluation as it elicited superior cellular and humoral responses in comparison to RTS,S/AS02; improved efficacy was also demonstrated in Phase II studies.[21, 22]

These results formed the basis to assess the RTS,S/AS malaria vaccine candidate in African children living in endemic regions. To date, eight Phase I/II clinical studies and one Phase III clinical study in subjects aged at least 5 months and three Phase I/II studies in infants aged less than 5 months of age have been completed. Efficacy data are presented above (section 3.1.2).

3.5.1 Phase III evaluation

From March 2009 through January 2011, 15460 children were recruited to the first large-scale randomised, controlled, double-blind trial evaluating the leading malaria vaccine candidate, RTS,S/AS01. There were two age categories: children 6 to 12 weeks of age and those 5 to 17 months of age at enrolment. Within each age category, there were three study groups: children who received all three doses of the immunisation schedule at 1-month intervals and scheduled to receive a booster dose at 18 months, children who received the primary RTS,S/AS01 vaccination series without a booster and a control group who received a comparator vaccine.[5, 6]

To date, four sets of results are available on the primary endpoint of the trial, vaccine efficacy.[5-7] (See Section 3.1.2 and Figure 4) Anti-CSP antibodies were measured in in the first 200 participants in each age category at each study site at enrolment and 1 month after the third dose of vaccine. The GMT was low at enrolment in all three study groups and

99.9% of children in the RTS,S/AS01 group were seropositive for anti-CSP Abs at 1 month after the third dose, with a GMT of 621 EU/ml (95% CI 592-652) in the older age category.[5]

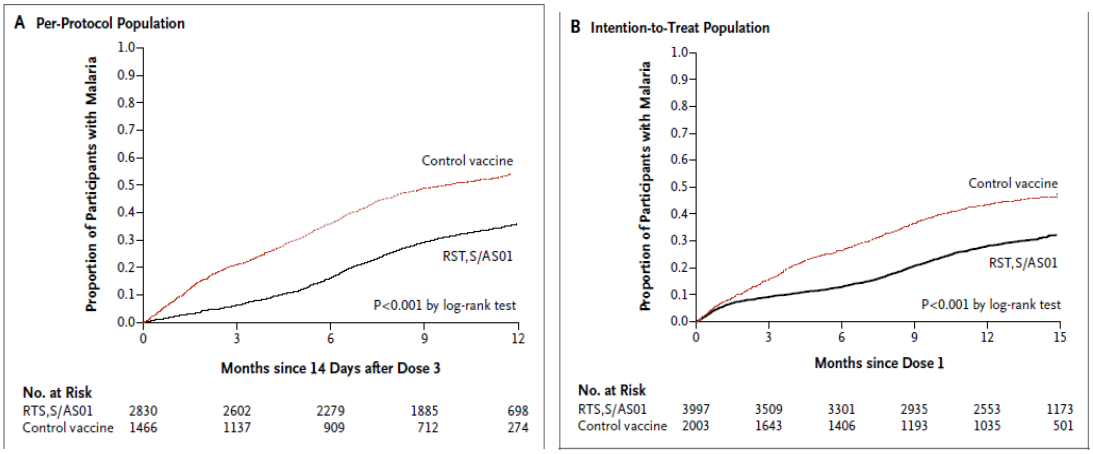


Figure 4. *Cumulative Incidence of First or Only Episodes of Clinical Malaria (Primary Case Definition) in the Older Age Category. The cumulative incidence of the primary case definition in children 5 to 17 months of age at enrollment is shown during 12 months of follow-up after the administration of the third dose of vaccine in the per-protocol population (Panel A) and during 14 months of follow-up after the administration of the first dose of vaccine in the intention-to-treat population (Panel B).[5]*

3.6 MATRIX-M1

Matrix-M1 (MM) 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*. The molecular weights of the different saponins range from 1800 - 2000 Da. In water, saponin in concentrations of 200-500 ppm exist as monomers; at higher concentrations they aggregate as micelles, with a molecular weight of approximately 100 000 Da. Saponins are surface-active compounds with a variety of applications including in agriculture, feed, food and beverage, mining, and veterinary vaccines, and are currently being investigated in human vaccine clinical trials. In aqueous solution, saponins are excellent adjuvants and are used in commercial veterinary vaccines, e.g., vaccines against foot-and-mouth disease, bovine mastitis, feline leukemia and equine influenza. An HPLC-purified fraction of the same saponin, called QS21, is a component of the AS01 adjuvant used in RTS,S/AS01.

3.6.1 Pre-clinical studies

In animal studies, MM has been shown to perform better than most other adjuvants, inducing a multifaceted response including antibody production, T cell responses and

recruitment of innate immune cells into draining lymph nodes.[25, 26] Mixed with a virosomal H9N2 avian influenza vaccine, Matrix-M1 induced enhanced antigen-specific humoral and CD8+ T cell responses.[27] Matrix-M1 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.[28]

In pre-clinical studies, R21 adjuvanted with both Matrix-M1 and MF59 has demonstrated good antibody responses.[29] In addition, there was no interference with induction of antibodies or T cells when R21/MF59 was combined with viral vectors.[29] BALB/c mice immunised with 2 doses of R21/MM showed excellent efficacy (91.3% sterile protection) against transgenic malaria parasite challenge.[29] Combining protein and viral-vectored vaccines in murine malaria models has also previously been shown to have a synergistic effect resulting in much higher sterile efficacy (90%) than either vaccine individually.[30]

3.6.2 Clinical studies

Phase I clinical trials have provided evidence that MM appears safe and well tolerated in humans.

Phase I human clinical trial of a Matrix M™-adjuvanted virosomal H5N1 vaccine[31]

An open-label human phase I dose escalating clinical trial was conducted to evaluate the safety and tolerability of a pandemic influenza A/H5N1 virosomal vaccine formulated with Matrix M™ . Matrix M™ was constituted from Matrix A and Matrix C using a ratio of Matrix A: Matrix C of 91:9.

In total 60 healthy adult volunteers (38 women and 22 males, 20-49 years old) were recruited. Volunteers were randomised into four groups of 15 subjects and vaccinated with two intramuscular (IM) injections into the deltoid muscle at an interval of 21 (±1) days. One group received 30 microgram of HA alone, the other groups received 50 microgram of Matrix M™ mixed with 1.5, 7.5 or 30 microgram HA (Table 1). Local and systemic adverse events (AE) were ascertained, and haematological, biochemical and immunological parameters were investigated. Local and systemic immunogenicity of the test items were assessed by ELISPOT (enzyme-linked immunospot assay) for antibody secreting cells and flow cytometry for detection of multifunctional CD4+ T cells. Serum samples were tested for detection of homologous and cross reactive HI antibodies using a modified haemagglutination inhibition assay.

Table 1. *Details for the respective treatment groups included in the phase I clinical trial.*

Group	Treatment	Number of Volunteers (female/male)	Mean age	Administration days
1	30 μg HA	15 (9/6)	32 (20-41)	d0, d21
2	1.5 μg HA + 50 μg Matrix M-2	15 (10/5)	29 (21-44)	d0, d21
3	7.5 μg HA + 50 μg Matrix M-2	15 (8/7)	31 (22-42)	d0, d21
4	30 μg HA + 50 μg Matrix M-2	15 (11/4)	31 (25-49)	d0, d21

There were no serious adverse events and no clinically relevant changes in haematological, biochemical, and immunological parameters. All four vaccine formulations were well tolerated with the majority of reported solicited adverse events described as mild to moderate in intensity usually resolving within 3 days of vaccination. The most common local reactions were pain or tenderness at the injection site, often lasting up to three days, and more frequent in volunteers receiving Matrix M™. Pain at the injection site was described as mild and transient, apart from in two volunteers who reported it as moderate (severe enough to affect daily activity). Systemic reactions were most commonly described as mild to moderate fatigue, headache and myalgia. Adverse events are detailed below in Fig 5.

Antibody responses were enhanced when adjuvanted with virosomal H5N1 vaccine, allowing significant dose-sparing.[31]

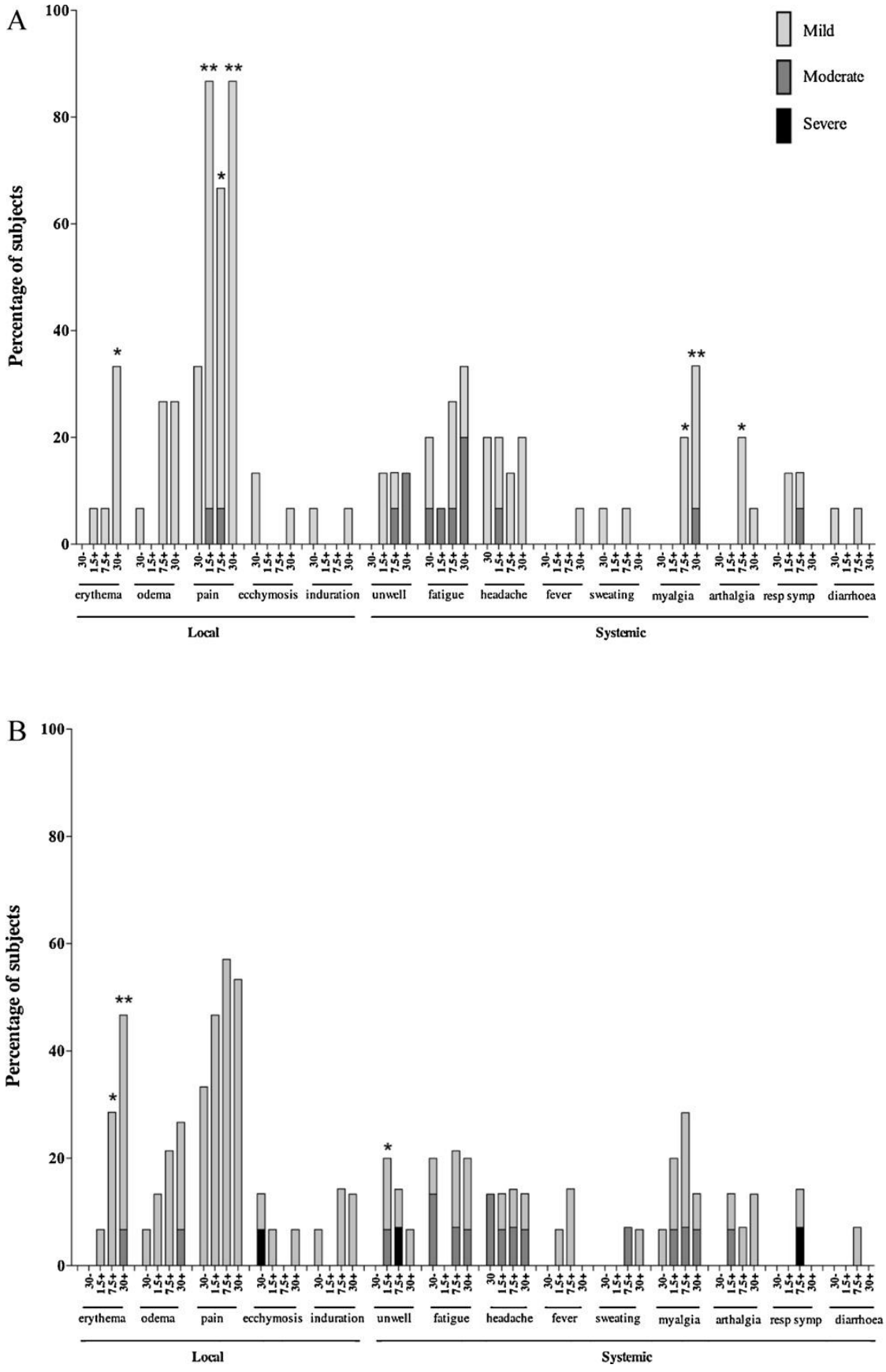


Figure 5. *Adverse events in volunteers receiving two doses of a virosomal influenza vaccine alone, or adjuvanted with Matrix M™. The vaccine dose and presence (+) or absence (−) of*

*Matrix M™ adjuvant is shown on the x-axis. * and ** indicates significantly more subjects reporting adverse events as compared to the 30 µg HA virosomal alone group, $p < 0.05$ and $p < 0.01$, respectively (Chi square test). Adopted from Cox et al.[31]*

Phase I clinical trial of a Matrix-M1-adjuvanted seasonal influenza vaccine (Vaxigrip)

A human phase I clinical trial was conducted to evaluate the safety and tolerability of Matrix-M1 (Matrix A and C ratio, 85:15) formulated with a seasonal influenza vaccine (Vaxigrip, Sanofi-Pasteur), in an elderly population. The efficacy was also evaluated with respect to serological and cell mediated immunological parameters. The commercial influenza vaccine, Vaxigrip was a marketed, inactivated, trivalent, split virus seasonal influenza vaccine (season 2010/2011). The primary endpoint of the study was to assess safety of the investigational vaccine (Matrix M-1 formulated with Vaxigrip) in both young adults and elderly adults in comparison with the commercial vaccine without adjuvant. The secondary endpoint of the study was to assess a comprehensive battery of immunological parameters.

The study started with vaccination of one cohort of 22 healthy adults aged 18-50 years, with one dose of the investigational vaccine to assess the adverse event profile. Vaccination of the elderly subjects started when the results from the first cohort were evaluated and found acceptable, no SAEs were detected. One cohort (age 65-75, n=44) received one dose of the commercial vaccine and another cohort (age 65-75, n=44) received one dose of the investigational vaccine (Table 2.1).

All test items were administered intramuscularly once and blood samples were taken at days 0, 7, 28, 90 and 150 post vaccination. Haematological parameters, blood biochemical and immunological parameters were investigated day 0 and 7.

Cohort	Age group	Subjects (n)	Vaccine	HA (µg)	Matrix M (µg)
1	Adults 18-50 y	22	Vaxigrip + Matrix-M1	3x15	50
2	Elderly 65-75 y	44	Vaxigrip	3x15	0
3	Elderly 65-75 y	44	Vaxigrip + Matrix-M1	3x15	50

Table 2.1. *Details for the respective treatment groups included in the phase I clinical trial with Matrix-M1/Vaxigrip.*

The Matrix M-1 adjuvanted investigational vaccine was well tolerated. An expected increase of side-effects compared to the unadjuvanted comparator vaccine was recorded. No severe side effects were found. The only local side effect with an increased incidence for

the investigational vaccine was pain. There were an overall increase of systemic side events, but all were predominantly mild and transient (Table 2.2).

		Vaxigrip + Matrix-M1 (n=44)	Vaxigrip (n=44)
Local side effects	Pain	27%	9%
	Erythema	14%	14%
	Induration	2%	2%
Systemic side effects	Malaise	2%	0%
	Fever	0%	0%
	Myalgia	7%	0%
	Headache	7%	5 %

Table 2.2. *Local and systemic side events for the elderly cohorts in the phase I clinical trial with Matrix-M1/Vaxigrip.*

In summary, this was the first clinical trial in which the Matrix M-1 formulation of Matrix M™ was administered to humans. Matrix M-1 appeared safe and well tolerated in 66 adult clinical trial volunteers, at a dose of 50µg, formulated with a commercial influenza vaccine. There was a tendency to more adverse events in the Matrix M-1- adjuvanted versus non adjuvanted vaccinations, but the adverse event profile was still excellent.

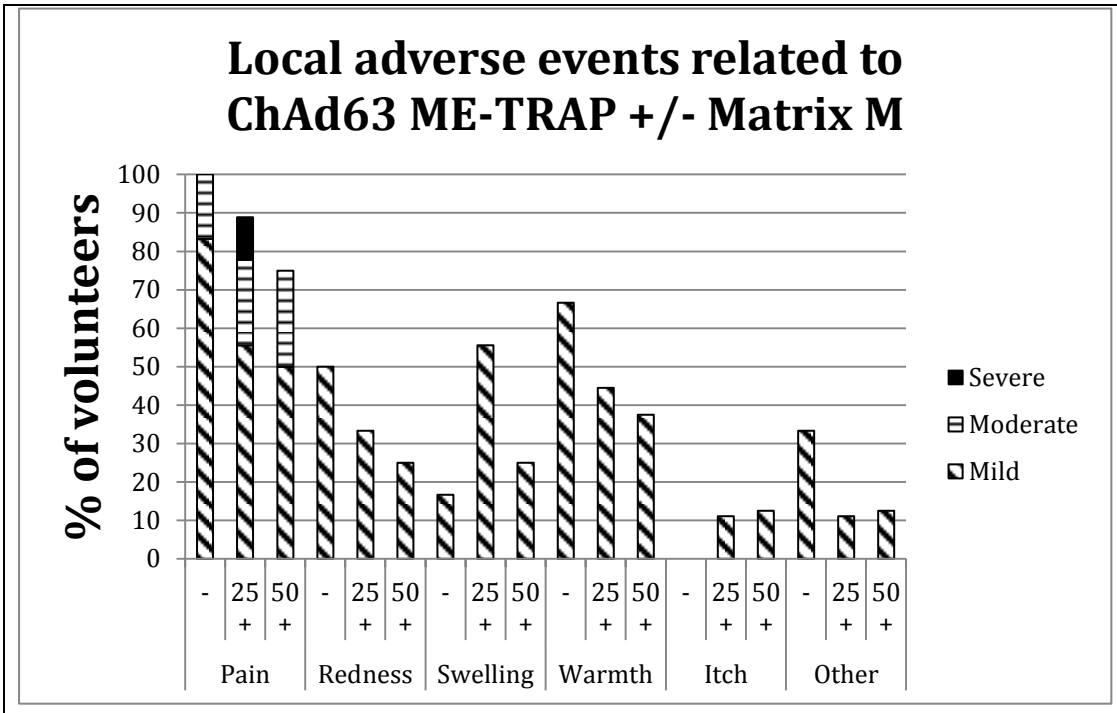
Phase I clinical trial of ChAd63 ME-TRAP / MVA ME-TRAP heterologous prime boost malaria vaccination adjuvanted with Matrix M™

VAC048 is an open-label Phase I study that has been undertaken at the University of Oxford evaluating the safety and immunogenicity of MM administered in combination with heterologous prime-boost vaccination with ChAd63 ME-TRAP and MVA ME-TRAP. The study groups are shown below.

Group Name	Vaccination Regimen		Number of Volunteers
	Vaccination 1 (Day 0)	Vaccination 2 (Day 56)	
Control	ChAd63 ME-TRAP 5 x 10^{10} vp	MVA ME-TRAP 2 x 10^{8} pfu	6
Low dose Matrix M™	ChAd63 ME-TRAP 5 x 10^{10} vp mixed with Matrix-M1 25µg	MVA ME-TRAP 2 x 10^{8} pfu mixed with Matrix-M1 25µg	8
Standard Dose Matrix M™	ChAd63 ME-TRAP 5 x 10^{10} vp mixed with Matrix-M1 50µg	MVA ME-TRAP 2 x 10^{8} pfu mixed with Matrix-M1 50µg	8

Table 3. *Study groups in VAC048*.

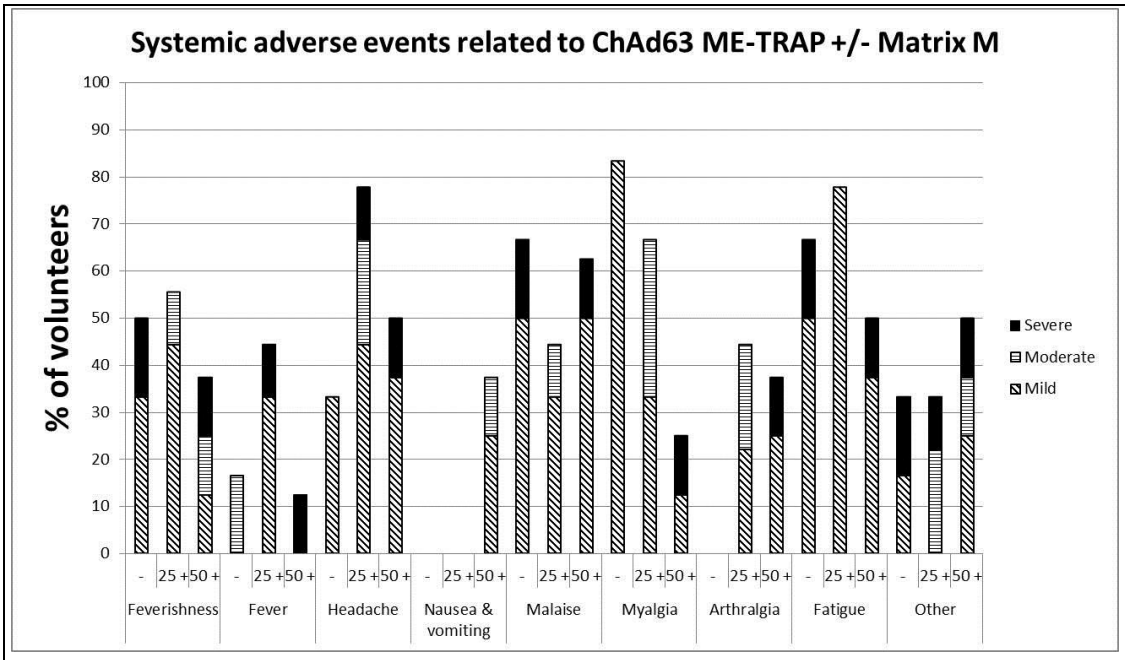
Overall, vaccines were safe and generally well tolerated. Most adverse events were mild in nature and resolved within 48 hours of vaccination. The local and systemic adverse event profile for the low dose (25μg) and standard dose (50μg) MM groups are shown below.



-	ChAd63 ME-TRAP 5 x 10^{10} vp IM only	n=6
25 +	ChAd63 ME-TRAP 5 x 10^{10} vp mixed with Matrix M-1 25μg IM	n=9
50 +	ChAd63 ME-TRAP 5 x 10^{10} vp mixed with Matrix M-1 50μg IM	n=8

'Other' (Control group) = bruising at vaccine site and sensation of sensitive skin
'Other' (25 +) = bruising at vaccine site
'Other' (50+) = shoulder pain

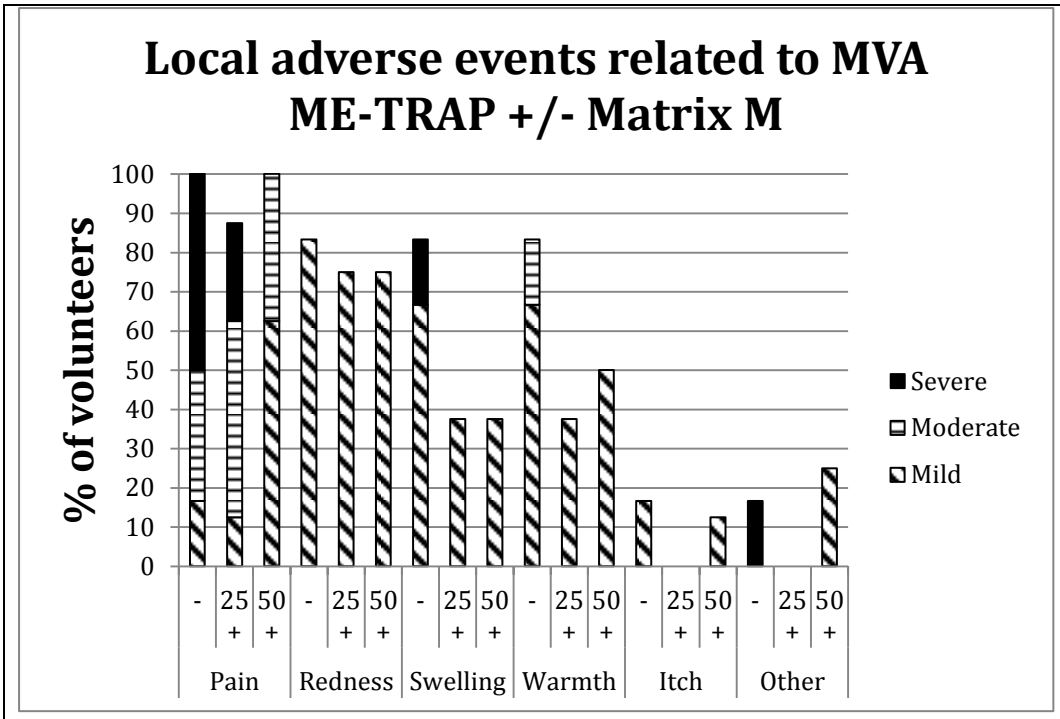
Figure 6.1: VAC048 Reactogenicity results. The figure shows the percentage of subjects that developed local adverse effects after vaccination with ChAd63 ME-TRAP mixed with Matrix M-1 (25μg or 50μg) IM in comparison to subjects who received ChAd63 ME-TRAP 5 x 10^{10} vp IM only. Only AEs deemed definitely, possibly or probably related to vaccination are reported. The highest intensity of each AE per subject is listed.



-	ChAd63 ME-TRAP 5 x 10^{10} vp IM	n=6
25 +	ChAd63 ME-TRAP 5 x 10^{10} vp mixed with Matrix M-1 25µg IM	n=9
50 +	ChAd63 ME-TRAP 5 x 10^{10} vp mixed with Matrix M-1 50µg IM	n=8

'Other' (control group) = back pain and postural dizziness
'Other' (25 +) = dizziness, rigors and lightheadedness
'Other' (50 +) = lower backache, cough, sore throat, runny nose, rigors and difficulty sleeping

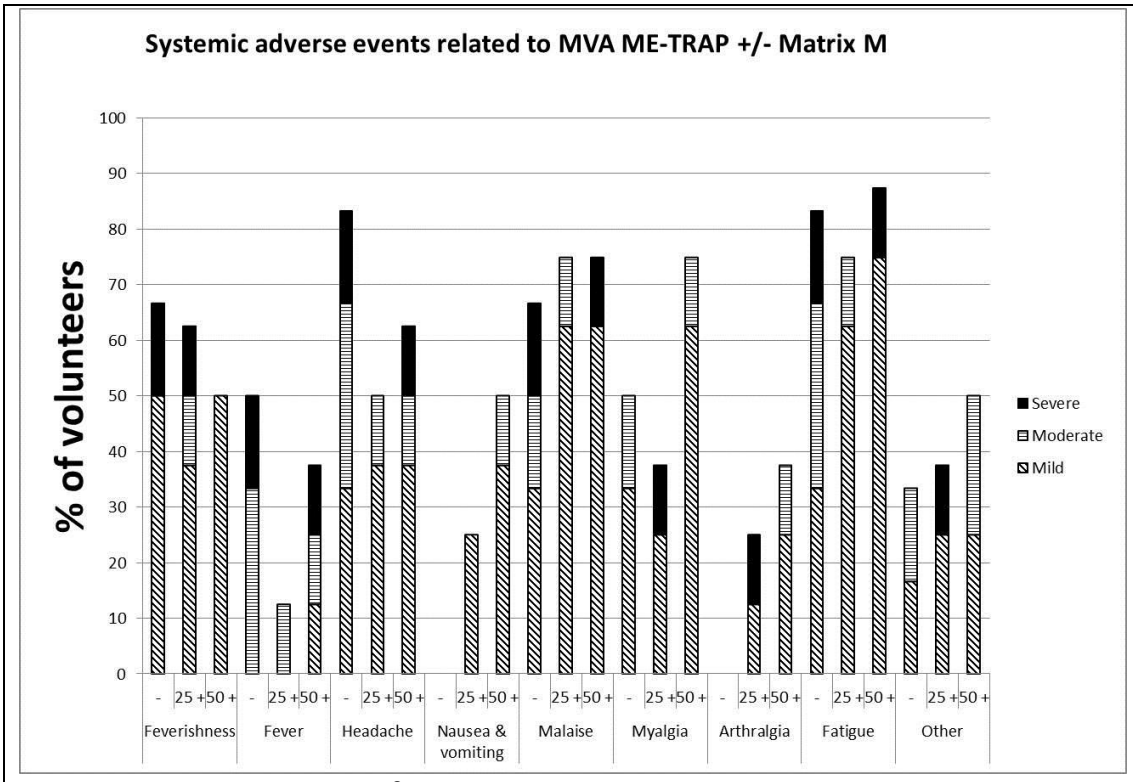
Figure 6.2: VAC048: Reactogenicity results. The figure shows the percentage of subjects that developed systemic adverse effects after vaccination with ChAd63 ME-TRAP mixed with Matrix M-1 (25µg or 50µg) IM in comparison to subjects who received ChAd63 ME-TRAP 5 x 10^{10} vp IM only. Only AEs deemed definitely, possibly or probably related to vaccination are reported. The highest intensity of each AE per subject is listed.



-	MVA ME-TRAP 2 x 10^8 pfu IM	n=6
25 +	MVA ME-TRAP 1.45 x 10^8 pfu or 2 x 10^8 mixed with Matrix M-1 25µg IM	n=8
50 +	MVA ME-TRAP 2 x 10^8 pfu mixed with Matrix M-1 50µg IM	n=8

'Other' (Control) severe burning sensation in arm
'Other' (50 +) two volunteeers reported mild bruising at the vaccine site

Figure 6.3: VAC048: Reactogenicity results. The figure shows the percentage of subjects that developed local adverse effects after vaccination with MVA ME-TRAP mixed with Matrix M-1 (25µg or 50µg) IM in comparison to subjects who received MVA ME-TRAP 2 x 10^8 pfu IM only. The first two volunteers in the low dose group only received 1.45 x 10^8 pfu instead of 2 x 10^8 pfu. Only AEs deemed definitely, possibly or probably related to vaccination are reported. The highest intensity of each AE per subject is listed.



-	MVA ME-TRAP 2 x 10^8 pfu IM	n=6
25 +	MVA ME-TRAP 1.45 x 10^8 pfu or 2 x 10^8 mixed with Matrix M-1 25µg IM	n=8
50 +	MVA ME-TRAP 2 x 10^8 pfu mixed with Matrix M-1 50µg IM	n=8

'Other' (Control) – moderate backache and mild abdominal cramps
'Other' (25+) – mild palpitations and feeling shaky; severe rigors
'Other' (50+) – moderate dizziness, feeling shaky, abdominal pain, chills and rigors; mild lower back ache

Figure 6.4: VAC048: Reactogenicity results. The figure shows the percentage of subjects that developed systemic adverse effects after vaccination with MVA ME-TRAP mixed with Matrix M-1 (25µg or 50µg) IM in comparison to subjects who received MVA ME-TRAP 2 x 10^8 pfu IM only. The first two volunteers in the low dose group only received 1.45 x 10^8 pfu instead of 2 x 10^8 pfu. Only AEs deemed definitely, possibly or probably related to vaccination are reported. The highest intensity of each AE per subject is listed.

3.7 Rationale

3.7.1 Vaccine Development Strategy

R21 has been developed at the Jenner Institute, University of Oxford. It is produced by using recombinant HBsAg particles expressing the central repeat and the C-terminus of the circumsporozoite protein (CSP) and has been GMP manufactured in *Pichia pastoris*. This is a biosimilar protein particle to RTS,S which also targets the pre-erythrocytic circumsporozoite protein, the major functional protein in sporozoite development and hepatocyte invasion. R21 lacks the excess of HBsAg in RTS,S (See Figure 2) and has been shown to be highly immunogenic (> 10^5 ELISA units after two immunisations) and have at least comparable immunogenicity and similar high level efficacy to RTS,S in animal studies. (K Collins and A Hill, unpublished data). To date, safety and immunogenicity observed in VAC053 is very promising and antibody levels observed are comparable to previous studies done in Oxford with the leading malaria vaccine candidate, RTS,S. Furthermore, comparable immunogenicity is observed at both the tested doses of R21 adjuvanted with Matrix M. (See Figure 7). Therefore, we are proposing to add an additional group to the study to test an even lower dose of R21 of 2μg as this could lead to significant dose-sparing if it is shown to be equally immunogenic.

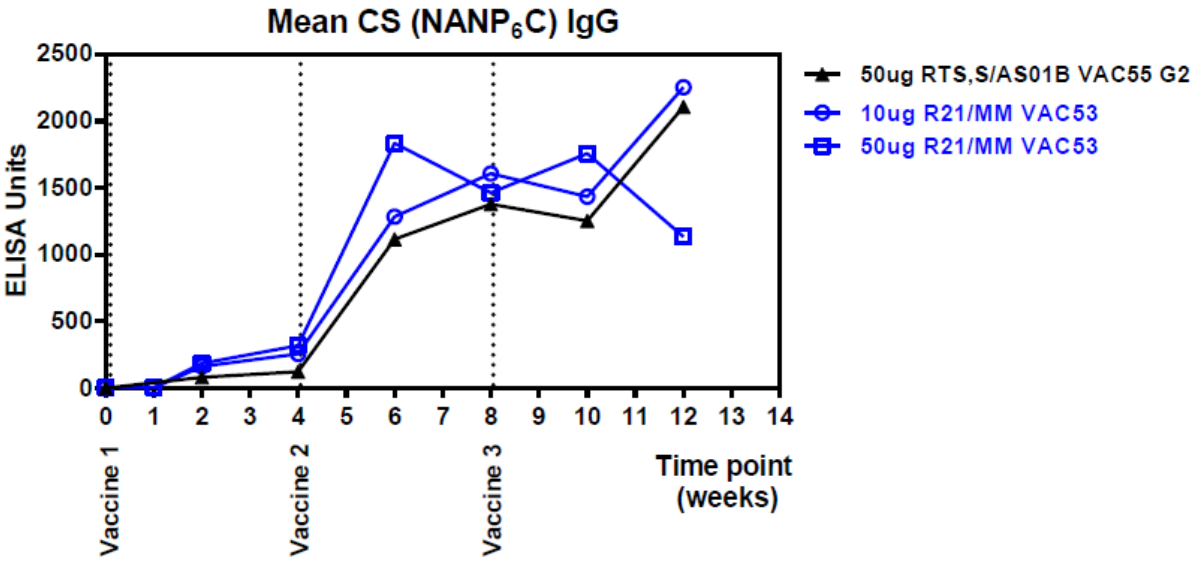


Figure 7. Mean IgG antibody responses observed in Groups 1 and 3 of the VAC053 trial in comparison to historical data with RTS,S.

Matrix-M1 is an attractive adjuvant, as it, and other matrix formulations of Quillaja saponins, show good safety profiles, and the ability to enhance both cellular and humoral immune responses to a range of vaccines. Preclinical data presented in Section 3.4 demonstrate the potential for Matrix-M1 to enhance the immunogenicity of R21.

In order to progress the development of a potentially more efficacious anti-sporozoite malaria vaccine, the proposed clinical trial, VAC053, has two objectives. The first is to assess the safety of R21 administered as a mixture with the Matrix-M1 adjuvant. The second is to determine the effects of Matrix-M1 on the immunogenicity of the vaccine.

If these studies show,

- Satisfactory safety of R21 adjuvanted with Matrix-M1, and
- Matrix-M1 generates sufficient enhancement of the humoral immunogenicity of R21 with acceptable safety,

this will support future clinical evaluation of the efficacy of the vaccine using controlled human malaria infection (CHMI) where sporozoites are delivered by mosquito bite.

4 STUDY OVERVIEW

This is an open label Phase I study of a protein particle malaria vaccine candidate in healthy volunteers. R21 will be administered intramuscularly alone or with the adjuvant Matrix-M1.

There will be 4 study groups, with Groups 1, 3 and 4 containing 10 volunteers and Group 2 containing 4 volunteers as shown in Table 4.

Volunteers will be first recruited to Group 1 and subsequently to Groups 2-4 with interim clinical safety reviews (See Section 8.5.3). There will also be staggered enrolment for the first three volunteers within Groups 1-3. Volunteers will be allocated to study group by selecting eligible volunteers for enrolment in the order in which they were deemed eligible following screening.

4.1 Study Groups

	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

Table 4: *Study Groups*

4.1.1 First volunteers

The first volunteer in Group 1 will be vaccinated alone and then reviewed 72 hours following vaccination. If there are no safety concerns, another two Group 1 volunteers may be immunised, at least one hour apart, and reviewed in a further 72 hours. Providing there are no safety concerns as assessed by the CI and LSM, the remaining volunteers in Group 1 and the first volunteer in Group 3 may be vaccinated.

The first volunteer in Group 3 will be vaccinated alone and then reviewed 72 hours following vaccination. If there are no safety concerns, another two volunteers in Group 3 may be immunised, at least one hour apart, and reviewed in a further 72 hours. Providing there are no safety concerns as assessed by the CI and LSM, the remaining volunteers in Group 3 may be vaccinated.

In parallel, following review of the first Group 3 volunteer at 72 hours post-vaccination, the first volunteer in Group 2 will be vaccinated alone and then reviewed 72 hours following vaccination. If there are no safety concerns, another two Group 2 volunteers may be immunised, at least one hour apart, and reviewed in a further 72 hours. Providing there are no safety concerns as assessed by the CI and LSM, the remaining volunteer in Group 2 may be vaccinated.

Group 4 has been added to the trial to assess whether a further reduction in dose would still elicit comparable immunogenicity to Groups 1 and 3. There is no requirement for staggered enrolment of the first three volunteers in Group 4 as R21 has already been administered to humans in much higher doses (10μg in Group1 and 50μg in Group 3).

All volunteers will be issued with the telephone number of the investigators and encouraged to contact the investigators if there are any problems. An investigator will be available 24 hours a day.

4.1.2 Duration of study

The total duration of the study will be 34 weeks from the day of enrolment for all volunteers.

4.1.3 Definition of Start and End of Trial

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

4.2 Potential Risks for volunteers

The potential risk to participants is considered as low. The potential risks are those associated with phlebotomy and vaccination.

Phlebotomy:

The maximum volume of blood drawn over the study period (approximately 620ml) should not compromise these otherwise healthy volunteers. There may be minor bruising, local tenderness or pre-syncopal symptoms associated with venepuncture, which will not be documented as AEs if they occur.

Vaccination:

R21 has not been used in humans before, and therefore will be initially administered at the lower dose of 10μg before progressing to the higher dose of 50μg in Groups 2 and 3. Potential expected risks from vaccination include local effects such as pain, redness, warmth and swelling and systemic effects including a mild self-limiting flu-like illness.

There has been previous experience with saponin-based adjuvants administered to humans with a range of vaccines. Matrix M™ (constituted of Matrix A : Matrix C at a ratio of 91:9) has been administered at a dose of 50 μg, mixed with a virosomal influenza vaccine, to 45 adult volunteers in the Phase I clinical trial presented above[31]. Vaccination was well tolerated, with adverse events related to vaccination generally being mild and resolving within three days. The formulation of Matrix M™ to be used in this study, Matrix-M1, is constituted of Matrix A : Matrix C at a ratio of 85:15. This formulation has been used in the VAC048 trial and the results presented above demonstrate an acceptable safety profile, despite the mixed administration with liver-stage viral vectors in that trial, with most symptoms being mild in nature.

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

4.3 Known Potential Benefits

Volunteers will not benefit directly from participation in this study. However, it is hoped that the information gained from this study will contribute to the development of a safe and effective malaria vaccine regime. The only benefits for participants would be information about their general health status.

5 OBJECTIVES AND ENDPOINTS

The number of volunteers has been chosen to generate adequate safety and immunogenicity data to meet these objectives, whilst minimising the number of volunteers exposed to a new vaccination regimen.

5.1 Primary Objective

To assess the safety and tolerability of R21 with and without adjuvant Matrix-M1 in healthy volunteers.

5.1.1 Primary Outcome Measures

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

The following parameters will be 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

Volunteers will undergo clinical follow up for adverse events for a further 182 days following completion of the vaccination regimen. The duration of follow up reflects the desire to obtain longer term safety data with the first use of R21 in humans.

5.2 Secondary Objective

To assess the cellular and humoral immunogenicity of R21 in humans with and without adjuvant Matrix-M1 in healthy volunteers.

5.2.1 Secondary Outcome Measures

Comparison of immunogenicity of the Matrix-M1 – adjuvanted vaccination regimens, versus R21 alone.

Measures of immunogenicity may include:

- ELISA to quantify antibodies to CSP and NANP

- ELISPOT to enumerate IFN-γ producing T cells
- Ex vivo ELISPOT responses to NANP
- Flow cytometry and intracellular cytokine staining to enumerate and functionally characterise immune cell populations such as; T cells (e.g. CD4+ and CD8+), B cells and dendritic cells
- ELISPOT for enumeration of antibody-secreting cells (e.g. B cell ELISPOT responses to NANP)
- Gene expression profiling including RNA analysis, DNA sequencing and other genotypic methods

The immunoassay of most interest is the antibody response to NANP because this correlates with vaccine efficacy after RTS,S/AS01 administration, and induction of antibody levels comparable to or greater than RTS,S/AS01 would suggest likely vaccine efficacy.

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

6 INVESTIGATIONAL PRODUCTS

The following vaccinations will be given in this study:

1. R21 10μg mixed with Matrix-M1 50μg
2. R21 50μg given alone without adjuvant
3. R21 50μg mixed with Matrix-M1 50μg
4. R21 2μg mixed with Matrix-M1 50μg

6.1 Formulation, Dose, Storage and Accountability of Investigational Medicinal Products

6.1.1 Description of R21c

R21c has been developed and produced at the Jenner Institute, University of Oxford. The R21 vaccine consists of recombinant HBsAg particles expressing the central repeat and the C-terminus of the circumsporozoite protein from *Plasmodium falciparum* strain NF54. R21 is a biosimilar to RTS,S expressed in the yeast *Pichia Pastoris.* It is 14 amino acids smaller than the RTS fusion protein at the C-terminus of the CSP sequence, has an additional four amino acids (EPEA, called C-tag) at the C-terminus, and lacks the excess of HBsAg in RTS,S (See Figure 2).

To increase the efficiency of biomanufacture and likely reduce the final cost of goods of this potential vaccine a C-tag has been encoded at the C-terminus of R21. The C-tag is a four amino acid tag (E-P-E-A) located at the C-terminus of the protein to allow immunoaffinity purification of the R21. To permit affinity purification of proteins carrying this C-tag, a chromatography resin utilising a cross-linked camelid nanobody developed by the company BAC BV (Naarden, The Netherlands, now part of Thermo Fisher) specifically binds the tag. This affinity chromatography resin was used for the purification of R21.

R21 will be used at a dose of 10μg in Group 1, 50μg in Groups 2 and 3 and 2μg in Group 4.

6.1.2 R21 formulation and packaging

R21 is formulated in formulation buffer at a target concentration to allow the extraction of 25μg from each vial. The drug product is 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 aluminium seal. The nitrogen filled vials are supplied sterile. The containers and closures are tested for compliance with defined specifications. The vials are made from Ph Eur Type 1 glass.

6.1.3 R21 Storage and Handling

Long term, R21 vaccine is stored frozen at a nominal temperature of -80°C.

6.2.1 R21 mixed with Matrix-M1

Matrix-M1 to be used in Group 1and 3 volunteers was manufactured in compliance with cGMP by Apoteket Produktion & Laboratorier AB (APL) Formvägen 5B, SE-903 03 Umeå, Sweden. It is supplied as a sterile 1mg/ml solution in 2ml glass vials. Matrix-M1 (85 parts Matrix A and 15 parts of Matrix C) is obtained by simply mixing Matrix A and C, followed by dilution in PBS to 1 mg/ml, filtration though filter 0.22 μm and filling into vials in a volume of 2 ml. Matrix M-1 is a slightly-opalescent non-viscous liquid.

Matrix-M1 to be used in Group 4 volunteers was manufactured in compliance with cGMP by Apotek Produktion & Laboratorier AB (APL) Formvägen 5B, SE-903 03 Umeå, Sweden. It is supplied as a sterile 0.75 mg/ml solution in 3ml glass vials. Matrix-M1 (85 parts Matrix A and 15 parts of Matrix C) is obtained by simply mixing Matrix A and C, followed by dilution in PBS to 0.75 mg/ml, filtration though filter 0.22 μm and filling into vials in a volume of 2 ml. Matrix M-1 is a colourless slightly-opalescent non-viscous liquid.

A mixture of R21 with Matrix M-1 50µg will be administered to volunteers in Groups 1, 3 and 4. Matrix-M1 and R21 will be mixed at the bedside immediately prior to administration and will be administered intramuscularly within one hour of thawing of R21.

6.2.2 Matrix-M1 formulation and packaging

Matrix-M1 to be used in Group 1and 3 volunteers is formulated at a concentration of 1 mg/mL in PBS. The drug product is filled into sterile brown glass vials.

Matrix-M1 to be used in Group 4 volunteers is formulated at a concentration of 0.75 mg/mL in PBS. The drug product is filled into sterile brown glass vials.

6.2.3 Matrix-M1 Storage and Handling

Matrix-M1 is stored refrigerated at 2 to 8°C and protected from light.

All movements of the vaccines and adjuvants will be documented. Accountability, storage, shipment and handling of R21 and Matrix-M1 will be in accordance with relevant local SOPs and forms.

6.3 Administration of Investigational Medicinal Products

On vaccination day, R21 will be allowed to thaw to room temperature. It will be administered within 1 hour of removal from the freezer, either alone, or mixed with Matrix-M1. The investigational products will be administered intramuscularly into the deltoid of the non-dominant arm. All volunteers will be observed in the unit for 1 hour (±10 minutes) after vaccination. During administration of the investigational products, Advanced Life Support drugs and resuscitation equipment will be immediately available for the management of anaphylaxis. Vaccination will be performed and the IMPs handled according to the relevant SOPs.

6.4 Vaccine Supply

R21 will be formulated and vialed under Good Manufacturing Practice conditions at the CBF, University of Oxford. At the CBF the vaccines will be certified and labelled for trial VAC053 by a Qualified Person (QP) before transfer to the clinical site.

7 RECRUITMENT AND WITHDRAWAL OF TRIAL VOLUNTEERS

7.1 Volunteers

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

- In public places, including buses and trains, with the agreement of the owner/proprietor.
- In newspapers or other literature for circulation.
- On radio via announcements.
- On a website or social media site operated by our group or with the agreement of the owner or operator (including on-line recruitment through our web-site).
- By e-mail distribution to a group or list only with the express agreement of the network administrator or with equivalent authorisation.
- By email distribution to individuals who have already expressed an interest in taking part in any clinical trial at the Oxford Vaccine Centre or the Hammersmith NIHR/Wellcome Trust Imperial Clinical Research Facility (CRF), London.
- On stalls or stands at exhibitions or fairs.
- Via presentations (e.g. presentations at lectures or invited seminars).
- Direct mail-out: This will involve obtaining names and addresses of adults via the most recent Electoral Roll. The contact details of individuals who have indicated that they do not wish to receive postal mail-shots would be removed prior to the investigators being given this information. The company providing this service is registered under the Data Protection Act 1998. Investigators would not be given dates of birth or ages of individuals but the list supplied would only contain names of those aged between 18-50 years (as per the inclusion criteria).
- Oxford Vaccine Centre databases: We may contact individuals from databases of groups within the CCVTM (including the Oxford Vaccine Centre database) of previous trial participants who have expressed an interest in receiving information about all future studies for which they may be eligible.
- Hammersmith NIHR Wellcome Trust Imperial CRF Database of Healthy Volunteers: We may contact individuals from this database who have previously expressed an interest in receiving information about future studies for which they may be eligible.

7.2 Informed consent

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

- Participation in the study is entirely voluntary
- Refusal to participate involves no penalty or loss of medical benefits
- The volunteer may withdraw from the study at any time

- The volunteer is free to ask questions at any time to allow him or her to understand the purpose of the study and the procedures involved
- The study involves research of an investigational vaccine
- There is no direct benefit from participating
- The volunteer's GP will be contacted to corroborate their medical history
- The volunteer's blood samples taken as part of the study will be stored indefinitely and samples may be sent outside of the UK and Europe to laboratories in collaboration with the University of Oxford. These will be anonymised.

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

7.3 Inclusion and exclusion criteria

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

7.3.1 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 (see below) during the study and a negative pregnancy test on the day(s) of screening and vaccination
- Agreement to refrain from blood donation during the course of the study
- Provide written informed consent

7.3.2 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

7.3.3 Effective contraception for female volunteers

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

Acceptable forms of contraception for female volunteers include:

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

7.3.4 Prevention of 'Over Volunteering'

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

7.3.5 Re-vaccination exclusion criteria

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

- Anaphylactic reaction following administration of vaccine
- Pregnancy

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

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

7.3.6 Withdrawal of Volunteers

In accordance with the principles of the current revision of the Declaration of Helsinki and any other applicable regulations, a volunteer has the right to withdraw from the study at

any time and for any reason, and is not obliged to give his or her reasons for doing so. The Investigator may withdraw the volunteer at any time in the interests of the volunteer's health and well-being. In addition the volunteer may withdraw/be withdrawn for any of the following reasons:

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

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

Any volunteer who fails to attend for two or more follow-up visits during the study will be deemed to have withdrawn from the study.

If a volunteer withdraws from the study, blood samples collected before their withdrawal from the trial will be used/ stored unless the volunteer specifically requests otherwise.

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

7.4 Compliance with Dosing Regime

All doses in this vaccine study will be administered by the Investigator and recorded in the CRF. The study medication will be at no time in the possession of the volunteer and compliance will not, therefore, be an issue.

7.5 Pregnancy

Should a volunteer become pregnant during the trial, she will be followed up as other volunteers and in addition will be followed until pregnancy outcome. We will not routinely perform venepuncture in a pregnant volunteer.

7.6 Safety Stopping/ Holding Rules

Safety holding rules have been developed considering the fact that this is a first-in-human dose escalation study.

'Solicited adverse events' are those listed as foreseeable adverse events in section 9.3 of the protocol, occurring within the first 7 days after vaccination (day of vaccination and six subsequent days). 'Unsolicited adverse events' are adverse events other than the foreseeable AEs occurring within the first 7 days, or any AEs occurring after the first 7 days after vaccination.

7.6.1 Group holding rules

For safety reasons the first volunteer to receive a new vaccine dose in Groups 1-3 will be vaccinated alone and we will wait 72 hours before vaccinating subsequent volunteers. Two further volunteers may be vaccinated 72 hours after the first, and then at least another 72 hours gap will be left before vaccinating the rest of the volunteers receiving that dose of vaccine.

- **Solicited local adverse events:**
    - If more than 25% of doses of a vaccine are followed by 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:**
    - If more than 25% of doses of a vaccine are followed by 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:**
    - If more than 25% of volunteers develop a Grade 3 unsolicited adverse event (including the same laboratory adverse event) that is considered possibly, probably or definitely related to vaccination and persists at Grade 3 for > 48hrs.
- **A serious adverse event considered possibly, probably or definitely related to vaccination occurs**
- **Death occurs**
- **A life-threatening reaction occurs**

If a holding rule has been met and following an internal safety review it is deemed appropriate to restart dosing, a request to restart dosing with pertinent data must be submitted to the regulatory authority as a request for a substantial amendment. The internal safety review will consider:

- The relationship of the AE or SAE to the vaccine.
- The relationship of the AE or SAE to the vaccine dose, or other possible causes of the event.

- If appropriate, additional screening or laboratory testing for other volunteers to identify those who may develop similar symptoms and alterations to the current Participant Information Sheet (PIS) are discussed.
- New, relevant safety information from ongoing research programs on the various components of the vaccine.

The local ethics committee and vaccine manufacturers will also be notified if a holding rule is activated or released.

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

7.6.2 Individual stopping rules (will apply to all vaccinated individuals)

In addition to the above stated group holding rules, stopping rules for individual volunteers will apply (i.e., indications to withdraw individuals from further vaccinations). If any of the events listed below occur and are considered possibly, probably or definitely related to vaccination the volunteer will be withdrawn from further vaccination.

- **Local reactions:** Injection site ulceration, abscess or necrosis
- **Laboratory AEs:**
  - the volunteer develops a Grade 3 laboratory adverse event considered possibly, probably or definitely related within 7 days after vaccination and persisting continuously at Grade 3 for > 72hrs.
- **Systemic solicited adverse events:**
  - the volunteer develops a Grade 3 systemic solicited adverse event considered possibly, probably or definitely related within 2 days after vaccination (day of vaccination and one subsequent day) and persisting continuously at Grade 3 for > 72hrs.
- **Unsolicited adverse events:**
  - the volunteer has a Grade 3 adverse event, persisting continuously at Grade 3 for >72hrs.
  - the volunteer has a serious adverse event.
  - the volunteer has an acute allergic reaction or anaphylactic shock following the administration of vaccine investigational product.

If a volunteer has an acute illness (moderate or severe illness with or without fever) or a fever (oral temperature greater than 37.5°C) at the scheduled time of administration of investigational product, the volunteer will not receive the vaccine at that time. The vaccine may be administered to that volunteer at a later date within the time window specified in the protocol (see Table 6) or they may be withdrawn from the study at the discretion of the Investigator.

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

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

8 TREATMENT OF TRIAL VOLUNTEERS

This section describes the clinical procedures for evaluating study participants and follow-up after administration of study vaccine.

8.1 Study procedures

Procedures will be performed on the visit time points indicated in the schedules of attendance (Table 6). All volunteers will have the same schedule of clinic attendances and procedures, except the first three volunteers in Groups 1-3 will have an extra visit at three days post Vaccination 1 for additional safety assessment. All subjects will receive three vaccinations, four weeks apart, and undergo follow-up for a total of 34 weeks (26 weeks following the final vaccination). The total volume of blood donated during the study will be 620ml. Additional visits or procedures may be performed at the discretion of the investigators, e.g., further medical history and physical examination, or urine microscopy in the event of positive urinalysis.

8.2 Observations

Pulse, blood pressure and temperature will be measured at the time-points indicated in the schedule of procedures and may also be measured as part of a physical examination if indicated at other time-points.

8.3 Blood Tests

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

1. At Oxford University Hospitals' NHS Trust, or Hammersmith Hospital using NHS standard procedures:
    - **Haematology;** Full Blood Count
    - **Biochemistry;** Sodium, Potassium, Urea, Creatinine, Albumin, Liver Function Tests
    - **Diagnostic serology;** HBsAg, HCV antibodies, HIV antibodies (specific consent will be gained prior to testing blood for these blood-borne viruses)
    - **Immunology;** Human Leukocyte Antigen (HLA) typing
2. At University of Oxford research laboratories:

- **Exploratory Immunology**; Immunogenicity will be assessed by a variety of immunological assays. This includes antibodies to CSP and NANP, *ex vivo* ELISpot assays for interferon gamma and flow cytometry assays, functional antibody assays and B cell analyses. Other exploratory immunological assays including cytokine analysis, other antibody assays, DNA analysis of genetic polymorphisms potentially relevant to vaccine immunogenicity and gene expression studies amongst others may be performed at the discretion of the Investigators.

3. **Urinalysis;** Urine will be tested for protein, blood and glucose at screening. For female volunteers only, urine will be tested for beta-human chorionic gonadotrophin (β-HCG) at screening and immediately prior to each vaccination.
4. Collaboration with other specialist laboratories in the UK, Europe and outside of Europe for further exploratory immunological tests may occur. This would involve the transfer of serum or plasma and/or PBMC to these laboratories, but these would remain anonymised. Informed consent for this will be gained from volunteers.

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

8.4 Vaccinations

Before each vaccination, the on-going eligibility of the volunteer will be reviewed. All vaccines will be administered intramuscularly according to SOP VC002 Vaccination as described in Section 6.3. The injection site will be covered with a sterile dressing and the volunteer will stay in the CCVTM for observation, in case of immediate adverse events. Observations will be taken 30 minutes after vaccination (+/- 5 minutes) and the sterile dressing removed and injection site inspected. Observations will also be taken at 60 minutes (+/- 10 minutes) before the volunteer leaves. An oral thermometer, tape measure and diary card (paper or electronic) will be given to each volunteer, with instructions on use, along with the emergency 24 hour telephone number to contact the on-call study physician if needed.

Diary cards will collect information on the timing and severity of the following solicited AEs:

Local solicited AEs	Systemic solicited AEs
Pain	Fever
Redness	Feverishness/Chills
Warmth	Joint pains
Itch	Muscle pains
	Fatigue

	Headache
	Malaise

Table 5: *Solicited AEs as collected on post vaccination diary cards*

Volunteers will be instructed on how to self-assess the severity of these AEs. There will also be space on the diary card to self-document unsolicited AEs, and whether medication was taken to relieve the symptoms.

8.5 Study visits

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

8.5.1 Screening visit

All potential volunteers will have a screening visit, which may take place up to 90 days prior to vaccination. Informed consent will be taken before screening, as described in section 7.2. If consent is obtained, the screening procedures indicated in the schedule of attendances will be undertaken. To avoid unnecessary additional venepuncture, if the appropriate blood test results for screening are available for the same volunteer from a screening visit for another Jenner Institute Clinical Trials group vaccine study, these results may be used for assessing eligibility (provided the results date is within the 3 months preceding enrolment in VAC053).

The subject's general practitioner will be contacted with the written permission of the subject after satisfactory screening as notification that the subject has volunteered for the study and to ascertain any significant medical history. During the screening the volunteers will be asked to provide their National Insurance or passport number so that this can be entered on to a national database which helps prevent volunteers from participating in more than one clinical trial simultaneously or over-volunteering for clinical trials (www.tops.org.uk).

Potential subjects will be informed that there may be leftover samples of their blood (after all testing for this study is completed), and that such samples may be stored indefinitely for possible future research (exploratory immunology), including genotypic testing of genetic polymorphisms potentially relevant to vaccine immunogenicity. Subjects will be able to decide if they will permit such future use of any leftover samples. If a subject elects not to permit this, all of that subject's leftover samples will be discarded after the required period of storage to meet Good Clinical Practice (GCP) and regulatory requirements.

Abnormal clinical findings from the medical history, physical examination, urinalysis or blood tests at screening will be assessed as detailed in Appendix A. Abnormal blood tests following enrolment will be assessed according to site-specific laboratory adverse event

grading tables which are filed in the trial master file (TMF). If a test is deemed clinically significant it may be repeated to ensure it is not a single occurrence. If an abnormal finding is deemed to be clinically significant, the volunteer will be informed and appropriate medical care arranged with the permission of the volunteer. Decisions to exclude the volunteer from enrolling in the trial or to withdraw a volunteer from the trial will be at the discretion of the Investigator.

8.5.2 Day 0: Enrolment and vaccination visit

The eligibility of the volunteer will be reviewed at the end of the screening visit and again when all results from the screening visit have been considered. If eligible, a day 0 visit will be scheduled for the volunteer to receive the vaccine. Volunteers will not be considered enrolled in the study until they have received a vaccine. The vaccine will be administered as described above in sections 6.3 and 8.4.

8.5.3 Sequence of Enrolment and Vaccination of Volunteers

The first Group 1 volunteer to receive Vaccination 1 will be vaccinated alone. If there are no safety concerns following review of this volunteer at 3 days post-vaccination, a further two Group 1 volunteers may receive Vaccination 1. If there are no safety concerns following review of these volunteers at 3 days post-vaccination as assessed by the CI and LSM, the remaining seven Group 1 volunteers and the first volunteer in Group 3 may receive Vaccination 1.

The first Group 3 volunteer to receive Vaccination 1 will be vaccinated alone. If there are no safety concerns following review of this volunteer at 3 days post-vaccination, a further two Group 3 volunteers may receive Vaccination 1. If there are no safety concerns following review of these volunteers at 3 days post-vaccination as assessed by the CI and LSM, the remaining seven Group 3 volunteers will receive Vaccination 1. In parallel, following review of the first Group 3 volunteer at 3 days post-vaccination, the first Group 2 volunteer to receive Vaccination 1 will be vaccinated alone. If there are no safety concerns following review of this volunteer at 3 days post-vaccination, a further two Group 2 volunteers will receive Vaccination 1. If there are no safety concerns following review of these volunteers at 3 days post-vaccination as assessed by the CI and LSM, the remaining Group 2 volunteer will receive Vaccination 1. There will be no staggered enrolment of volunteers in Group 4 as R21 has already been previously administered to humans in much higher doses (10μg in Group1 and 50μg in Group 3).

8.5.4 Subsequent Visits: Day 1, 7, 14, 29, 35, 42, 57, 63, 70, 84, 238

On subsequent visits, the volunteers will be assessed for local and systemic adverse events, using diary cards (paper or electronic), interim history, physical examination and blood tests

at the time-points indicated in the schedule of attendances (Table 6). Blood will also be taken for exploratory immunology analysis.

8.5.5 First three volunteers (Groups 1-3)

The first three volunteers in Groups 1-3 will be required to attend an additional visit at Day 3 post-vaccination 1 to assess for local and systemic adverse events.

Attendance Number	1[S]	2	3	3a*	4	5	6	7	8	9	10	11	12	13	14	15
Timeline** (days)	≤ 90	0	1	3	7	14	28	29	35	42	56	57	63	70	84	238
(weeks)		0			1	2	4		5	6	8		9	10	12	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[%]

Table 6. *Schedule of attendances for Groups 1-4.* [S] *= screening visit; (X) = if considered necessary ^ = Vital signs includes pulse, blood pressure and temperature;* [$] *= Biochemistry will include Sodium, Potassium, Urea, Creatinine, Albumin and Liver function tests.* [£] *=*

Exploratory immunology includes antibodies to CSP and NANP, B cell ELISPOT to NANP, ex vivo ELISPOT responses to interferon-γ.

**The first three volunteers in Groups 1-3 will be 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 $\pm$ 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.*

9 ASSESSMENT OF SAFETY

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

9.1 Interim Safety Review

Prior to dose escalation of each vaccine the local safety monitor will be consulted to provide a review of safety data and adverse events in volunteers before proceeding to the next vaccine dose. Interim safety data may also be made available to manufacturers (in coded format) as specified in the contract with the manufacturer(s).

9.2 Definitions

9.2.1 Adverse Event (AE)

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

9.2.2 Adverse Reaction (AR)

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

9.2.3 Unexpected Adverse Reaction

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

9.2.4 Serious Adverse Event (SAE)

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

- Death
- Life-threatening event (i.e., the volunteer was, in the view of the Investigator, at immediate risk of death from the event that occurred). This does not include an AE that, if it occurred in a more severe form, might have caused death.
- Persistent or significant disability or incapacity (i.e., substantial disruption of one's ability to carry out normal life functions).
- Hospitalisation, regardless of length of stay, even if it is a precautionary measure for continued observation. Hospitalisation (including inpatient or outpatient

hospitalisation for an elective procedure) for a pre-existing condition that has not worsened unexpectedly does not constitute a serious AE.

- An important medical event (that may not cause death, be life threatening, or require hospitalisation) that may, based upon appropriate medical judgment, jeopardise the volunteer and/or require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic reaction requiring intensive treatment in an emergency room or clinic, blood dyscrasias, or convulsions that do not result in inpatient hospitalisation.
- Congenital anomaly or birth defect.

9.2.5 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.

9.2.6 Suspected Unexpected Serious Adverse Reaction (SUSAR)

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

9.3 Foreseeable Adverse Reactions:

The foreseeable ARs following vaccination with R21 and Matrix-M1 include injection site pain, erythema, warmth, swelling, pruritus, myalgia, arthralgia, headache, fatigue, fever, feverishness, malaise and nausea.

9.4 Expected Serious Adverse Events

No serious adverse events are expected in this study.

9.5 Causality Assessment

For every unsolicited AE, an assessment of the relationship of the event to the administration of the vaccine will be undertaken. An intervention-related AE refers to an AE for which there is a probable or definite relationship to administration of a vaccine. An interpretation of the causal relationship of the intervention to the AE in question will be made, based on the type of event; the relationship of the event to the time of vaccine administration; and the known biology of the vaccine therapy (Table 7).

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 7. *Guidelines for assessing the relationship of vaccine administration to an AE.*

9.6 Reporting Procedures for All Adverse Events (see SOP VC027)

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

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

In order to comply with current regulations on serious adverse event reporting to regulatory authorities, the event will be documented accurately and notification deadlines respected. SAEs will be reported on the SAE forms to members of the study team immediately the Investigators become aware of their occurrence, as described in SOP

OVC005. Copies of all reports will be forwarded for review to the Chief Investigator (as the Sponsor's representative) within 24 hours of the Investigator being aware of the suspected SAE. The local safety committee (LSC) will be notified of SAEs that are deemed possibly, probably or definitely related to study interventions; the LSC will be notified immediately (within 24 hours) of the Investigators' being aware of their occurrence. SAEs will not normally be reported immediately to the ethical committee(s) unless there is a clinically important increase in occurrence rate, an unexpected outcome, or a new event that is likely to affect safety of trial volunteers, at the discretion of the Chief Investigator and/or LSC. In addition to the expedited reporting above, the Investigator shall include all SAEs in the annual Development Safety Update Report (DSUR) report.

9.6.2 Reporting Procedures for SUSARS

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

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

9.6.3 Development Safety Update Report

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

9.7 Assessment of severity

The severity of clinical and laboratory adverse events will be assessed according to the scales in Tables 8-10.

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	>1 - ≤20 mm

	2	>20 - ≤50 mm
	3	>50 mm

Table 8. *Severity grading criteria for local adverse events.*

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

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

Table 9. *Severity grading criteria for physical observations*

**Taken after ≥10 minutes at rest*

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

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

GRADE 0	None
GRADE 1	Mild: Transient or mild discomfort (< 48 hours); no medical intervention/therapy required
GRADE 2	Moderate: Mild to moderate limitation in activity – some assistance may be needed; no or minimal medical intervention/therapy required
GRADE 3	Severe: Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalisation possible

Table 10. *Severity grading criteria for local and systemic AEs.*

9.8 Procedures to be followed in the event of abnormal findings

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

9.9 Local Safety Committee

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

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

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

9.9.1 Safety Profile Review

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

10 STATISTICS

This is an observational and descriptive safety study, where volunteers will be vaccinated with R21 alone or in combination with adjuvant Matrx-M1. 34 volunteers will be vaccinated in total - 10 with 10μg R21 adjuvanted with 50μg Matrix-M1, 4 with 50μg R21 alone, 10 with 50μg R21 adjuvanted with 50μg Matrix-M1 and a further 10 with 2μg R21 adjuvanted with 50μg Matrix-M1. This sample size should allow an initial estimation to be made of the frequency and magnitude of outcome measures, rather than aiming to obtain statistical significance for differences between groups.

Sample Size Selection

This is a descriptive phase I first in human trial that will balance the safety of volunteers with the aims to assess the vaccine's safety profile and immunogenicity after selected doses of the vaccines. The primary dose comparison will be between Groups 1, 3 and 4, which will each have 10 subjects. Group 2 is a smaller group because we anticipate that the immunogenicity of the R21 without adjuvant is very likely indeed to be considerably lower than R21 with adjuvant, based on both pre-clinical data and general performance of non-adjuvanted protein-based vaccines. This should be demonstrable with just four vaccinees being administered vaccine without adjuvant. CSP-specific immunogenicity will be the key immunological readout assessed by a variety of immunological assays.

11 QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

11.1 Investigator procedures

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

11.2 Monitoring

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

11.3 Modification to protocol

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

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

11.4 Protocol deviation

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

11.5 Audit & inspection

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

The Sponsor, trial sites, and ethical committee(s) may carry out audit to ensure compliance with the protocol, GCP and appropriate regulations. GCP inspections may also be

undertaken by the MHRA to ensure compliance with protocol and the Medicines for Human Use (Clinical Trials) Regulations 2004. The Sponsor will assist in any inspections and will formally respond to the MHRA as part of the inspection procedure.

11.6 Serious Breaches

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

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

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

(b) the scientific value of the trial”.

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

11.7 Trial Progress

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

11.8 Publication Policy

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Data from the study may also be used as part of a thesis for a PhD or MD.

12 ETHICS

12.1 Declaration of Helsinki

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

12.2 ICH Guidelines for Good Clinical Practice

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

12.3 Informed Consent

Written, informed consent will be obtained, as described in section 6.2

12.4 Research Ethics Committee (REC)

A copy of the protocol, proposed informed consent form, other written volunteer information and the proposed advertising material will be submitted to a REC for written approval. The Chief Investigator will submit and, where necessary, obtain approval from the REC for all subsequent substantial amendments to the protocol and informed consent document.

12.5 Volunteer Confidentiality

All data will be anonymised: volunteer data will be identified by a unique study number in the CRF and database. A separate confidential file containing identifiable information will be stored in a secured location in accordance with the Data Protection Act 1998. Only the Sponsor representative, Investigators, the clinical monitor, the REC and the MHRA will have access to the records. Photographs taken of vaccination sites (if required, with the volunteer's written, informed consent) will not include the volunteer's face and will be identified by the date, trial code and subject's unique identifier. Once developed, photographs will be stored as confidential records, as above. This material may be shown to other professional staff, used for educational purposes, or included in a scientific publication.

13 DATA HANDLING AND RECORD KEEPING

13.1 Data Handling

The Chief Investigator will be the data manager with responsibility for receiving, entering, cleaning, querying, analysing and storing all data that accrues from the study. The data will be entered into the volunteers' CRFs in a paper and/or electronic format (using OpenClinica™ database). Electronic data will be stored on secure servers which are outsourced by OpenClinica™. Data will be entered in a web browser on PCs in the CCVTM building and then transferred to the OpenClinica Database by encrypted (Https) transfer. OpenClinica™ meets FDA part 11B standards. This includes safety data, laboratory data (both clinical and immunological) and outcome data.

13.2 Record Keeping

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

13.3 Source Data and Case Report Forms (CRFs)

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

13.4 Data Protection

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

14 FINANCING AND INSURANCE

14.1 Financing

The study is funded through MultiMalVax, an EC FP7 funded project which intends to develop a highly effective multi-stage malaria vaccine to the point of proof-of-concept Phase II testing in Europe, prior to clinical trials in malaria-endemic areas.

14.2 Insurance

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

14.3 Compensation

Volunteers will be compensated for their time and for the inconvenience caused by procedures. They will be compensated £25 for attending the screening visit. For all other trial visits as outlined in Table 6, compensation will be calculated according to the following:

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

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APPENDIX A LABORATORY VALUES FOR EXCLUSION

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

Urinalysis at screening will be assessed as per the table below:

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

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

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