Clinical trial protocol

Safety, Tolerability and Plasmodium falciparum transmission-reducing activity of R0.6C vaccine adjuvanted with Alhydrogel alone or combined with Matrix-M in healthy malaria-naïve adults in the Netherlands

(STOP-TRANS)

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CONFIDENTIAL

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PROTOCOL SIGNATURE SHEET

The signature below constitutes approval of this protocol and the attachments and provides required assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements, applicable to ICH E6 [R2] guidelines.

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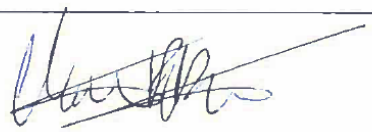
Principal Investigator ***Matthew McCall, MD, PhD*** *Department of Medical Microbiology* *Radboud university medical center*		30-06-2021

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)**
AE	**Adverse Event**
AR	**Adverse Reaction**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
ELISA	**Enzyme-linked immunosorbent assay**
EPD	**Electronic Patient Dossier**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
GP	**General Practicioner**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IM	**Intramuscular(ly)**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)**
PBMC	**Peripheral Blood Mononuclear Cells**
(S)AE	**(Serious) Adverse Event**
SMFA	**Standard Membrane Feeding Assay**
SMC	**Safety Monitoring Committee**
SPC	**Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical**

	company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
TBV	**Transmission blocking vaccine**
TBA	**Transmission blocking activity**
TRA	**Transmission reducing activity**
UAVG	**Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG**
WMO	**Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: Malaria, a disease caused by *Plasmodium* parasites, is one of the most important infectious diseases worldwide. After a period of success in global malaria control, progress has stalled in 2015-2018. The availability of a transmission blocking vaccine would be a critical step to move towards malaria elimination[1, 2]. The R0.6C fusion protein, consisting of the N-terminal region of Glutamate Rich Protein GLURP (R0) and the 6-cysteine C-terminal fragment of the well-established Pfs48/45 antigen (6C), is a lead candidate for a transmission blocking vaccine.

Objectives

Primary safety objective:

1) To evaluate safety of R0.6C immunizations in healthy malaria-naïve volunteers in four dose-adjuvant combinations.

Primary efficacy objective:

1) To assess transmission reducing activity (TRA) to mosquitoes in the standard membrane feeding assay of sera after full course of R0.6C immunizations in each of the four dose-adjuvant combinations.

Secondary safety objective;

1) To evaluate tolerability of R0.6C immunizations in healthy malaria-naïve volunteers in four dose-adjuvant combinations.

Secondary efficacy objectives:

1) To assess the dynamics of TRA in the standard membrane feeding assay of sera collected during and after R0.6C immunizations in each of the four dose-adjuvant combinations.
2) To assess the dynamics of anti-6C antibody quantities during and after R0.6C immunizations in each of the four dose-adjuvant combinations.

Exploratory objectives:

1) To assess transmission blocking activity (TBA) in the standard membrane feeding assay in each of the four dose-adjuvant combinations.
2) To estimate antibody decay rate against the 6C fraction of R0.6C following R0.6C immunizations for each of the four dose-adjuvant combinations.
3) To quantify antibody quantities against R0.6C and the R0 fraction of R0.6C after immunization with R0.6C in each of the four dose-adjuvant combinations.
4) To analyse cellular immune responses after R0.6C immunization.

5) To compare TRA between the two dose groups (30 or 100µg R0.6C) and/or between adjuvant groups (alhydrogel and alhydrogel+Matrix-M).
6) To compare anti-6C antibody quantities between the two dose groups (30 or 100µg R0.6C) and/or between adjuvant groups (alhydrogel and alhydrogel+Matrix-M).

Study design: R0.6C is a first-in-human phase I, open-label, single-site, dose escalation study to determine the safety, tolerability and transmission reducing activity of the R0.6C vaccine in two different adjuvant combinations.
Study population: 32 healthy, malaria naïve adults (males and females), aged 18 – 55 years.
Intervention: Each of the study arms will receive four intramuscular vaccinations on days 0, 28, 56 and 168 with R0.6C adsorbed to Alhydrogel alone (Groups 1-4A), or combined with an additional adjuvant Matrix-M (groups 1-4B). Dose escalation with the two adjuvant arms will take place in parallel: first, a sentinel group (1A and 1B, n=3 per arm) will receive the low dose of 30µg R0.6C; subsequently the additional subjects (groups 2A and 2B, n=5 per arm) will receive the low dose of 30 µg R0.6C; if considered safe, a sentinel group (3A and 3B, n=3 per arm) will receive the high dose of 100µg R0.6C; and finally, the remainder of subjects will receive the high dose of 100µg R0.6C (groups 4A and 4B, n=5 per arm).

Main study endpoints:
Primary safety endpoints:
1) The number of serious adverse events and solicited and unsolicited grade 3 adverse events possibly, probably or definitely related to the vaccine in the period from first R0.6C administration up to 84 days after the last immunization.

Primary efficacy endpoints:
1) The functional TRA in the standard membrane feeding assay of volunteer sera collected two weeks after the fourth R0.6C immunization (I4+14), compared to baseline (I1-1) within each of the four dose-adjuvant groups.

Secondary safety endpoints:
1) The number of solicited and unsolicited grade 1 and 2 adverse events possibly, probably or definitely related to the vaccine in the period from first R0.6C administration up to 84 days after the last immunization.

Secondary efficacy endpoints:
1) The TRA at other timepoints (I1+14, I2+14, I3+14, I3+111 [I4-1], and I4+84) compared to baseline (I1-1) in each of the four dose-adjuvant groups.

2) The anti-6C antibody quantity in volunteer sera collected two weeks after fourth R0.6C immunization (I4+14) and at other time points (I1+14, I2+14, I3+14, I3+111 [I4-1], and I4+84) compared to baseline (I1-1) in each of the four dose-adjuvant combinations, as determined by ELISA.

Exploratory endpoints:

1) The functional TBA in the SMFA of volunteer sera collected at different time points compared to baseline (I1-1) in each of the four dose-adjuvant combinations.
2) The anti-6C antibody decay rate following R0.6C immunization for each of the four dose-adjuvant combinations.
3) The anti R0.6C and anti-R0 antibody quantity in volunteer sera collected at different time points in each of the four dose-adjuvant combinations.
4) The cellular immune responses in volunteers samples collected at different time points in each of the four dose-adjuvant combinations.
5) The difference in TRA between the two dose groups (30 or 100µg R0.6C) and/or between adjuvant groups (alhydrogel and alhydrogel+Matrix-M).
6) The difference in peak anti-6C antibody quantity between the two dose groups (30 or 100µg R0.6C) and/or between adjuvant groups (alhydrogel and alhydrogel+Matrix-M).

Nature and extent of discomfort and risks associated with participation, benefit and group relatedness: There are no individual benefits to participating in this study. In case that the vaccine is effective, vaccination campaigns will reduce the risk of transmission in a population. Subjects will not be protected against malaria after participating. The vaccine platform (*Lactococcus lactis* expressed recombinant protein vaccine) and both adjuvants (Alhydrogel, Matrix M) have been used extensively before. However, R0.6C has not been administered in humans before, therefore there is a theoretic risk of novel side effects to occur. Participating in this trial includes the discomfort of R0.6C vaccine administrations with potential adverse reactions, multiple blood sampling tests, follow-up visits, physical examinations, screening for HIV, Hepatitis B and Hepatitis C, pregnancy tests (for females), filling out a diary and abiding to all study rules.

1. INTRODUCTION AND RATIONALE

Malaria is one of the most devastating infectious diseases worldwide. After a period of success in global malaria control, progress has stalled. Data from 2015-2018 highlight that no significant progress in reducing global malaria cases was made in this period. There were an estimated 228 million cases and 405 000 related deaths in 2018, mainly in children below five years of age[3]. In addition to the this burden of morbidity and mortality, this disease forms a profound economic burden for the affected countries, many of which are in any case characterised as Low and Middle Income Countries (LMIC)[4]. The urgency of the situation is further emphasized by the waning effectiveness of currently registered anti-malarials due to fast emergence and spread of resistance and the absence of a highly effective vaccine[1, 5].

Human malaria is caused by protozoa of the genus Plasmodium: P. *falciparum*, P. *vivax*, P. *ovale*, P. *malariae*, and P. *knowlesi*, of which P. *falciparum* is responsible for the greatest global burden of morbidity and mortality. Parasites (sporozoite stage) are injected into the skin by an infected female Anopheles mosquito when taking a blood meal. After penetration of the skin capillaries, sporozoites migrate to the liver, where they develop and multiply in liver cells before release into the blood (merozoite stage) and invading red blood cells for further maturation and multiplication. The cyclical proliferation of asexual stages within the human red blood cells is responsible for the occurrence of clinical symptoms. A small fraction of asexual stages commits to enter sexual development and ultimately form mature male and female gametocytes. These male and female gametocytes do not cause clinical pathology or symptoms, but can be taken up by a new Anopheline vector, in whose midgut they transform into male and female gametes, which fuse to form a zygote that in turn becomes motile and elongated (ookinete). This ookinete invades the mosquito midgut wall, developing into an oocyst which in turn releases new sporozoites. This ultimately renders the mosquito infectious upon its next bite, leading to onward transmission of malaria infections.
Malaria transmission blocking vaccines (TBVs) and transmission-blocking drugs aim to interrupt transmission to or the development of parasites in the mosquito vector by an intervention applied to the human intermediate host[5]. Deployment of transmission-blocking drugs and TBVs will be an efficient complementary element in an integrated program of anti-malarial interventions. With the implementation of TBVs we will be able to reduce the malaria burden, contain drug resistance and to move towards malaria elimination[2, 6].

The sexual stage Pfs48/45 antigen is a well-established lead candidate for a *P. falciparum* transmission blocking vaccine because of its critical role in parasite fertilisation. Male gametes lacking Pfs48/45 are unable to bind female gametes in the mosquito midgut, thus preventing ookinete, oocyst and ultimately sporozoite development. The R0.6C fusion protein

is a chimera consisting of the 6-cysteine C-terminal fragment of PfS48/45 (6C) coupled to the N-terminal region of asexual stage Glutamate Rich Protein GLURP (R0) produced in *Lactococcus lactis*. Immunisation with R0.6C in rodents induces functional antibodies against the 6C subunit[7]. Anti-6C antibodies (Abs) are ingested during the blood meal and can bind male sexual forms in the mosquito gut, preventing their fertilisation of female gametes and thus ookinete and oocyst development. Sera of vaccinated animals were able to reduce transmission in the standard membrane feeding assay (SMFA) with cultured gametocytes. Anti-6C antibody titres were further increased by immunising with R0.6C adjuvanted with Alhydrogel or Alhydrogel and Matrix-M[8].

The SMFA is the most widely used assay to determine the TRA and an accepted surrogate of TRA for public health interventions [5]. Laboratory reared Anopheles mosquitoes are fed through membrane feeders with in vitro cultured gametocytes to which serum or purified anti-R0.6C antibodies in various concentrations are added. Subsequently, the oocyst densities in mosquitoes are compared between the experimental and control group by means of microscopy of the mosquito gut, parasite DNA detection or immune-assays [5, 9]. The SMFA can also be used to determine the transmission blocking activity (TBA) by comparing the number of infected mosquitoes between the experimental and control group.

Because the mode of action of TBVs is so explicit, there is a highly informative endpoint in the SMFA that is a clear indicator of later public health impact. For other vaccines, there is no such clear in vitro assay. The TRA measured in the SMFA assay around which there is confidence that blocking activity is present was set by consensus at 80% reduction in oocyst intensity, however even TBVs with a TRA of less than 80% could eliminate plasmodium at low transmission levels over consecutive transmission seasons[10].

2.1.2 Rationale

The renewed focus on malaria elimination has increased the priority of research towards development of interventions to block malaria transmission [10, 11]. By interrupting transmission of malaria parasites in mosquito vectors, a reduction in the number of secondary infections in the community is expected with an overall reduction in disease and mortality[12].

The lead TBV candidate R0.6C has shown good transmission reducing efficacy in rodent models. Also, a monoclonal antibody TB31F against the same Pfs48/46 6C region has shown excellent safety and transmission reducing activity in humans in a recent clinical trial (van der Boor et al, manuscript in preparation). Efficacious TBVs would be a critical step on

the path towards malaria elimination and their safety and preliminary efficacy data can be acquired in malaria-naïve volunteers.

3. OBJECTIVES

Primary safety Objective:

1) To evaluate safety of R0.6C immunizations in healthy malaria-naïve volunteers in four dose-adjuvant combinations.

Primary efficacy objective:

1) To assess TRA in the standard membrane feeding assay of sera after full course of R0.6C immunizations in each of the four dose-adjuvant combinations.

Secondary safety objective;

1) To evaluate tolerability of R0.6C immunizations in healthy malaria-naïve volunteers in four dose-adjuvant combinations.

Secondary efficacy objectives:

1) To assess the dynamics of TRA in the standard membrane feeding assay of sera collected during and after R0.6C immunizations in each of the four dose-adjuvant combinations.
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Exploratory Objectives:

1) To assess TBA in the standard membrane feeding assay in each of the four dose-adjuvant combinations.
2) To estimate antibody decay rate against the 6C fraction of R0.6C following R0.6C immunizations for each of the four dose-adjuvant combinations.
3) To quantify antibody quantities against R0.6C and the R0 fraction of R0.6C after immunization with R0.6C in each of the four dose-adjuvant combinations.
4) To analyse cellular immune responses after R0.6C immunization.
5) To compare TRA between the two dose groups (30 or 100µg R0.6C) and/or between adjuvant groups (alhydrogel and alhydrogel+Matrix-M).
6) To compare anti-6C antibody quantities between the two dose groups (30 or 100µg R0.6C) and/or between adjuvant groups (alhydrogel and alhydrogel+Matrix-M).

4. STUDY DESIGN

This is a first-in-human, phase I, open-label, single-site, dose escalation study to determine the safety tolerability and immunogenicity of the R0.6C vaccine in two different adjuvant combinations. Administration of R0.6C to volunteers, follow up and clinical chemical, microbiological analysis and storage of the samples will take place in the Radboudumc in Nijmegen.

Thirty-two healthy adult volunteers will be recruited and divided over the study arms that will receive four vaccinations on days 0, 28, 56 and 168 with either 30µg or 100µg of R0.6C adjuvanted with Alhydrogel alone, or combined with Matrix-M (Table 1).

Three volunteers (Group 1A, n=3) will receive four vaccinations with the lower dose of 30µg R0.6C with Alhydrogel, and, in parallel, three volunteers (Group 1B, n=3) will receive four vaccinations with the lower dose of 30µg R0.6C with Alhydrogel and Matrix-M. Volunteers will be closely monitored for adverse events for a period of minimally 14 days after the first vaccination. If safe, an additional 5 volunteers per adjuvant arm (groups 2A and 2B) will then receive four vaccinations with the lower dose (30µg R0.6C). If considered safe following a minimum of 14 days of follow-up after the first R0.6C administration of groups 2A and 2B, three volunteers per adjuvant arm (groups 3A and 3B) will start the vaccination regimen with the higher dose of 100µg R0.6C. Finally, a minimum of 14 days after administration of the first vaccination in groups 3A and 3B, if considered safe, an additional 5 volunteers per adjuvant arm (groups 4A and 4B) will initiate the vaccination regimen with the higher dose of 100µg R0.6C. There will be no placebo group; TRA endpoints will be compared to pre-vaccination values. All volunteers will be followed up for adverse events until 84 days after the last immunisation. Total trial duration is approximately 8 months for each subject. Blood will be collected to assess the functional TRA, TBA and immunogenicity at pre-specified time points after R0.6C vaccinations (also see section 8.3.17). If one of the volunteers is considered not eligible for participation on day 0, an different ('back-up') volunteer will replace him/her. For this purpose up to two additional volunteers may be screened and kept as back up in each group.

Table 1: study arms.

Study arm	***Number of volunteers***	***Candidate vaccine dose and adjuvant combination***
1A	*n=3*	*4x 30µg R0.6C Alhydrogel*
1B	*n=3*	*4x 30µg R0.6C Alhydrogel + Matrix M*
2A	*n=5*	*4x 30µg R0.6C Alhydrogel*
2B	*n=5*	*4x 30µg R0.6C Alhydrogel + Matrix M*
3A	*n=3*	*4x 100µg R0.6C Alhydrogel*

3B	*n=3*	*4x 100µg R0.6C Alhydrogel + Matrix M*
4A	*n=5*	*4x 100µg R0.6C Alhydrogel*
4B	*n=5*	*4x 100µg R0.6C Alhydrogel + Matrix M*

5. STUDY POPULATION

5.1 Population (base)

The study population will be comprised of adult male and female healthy subjects aged 18-55 at time of first R0.6C administration. A total of 32 subjects will be enrolled to participate in the study as well as up to 8 reserve subjects (up to 2 reserve subject per dosing group). The investigator will ensure that all subjects being considered for the study meet the eligibility criteria described below. Subject eligibility is to be established and confirmed by checking all inclusion/exclusion criteria at both screening and inclusion (baseline). A relevant record of the eligibility criteria will be stored with the source documentation at the study site.

5.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

1. Subject must sign written informed consent to participate in the trial.
2. Subject is a male or non-pregnant and non-lactating female age ≥ 18 and ≤ 55 years and in good health.
3. Subject is able to understand planned study procedures and demonstrate comprehension of the protocol procedures and knowledge of study by passing a quiz (assessment of understanding).
4. In the opinion of the investigator, the subject can and will comply with the requirements of the protocol.
5. Subjects are available to attend all study visits and are reachable by phone throughout the entire study period from day -1 until day 224 (end of study).
6. The subject will remain within reasonable travelling distance from the study center from day -1 until day 7 after each R0.6C administration and agrees not to travel to a malaria-endemic area during the study period
7. Subject agrees to their general practitioner (GP) being informed about participation in the study and agrees to sign a form to request the release by their GP, and medical specialist when necessary, of any relevant medical information concerning possible contra-indications for participation in the study to the investigator(s).
8. The subject agrees to refrain from blood donation to Sanquin or for other purposes throughout the study period according to current Sanquin guidelines.

9. Female subjects of non-childbearing potential may be enrolled in the study. Non-childbearing potential is defined as pre-menarche, current bilateral tubal ligation or occlusion, hysterectomy, bilateral ovariectomy or post-menopause. All subjects of childbearing potential must agree to use continuous adequate contraception* until 2 months after completion of the study. Female subjects must agree not to breastfeed from 30 days prior to R0.6C administration until 2 months after completion of the study. Female subjects must have a negative pregnancy test at the inclusion visit.

**Acceptable forms of female contraception include: established use of oral, injected or implanted hormonal contraceptives; intrauterine device or intrauterine system; barrier methods (condoms or diaphragm with additional spermicide); male partner's sterilization (with appropriate post-vasectomy documentation of absence of sperm in the ejaculate); true abstinence when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception. Adequate contraception does not apply to subjects of child bearing potential with partners of the same sex.*

5.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

1. Acute or chronic disease at time of R0.6C administration, clinically significant pulmonary, cardiovascular, hepatic, renal, neurological or immunological functional abnormality, as determined by medical history, physical examination or laboratory screening tests:
    a. Acute disease is defined as the presence of a moderate or severe illness with or without fever. For subjects with an illness on the day of R0.6C administration, the vaccination may be postponed up to 7 days.
    b. Fever is defined as an oral, axillary or tympanic temperature ≥ 38.0°C.
    c. Any abnormal and clinically significant baseline laboratory screening tests of ALT, AST, creatinine, hemoglobin, platelet count or total white blood cell count, as defined in the protocol according to the FDA Toxicity Grading Scale for Healthy Adult and Adolescent Subjects Enrolled in Preventative Vaccine Clinical Trials (appendix 1).
2. History of malignancy of any organ system (other than localized basal cell carcinoma of the skin), treated or untreated, within the past 5 years.
3. Chronic use of i) immunosuppressive drugs, iii) or other immune modifying drugs within three months prior to study onset (inhaled and topical corticosteroids and oral anti-histamines exempted) or expected use of such during the study period.
4. History of drug or alcohol abuse interfering with normal social function in the period of one year prior to study onset, positive urine toxicology test for cocaine or amphetamines at screening or at inclusion.
5. Screening tests positive for Human Immunodeficiency Virus (HIV), active Hepatitis B Virus (HBV), Hepatitis C Virus (HCV).

6. Use of any other investigational or non-registered product (drug or vaccine) during the study period.
7. Known hypersensitivity to macrolides.
8. Participation in any other clinical study involving an investigational product in the 30 days prior to the start of the study or during the study period.
9. Receipt of any other vaccination within 30 days prior to or up to 14 days after any R0.6C vaccination. Exceptions are made for vaccination against influenza and the novel coronavirus SARS-CoV2.
10. Any history of malaria, positive serology for *P. falciparum,* or previous participation in any malaria (vaccine) study or CHMI.
11. Body weight ≥ 115 kg
12. Being an employee or student of the department of Medical Microbiology of the Radboudumc at the time of screening, or a person otherwise related to the investigator.
13. Any other condition or situation that would, in the opinion of the investigator, place the subject at an unacceptable risk of injury or render the subject unable to meet the requirements of the protocol, or affects the interpretability of the trial results.

5.4 Sample size justification

R0.6C is a phase 1 safety and efficacy trial in healthy volunteers, designed to identify safety concerns associated with R0.6C administration at different dosages and/or adjuvants. A total of 32 healthy adults (males and females) aged 18 – 55 will be divided over four groups. Each group will include eight subjects.
Sample size for each group is appropriate based on similar trial designs in malaria protein vaccination studies. This is a small Phase 1 study; therefore, it is largely descriptive and is intended to provide primarily safety data of 2 doses of R0.6C combined with 2 adjuvants. Comparative statistics will be performed but will have low power to detect anything other than very large differences between the groups.

Furthermore, if we assume an effect size of 75% TRA at 14 days after the fourth R0.6C vaccination (I4+14) compared to baseline (I1-1), with the subject size of n=8 per dose/adjuvant arm we will have an empirical power of >99%, based on calculations and assumptions as described below in paragraph 5.4.1.

5.4.1 Estimation of empirical power for an analysis of transmission reducing activity under different assumptions

The simulation algorithm

1. We generate n_0 subjects in the control (pre-treatment) group and n_1 subjects in the treatment (post-treatment) group with a variable x_i to indicate whether the subject is in the control group $(x_i = 0)$ or in the treatment group $(x_i = 1)$.
2. We generate m mosquito dissections for each subject.

3. We simulate subject-specific random intercepts (z_i) from a normal distribution with a mean of 0 and a specified standard deviation σ, i.e. $Z \sim N(0, \sigma)$.
4. Lastly we generate the oocyst counts (y_{ij}) for each subject i and mosquito j using a negative binomial distribution such that $Y \sim NB(\mu, \theta)$ where θ is the anticipated dispersion parameter and $\mu_i j = \exp(\beta_0 + \beta_1 x_{ij} + z_i)$ where β_0 is the anticipated mean of the log oocyte counts in the control group and β_1 is the anticipated regression coefficient used to estimate the TRA, such that

$$\beta_1 = \log\left(1 - \frac{TRA}{100}\right).$$

The model

To analyze the simulated data, we make the of the $mgcv$ package in R. We run a Generalized Additive Mixed Model such that,

$$\log(y) = \beta_0 + \beta_1 x + z_i.$$

From each simulated dataset, we estimate the $TRA = \left(1 - \exp\left(\hat{\beta}_1\right)\right) \times 100$ and p (the p-value) of the test for whether β_1 is significantly different from 0. We then report the average TRA across simulations, which should be close to the anticipated value, and the emperical power which we define as the percentage of p's less than 0.05.

Simulation results

We chose $m = 20$ as the standard amount of mosquito dissections per subject. We used a previous study (NL69779.091.19) to extract anticipated values for the mean log oocyst count in the control group, the dispersion parameter θ and the standard deviation of the random effects σ. i.e. $\beta_0 = 3.7037$, $\theta = 4.451$ and $\sigma = 0.64$. For the anticipated TRA (and thus anticipated β_1) we used a range of assumed values, i.e. $TRA = (25\%, 50\%, 75\%)$. We also varied the number of people in the control and treatment groups, i.e. n_0 and n_1. The tables below show the emperical power under these scenarios.

$\boldsymbol{TRA = 25\%}$	$\boldsymbol{n_1}$					
$\boldsymbol{n_0}$	**5**	**6**	**7**	**8**	**9**	**10**
10	17.0	18.4	17.4	18.4	18.2	19.0
11	17.2	15.8	16.6	17.6	19.6	19.4
12	17.0	17.6	18.2	18.4	19.4	19.2
13	17.2	16.4	17.6	17.4	20.6	19.6
14	16.6	17.8	19.8	20.2	19.4	20.2
15	15.4	17.4	18.8	19.4	20.8	22.6
16	14.6	15.2	17.8	19.8	20.4	21.0
17	14.4	15.8	18.6	19.0	20.6	22.4
18	14.4	16.2	18.2	18.8	21.0	22.6
19	17.4	19.0	18.6	19.4	22.2	24.8
20	15.8	17.8	19.4	22.2	25.8	26.8

$TRA = TRA = 50\%$	n_1					
n_0	**5**	**6**	**7**	**8**	**9**	**10**
10	52.0	57.0	60.4	63.8	64.4	67.0
11	54.2	58.8	62.4	63.8	65.2	69.6
12	55.0	59.4	62.8	65.4	69.2	70.6
13	54.6	58.2	63.6	67.0	69.4	71.8
14	58.4	62.4	64.8	68.2	70.4	73.0
15	55.6	59.2	65.0	66.6	70.4	74.0
16	55.2	60.4	64.0	68.0	71.6	75.8
17	54.6	60.6	66.8	69.8	75.8	78.2
18	54.2	61.6	67.8	72.2	75.6	78.4
19	59.6	66.8	71.0	76.8	78.8	81.6
20	61.8	68.2	72.8	78.6	80.6	82.8

$TRA = 75\%$	n_1					
n_0	**5**	**6**	**7**	**8**	**9**	**10**
10	97.2	99.0	99.6	99.8	99.8	100.0
11	98.2	99.6	99.8	99.6	99.6	100.0
12	98.4	99.2	99.2	99.8	100.0	100.0
13	97.4	99.0	99.6	99.8	100.0	100.0
14	96.6	98.8	99.2	99.8	100.0	100.0
15	97.8	99.2	99.8	100.0	100.0	100.0
16	98.4	98.8	99.6	99.8	100.0	99.8
17	99.4	99.8	100.0	100.0	100.0	100.0
18	99.0	100.0	100.0	99.8	100.0	100.0
19	99.2	99.6	99.6	100.0	100.0	100.0
20	99.0	99.2	99.8	100.0	100.0	100.0

6. TREATMENT OF SUBJECTS

6.1 Investigational product/treatment

Volunteers will sequentially receive four administrations of R0.6C intramuscularly in the deltoid muscle on alternating sides on days 0, 28, 56 and 168. A total of 8 subjects will receive four times 30µg R0.6C adjuvated with 240 µg Alhydrogel (Alhydrogel, groups 1A and 2A), eight subjects will receive four times 30µg R0.6C adjuvated with 240 µg Alhydrogel and 15 µg Matrix-M ('Alhydrogel/MM' groups 1B and 2B), eight subjects will receive four times 100µg R0.6C adjuvated with 800 µg Alhydrogel (groups 3A and 4A) and eight subjects will receive four times 100µg R0.6C adjuvated with 800 µg Alhydrogel and 49 µg Matrix-M (groups 3B and 4B). There will be no placebo group.

6.2 Escape medication

Escape medication is not applicable, appropriate medication (e.g. paracetamol or antihistamines) for symptomatic relief of any untoward side effects will be advised as necessary.

7. INVESTIGATIONAL PRODUCT

7.1 Name and description of investigational product(s)

The R0.6C fusion protein is a chimera consisting of the 6-cysteine C-terminal fragment of PfS48/45 (6C) coupled to the N-terminal region of asexual stage Glutamate Rich Protein GLURP (R0) produced in Lactococcus lactis (Singh et al. 2020, Singh et al. 2017; Singh et al. 2015). The carrier protein R0 helps to enhance the immune response against Pfs48/45 (Theisen et al. 2014). R0.6C and the position of the Pfs48/45-6C domain is shown in Figure 1.

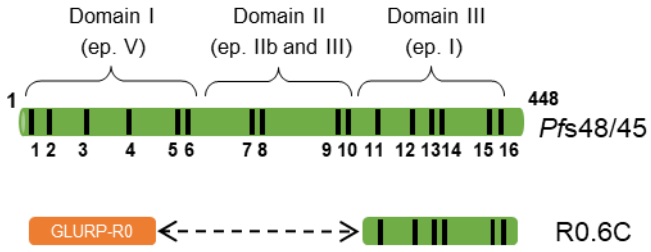

Figure 1: Schematic representation of Pfs45/48 and the fragment that is cloned in the R0.6C fusion protein

The recombinant R0.6C protein is formulated in 10 mM HEPES, 2.5% glucose, 0.5 mM EDTA, 155 mM NaCl and absorbed to 1.6 mg/mL Alhydrogel [$Al(OH)_3$], at a fill volume of 0.8 mL in a 2 mL borosilicate glass vial and stored at 2-8°C.

Alhydrogel [$Al(OH)_3$] is an aluminium hydroxide adjuvant supplied by Croda (Denmark, USA. It has been used as a vaccine adjuvant to enhance immune responses to vaccines. The final aluminium composition in the R0.6C/Alhydrogel product is 0.8 mg/mL Aluminum.

Matrix-M is a saponin based adjuvant manufactured by Novavax (Sweden). The saponin-based Matrix-M improves immune responses and enables vaccine dose-sparing. Matrix-M is supplied ready-to-mix as a refrigerated solution (stored 2-8°C) at a concentration of 0.375 mg/mL and a fill volume of 0.75 mL. Although it is not yet fully understood how the Matrix-M adjuvant achieves its stimulatory effects, this adjuvant is known to transiently enhance the number of activated immune cells in the draining lymph nodes which may in turn lead to increased uptake and presentation of vaccine antigens to elicit a competent immune response (Reimer et al. 2012). Specifically, it has been shown that there is an increase of CD169+ macrophages, as well as activated dendritic cells, to the draining lymph nodes after immunization with Matrix-M adjuvanted vaccines, which may help to increase antigen presentation (Magnusson et al. 2018). Hence, CD169+ macrophages has previously been shown to have a role in transporting antigens to B lymphocytes by trapping them in the draining lymph node and to facilitate cross-presentation of the antigen to CD8+ T lymphocytes (Gray and Cyster 2012; Carrasco and Batista 2007). This may lead to increased humoral and cellular immune responses, manifested by cross-reactive antibodies and multi-

functional CD4+ T lymphocytes (Bengtsson et al. 2016; Shinde et al. 2020). Consequently, Matrix-M has been shown to contribute to antigen dose-sparing and increased duration of humoral and cellular vaccine responses (42).

7.2 Summary of findings from non-clinical studies

Preclinical studies focused on safety, immunogenicity, and functional activity of the R0.6C vaccine candidate. Table 2 summarizes the preclinical studies conducted in various animal species. For more detailed information, please refer to D1. Investigator's Brochure R0.6C_AlOH, section 8.0 preclinical studies.

Table 2: Summary of Preclinical Animal Studies for R0.6C candidate Vaccines

Animal Species	Study Protocol	Material	Purpose	Dose, Route, & Regimen	Summary
Rat	Radboud UMC under approval number 2016-0020 [13]	Small scale lab-produced R0.6C	Perform dose-titration studies within suspected dose range to demonstrate that R0.6C elicit TB antibodies and to provide data for potency evaluation.	0.03, 1.2, 4.7, 9.4, 18.9, and 37.7µg of R0.6C adjuvanted on Alhydrogel, 3 SC injections on D0, D14, and D28. Terminal bleed on SD43.	Doses are within the suspected range for potency evaluation. Pfs48/45-6C antibody titers are associated with TRA.
Rat	MVI-01-2015/2016 under P 20-204 [7]	Up-scaled lab-produced R0.6C	Perform dose-titration studies to demonstrate that up-scaled R0.6C product elicit TB antibodies.	2.5, 10, 25µg of R0.6C adjuvanted in Montanide ISA720, 3 SC injections on D0, D21, and D42. Terminal bleed on D63.	Doses are within the suspected range for potency evaluation. Pfs48/45-6C antibody titers are associated with TRA.
Mice	Radboud UMC under approval number 2016-0020	Up-scaled R0.6C reference material	Perform two studies within suspected dose ranges and at the intended route of administration to confirm immunogenicity of DP formulations.	0.4, 2, 10µg of R0.6C adjuvanted on Alhydrogel or Alhydrogel + Matrix M, 2 IM injections on D0, and D28. Terminal bleed on D63.	Both R0.6C/AlOH and R0.6C/AlOH + Matrix M elicit high levels of functional antibodies when injected twice by the intended route of immunization.

Rabbits	CRL Study no. 78813	Tox Grade Material	Toxicity of repeated dose of R0.6C/AlOH and R0.6C/AlOH + Matrix-M	A total of 4 doses by IM, with 100 µg R0.6 with or without 50 µg Matrix-M	No systemic toxicological changes. Local test item related histopathology changes were observed at the injection sites and consisted of an up to marked focal inflammatory response. Partial recovery was recorded when comparing the site dosed 3 days prior to necropsy with the site dosed 46 days prior to necropsy.

7.3 Summary of findings from clinical studies

There is no past human experiences with the R0.6C/AlOH and R0.6C/AlOH + Matrix-M vaccines. However, the GLURP-R0 region, which constitute 76.7% of R0.6C, forms part of the malaria vaccine candidate, GMZ2, which has been tested in both European and African clinical trials listed in Table 3. Notably, GMZ2, adjuvanted with aluminum hydroxide (GMZ2/AlOH) was tested in 880 children 12-60 month of age in Burkina Faso (Banfora; n=580, Sapone; n=300).

To date 994 individuals, of whom the majority are African children below 5 years of age, have been immunized with GMZ2/AlOH. The GMZ2/AlOH formulation has shown excellent safety and tolerability confirming the safety of the expression system and the safety of the GLURP-R0 portion of R0.6C.

Table 3: Clinical experience of GMZ2/AlOH.

Trial registration number (Reference)	**Title**	**Interventions**	**Characteristics**	**Population**
NCT00397449 [14]	Safety and immunogenicity of GMZ2 - a MSP3-GLURP fusion protein malaria vaccine candidate	Biological: • 10µ GMZ2/AlOH • 30µ GMZ2/AlOH • 100µg GMZ2/AlOH	Phase 1a	30 healthy malaria-naïve German adults; 10 / group
NCT00424944 [14]	Safety and immunogenicity of the malaria vaccine candidate GMZ2 in malaria-exposed,	Biological: • 100µg GMZ2/AlOH • VerorabTM	Phase 1b	40 healthy malaria-exposed Gabonese adults; 20 / group

	adult individuals from Lambaréné, Gabon			
NCT00703066 [15]	A Randomized Controlled Phase Ib Trial of the Malaria Vaccine Candidate GMZ2 in African Children	Biological: • 30µ GMZ2/AlOH • 100µg GMZ2/AlOH • VerorabTM	Phase 1b	30 healthy malaria-exposed Gabonese children one to five years of age; 10 / group
PACTR201006 0002033537 [16]	A phase 2b randomized, controlled trial of the efficacy of the GMZ2 malaria vaccine in African children	Biological: • 100µg GMZ2/AlOH • VerorabTM	Phase 2b	1735 healthy malaria-exposed Gabonese children received three doses of vaccine (868 GMZ2, 867 control-vaccine).

The Matrix-M adjuvant technology [17] is a promising technology which has been explored for various infectious diseases and has a good safety profile in humans [18]. To-date Matrix-M has been utilized in more than 25+ clinical trials, including multiple Phase III trials (Table 4). In these studies, Matrix-M has been combined with multiple malaria vaccine candidates, influenza, covid-19, and other disease indications. The most common dosage of Matrix-M has been 50 µg in adults, with a maximum dosage of 75 µg. Further, Matrix-M is currently being evaluated with another malaria vaccine candidates (R21) In Burkina Faso under Phase I and Phase II clinical studies (NCT02925403) in children. There the tested dosages of Matrix-M are 25 and 50 µg.

In one recent example (NCT01444482), Matrix-M adjuvant has also been tested in a randomized, observer-blinded, active-comparator controlled trial during the 2019-2020 influenza season (doi: https://doi.org/10.1101/2020.08.07.20170514). In brief, 2654 clinically-stable, community-dwelling adults ≥65 years of age were randomized to receive a single intramuscular dose of either Matrix-M-adjuvanted quadrivalent nanoparticle influenza vaccine (qNIV) or a licensed inactivated influenza vaccine (IIV4). Local reactogenicity, primarily mild to moderate and transient pain, was higher in the qNIV group. Interpretation: qNIV was well tolerated and produced a qualitatively and quantitatively enhanced humoral and cellular immune response in older adults.

Table 4: Key clinical experience of Matrix-M with various vaccine antigens.

NCT Number	Title	Conditions	Interventions	Characteristics	Population
NCT04201431	Safety, Immunogenicity and Efficacy of the Blood-stage Plasmodium Vivax Malaria Vaccine Candidate PvDBPII in Matrix M1	Malaria, Vivax	• Biological: PvDBPII/Matrix M1	Phase 1 Phase 2	18 Years to 45 Years (Adult)
NCT01669512	Adjuvanting Viral Vectored Malaria Vaccines With Matrix M	Malaria	• Biological: Low Dose Matrix M Regimen • Biological: Standard Dose Matrix M Regimen	Phase 1	18 Years to 50 Years (Adult)
NCT04130282	VAC077: Safety and Immunogenicity of the Pfs25-IMX313/Matrix-M Vaccine	Malaria	• Biological: Pfs25-IMX313/ Matrix-M1	Phase 1	18 Years to 45 Years (Adult)
NCT04318002	Safety and Immunogenicity of RH5.1/ Matrix-M in Adults and Infants Living in Tanzania	Malaria	• Biological: RH5.1/Matrix-M	Phase 1	6 Months to 45 Years (Child, Adult)
NCT03896724	Safety, Immunogenicity and Efficacy of R21 Matrix-M in 5-17 Month Old Children in Nanoro, Burkina Faso	Malaria	• Biological: R21 adjuvanted with 25mcg Matrix-M • Biological: R21 adjuvanted with 50mcg Matrix-M	Phase 1 Phase 2	5 Months to 17 Months (Child)
NCT04271306	Safety, Immunogenicity and ex Vivo Efficacy of Pfs25-IMX313/Matrix-M in Healthy Volunteers in Bagamoyo, Tanzania.	Malaria	• Biological: Pfs25-IMX313 (10ug)/Matrix-M (50ug) • Biological: Pfs25-IMX313 (50ug)/Matrix-M (50ug) • Biological: Pfs25-IMX313 (50ug)/Matrix-M (50ug) & Pfs25-IMX313 (10ug)/ Matrix-M (50ug)	Phase 1	5 Years to 45 Years (Child, Adult)
NCT02572388	A Study to Assess the Safety and Immunogenicity of the Malaria Vaccine, R21, Administered With and Without Matrix-M1	Malaria	• Biological: R21 • Biological: Matrix-M1	Phase 1	18 Years to 50 Years (Adult)
NCT02925403	A Study to Assess the Safety and Immunogenicity of the Malaria Vaccine, R21, With Matrix-M1 Adjuvant	Malaria	• Biological: R21/Matrix-M1 • Other: Saline	Phase 1 Phase 2	18 Years to 45 Years (Adult)
NCT01444482	Study of Parenterally Administrated Adjuvanted Seasonal Influenza Vaccine in Healthy Elderly Volunteers	Influenza	• Biological: Matrix M • Biological: Seasonal influenza vaccine	Phase 1	65 Years to 75 Years (Older Adult)

NCT Number	Title	Conditions	Interventions	Characteristics	Population
NCT04611802	A Study Looking at the Efficacy, Immune Response, and Safety of a COVID-19 Vaccine in Adults at Risk for SARS-CoV-2	COVID-19	• Biological: SARS-CoV-2 rS/ Matrix-M1 Adjuvant • Other: Placebo	Phase 3	18 Years and older (Adult, Older Adult)
NCT04368988	Evaluation of the Safety and Immunogenicity of a SARS-CoV-2 rS Nanoparticle Vaccine With/Without Matrix-M Adjuvant	COVID-19	• Biological: SARS-CoV-2 rS - Phase 1 • Biological: SARS-CoV-2 rS/ Matrix-M Adjuvant, Days 0 and 21 - Phase 2 • And more	Phase 1 Phase 2	18 Years to 84 Years (Adult)
NCT04533399	A Study Looking at the Effectiveness and Safety of a COVID-19 Vaccine in South African Adults	COVID-19	• Biological: SARS-CoV-2 rS/ Matrix-M1 Adjuvant • Other: Placebo	Phase 2	18 Years to 84 Years (Adult)
NCT03580824	A Study to Determine if a New Malaria Vaccine is Safe and Induces Immunity Among Kenyan Adults, Young Children and Infants	Malaria	• Biological: R21 in Matrix- M adjuvant vaccine	Phase 1 Phase 2	5 Months to 45 Years (Child, Adult)
NCT04583995	A Study Looking at the Effectiveness, Immune Response, and Safety of a COVID-19 Vaccine in Adults in the United Kingdom	COVID-19	• Biological: SARS-CoV-2 rS/ Matrix M1-Adjuvant • Other: Placebo • Biological: Licensed seasonal influenza vaccine	Phase 3	18 Years to 84 Years (Adult, Older Adult)
NCT02078674	A(H7N9) VLP Antigen Dose-Ranging Study With Matrix-M1™ Adjuvant	Influenza (Pandemic)	• Biological: Monovalent Avian Influenza VLP (H7N9) • Biological: Matrix-M1™ adjuvant	Phase 1 Phase 2	18 Years to 64 Years (Adult)
NCT02300142	Rollover Trial for Placebo Subjects Previously Enrolled Into GEN-003-002 Study	Genital Herpes	• Biological: GEN-003 Vaccine (30-60µg of each antigen) • Biological: Matrix-M2 Adjuvant (25-75µg)	Phase 2	18 Years to 50 Years (Adult)
NCT01667341	Safety and Immunogenicity Study of Therapeutic HSV-2 Vaccine	Genital Herpes	• Biological: GEN-003 with Matrix M-2 • Biological: GEN-003	Phase 1 Phase 2	18 Years to 50 Years (Adult)
NCT03026348	Safety and Immunogenicity Study to Evaluate Single- or Two-Dose Regimens Of RSV F Vaccine With and Without Aluminum Phosphate or Matrix-M1™ Adjuvants In Clinically-Stable Older Adults	Respiratory Syncytial Viruses	• Biological: RSV F Vaccine with Aluminum Phosphate Adjuvant • Biological: RSV F Vaccine • Biological: Matrix-M1 Adjuvant	Phase 2	60 Years to 80 Years (Adult, Older Adult)
NCT02114060	Dose Ranging Safety and Efficacy of Therapeutic HSV-2 Vaccine	Genital Herpes	• Biological: GEN-003 Vaccine (30µg of each antigen) • Biological: Matrix-M2 Adjuvant (75µg) • And more	Phase 2	18 Years to 50 Years (Adult)

NCT Number	Title	Conditions	Interventions	Characteristics	Population
NCT03947190	A Study to Determine if New Types of Malaria Vaccines Are Safe, Effective and Lead to Immunity in Kenyan Adults	Malaria	• Biological: R21/Matrix-M • Biological: ChAd63/MVA ME-TRAP • Biological: intradermal injection (ID) or direct venous injection (DVI) of PfSPZ Challenge	Phase 2	18 Years to 45 Years (Adult)
NCT03293498	Evaluation of the Safety and Immunogenicity of a Recombinant Trivalent Nanoparticle Influenza Vaccine With Matrix M-1 Adjuvant (NanoFlu)	Influenza	• Biological: NanoFlu • Biological: Fluzone HD - Day 0 • Biological: Fluzone HD - Day 21 • Other: Saline - Day 21	Phase 1 Phase 2	60 Years and older (Adult, Older Adult)
NCT03658629	Phase 2 Dose and Formulation Confirmation of Quad-NIV in Older Adults	Influenza, Human	• Biological: NanoFlu (Quad - NIV) • Other: Matrix-M Adjuvant • Biological: Fluzone HD • Biological:Flublok	Phase 2	65 Years and older (Older Adult)
NCT03970993	VAC 072-An Efficacy Study of R21/MM in Different Dose Schedules	Malaria	• Biological: R21 Matrix-M vaccination • Biological: R21 Matrix-M vaccination and CHMI	Phase 1 Phase 2	18 Years to 45 Years (Adult)
NCT02370589	Study to Evaluate the Immunogenicity and Safety of an Ebola Virus (EBOV) Glycoprotein (GP) Vaccine in Healthy Subjects	Ebola	• Biological: Base Dose EBOV GP Vaccine • Biological: 2-8x Base Dose EBOV GP Vaccine • Biological: Matrix-M Adjuvant	Phase 1	18 Years to 50 Years (Adult)
NCT02515175	Evaluating New Formulation of Therapeutic HSV-2 Vaccine	Genital Herpes	• Biological: Matrix-M2 • Biological: GEN-003	Phase 2	18 Years to 50 Years (Adult)
NCT04120194	Phase 3 Pivotal Trial of NanoFlu™ in Older Adults	Influenza, Human	• Biological: NanoFlu • Biological: Fluzone Quadrivalent	Phase 3	65 Years and older (Older Adult)
NCT04645147	Safety and Immunogenicity of an Epstein-Barr Virus (EBV) gp350-Ferritin Nanoparticle Vaccine in Healthy Adults With or Without EBV Infection	EBV	• Biological: EBV gp350-Ferritin Vaccine • Other: Matrix-M1	Phase 1	18 Years to 29 Years (Adult)
NCT03146403	Maintenance Dose Study of GEN-003 in Subjects With Genital Herpes Infection	Genital Herpes	• Biological: GEN-003 • Biological: Matrix-M	Phase 2	Child, Adult, Older Adult
NCT02905019	A Safety and Efficacy Study of R21 +/- ChAd63/MVA ME-TRAP	Malaria	• Biological: R21 with Matrix- M1 • Biological: ChAd63 ME-TRAP • Biological: MVA ME-TRAP	Phase 1 Phase 2	18 Years to 45 Years (Adult)

7.4 Summary of known and potential risks and benefits

7.4.1 Risk Assessment

The following section (Table 5) outlines the initial risk assessment and mitigation strategy for this study protocol.

Table 5. Risk Assessment and mitigation measures

Important potential/identified risk	Data/rationale for risk	Mitigation strategy
Investigational study product (R0.6C vaccine)		
Important potential risk: **First-in-human unknown risk**	There is no prior experience of R0.6C vaccine in human subjects.	Because of lack of in human experience, the vaccine will be administered with a staggered dose escalation step of two dosages (30 µg and 100 µg). Subjects will be observed closely by qualified clinicians, and emergency care will be immediately available to subjects. A minimum of 14 days is required between product administration to each group. Dose-escalation will be stopped if any of the safety holding criteria are met (section 9.5.5).
Important potential risk: **Hypersensitivity (including anaphylaxis)**	As with other vaccines, hypersensitivity and anaphylaxis to one or several components of the vaccine can rarely occur.	Subjects who report allergic reaction against constituents of the vaccine in the past will not be included. All vaccinated volunteers will be observed closely for at least 1 hour following administration of R0.6C with appropriate medical treatment readily available in case of severe adverse events.
Local and systemic reactogenicity to intramuscular administration of R0.6C	Generally, risks associated with vaccination include local inflammatory reactions to the injected product, such as pain and swelling at the injection site. Up to marked focal histopathological inflammation was reported in the animal toxicity studies, with partial recovery in recovery animals. Systemic effects generally associated with vaccination may include flu-like symptoms, fever,	Experienced and qualified medical personnel will administer the vaccination intramuscularly. Subjects will be informed about the local and systemic side effects that may occur and will be monitored closely for local and systemic adverse events. Subjects will be advised to inform or call the study doctor immediately if they experience any adverse events. Appropriate medication (e.g. paracetamol or antihistamines) for

	chills, nausea, gastrointestinal symptoms, headache, malaise, myalgia and arthralgia. No significant systemic adverse effects were observed in repeat dose animal toxicity studies.	symptomatic relief of any untoward side effects may be advised as necessary.
Study procedures		
Pain when taking blood samples	Because of the necessity to obtain frequent blood sampling for laboratory safety analysis, determining the concentration of R0.6C-specific antibodies, and measuring cellular immune responses, there is a risk of feeling faint, or experience mild pain, bruising, irritation or redness at the site where blood was taken. In rare cases arteries or nervous tissue may be injured or a punctured vessel may occlude and induce inflammation of the surrounding tissue.	Experienced and qualified medical personnel will draw blood. The amount of blood to be taken for sampling will not be harmful to the subject's health. Total blood volume taken over the whole study period will not exceed 500 mL.
Pregnancy risks		
Pregnancy and lactating females	Risks of the R0.6C vaccine to unborn babies are unknown at this time; pregnant females will be excluded from this study.	Females are only eligible for participation if they agree to use efficient contraception and the pregnancy test at the inclusion visit is negative. In case effective contraceptives are not available the clinical team may provide them. Volunteer who becomes pregnant during the trial will be withdrawn from any subsequent investigational product administration. Pregnant volunteers will not be withdrawn from the trial. Conditional on their agreement, they will be followed until the end of the pregnancy. Immunological and other exploratory blood samplings will be reduced to a minimum. Lactating females will be excluded from this study.
Risks to study personnel		

Needlestick injuries	The principal risk in the clinical setting is the handling of needles that may be contaminated with blood or body fluids and the associated risk of acquiring a blood-borne pathogen (including hepatitis B and C viruses and human immunodeficiency virus (HIV)).	Adherence to standard operating procedures (SOP) for working with infectious agents and universal precautions will reduce the risk of exposure. Subjects will be screened for hepatitis B and C and HIV prior to inclusion. Individuals positive for HIV, hepatitis B and C are excluded from the study.

7.4.2 Benefit assessment

There is no direct benefit for study subjects from participation in the trial. It will be made clear that the vaccine is experimental and is not protective against malaria. Subjects may indirectly benefit from general medical evaluation and health screening procedures including testing for HIV, hepatitis B, and hepatitis C. Subject will be informed about the results of the screening and if necessary, they will be referred to their primary physician where they will receive counselling and further medical attention earlier than if they did not know of their disease status. Subjects will receive a financial compensation which is reasonable and in line with Dutch common practice (see below).

7.5 Description and justification of route of administration and dosage

Enrolled participants will receive four intra-muscular injections of the vaccine in the deltoid muscle, on alternating sides. The administration site minimizes the risk that the product is injected into a blood vessel. In case of suspicion that a blood vessel is hit, the vaccine will not be administered. In case of trauma or anatomical abnormalities the same arm may be used for subsequent injections.
Each volunteer will receive four equal doses of the vaccine on days 0, 28, 56, and 168.The trial is designed to evaluate the safety and tolerability in with a staggered dose escalation step of two dosages (30 µg and 100 µg). The interval of 4 weeks between immunizations 1, 2 and 3 is the most commonly used interval between malaria vaccine administrations, but in this trial also chosen to align with the RTS,S vaccination scheme. An additional fourth vaccination is given on day 168 as a booster, as promising effects of this fourth, delayed dose were seen in a trial with the Pfs25 transmission blocking vaccine candidate[19]. The low dose in this protocol is based on pre-clinical data (see above), and similar to starting concentrations in other malaria protein vaccine trials[20, 21].

7.6 Dose preparation and administration

Dose preparation, as described in the Pharmacy Manual, will be carried out by qualified personnel. The investigational product will be administered by intramuscular injection in the deltoid muscle in 2 different doses, 30µg R0.6C for groups 1 and 2, and 100µg R0.6C for groups 3 and 4. R0.6C is formulated pre-adsorbed to Alhydrogel (100ug R0.6C per 500uL of

[200µg R0.6C/mL, 1.6 mg $Al(OH)_3$/mL). Each vial contains 0.8 mL vaccine. Matrix-M is formulated as 750 µL per vial at a concentration of 375 µg/mL.
In groups A, a volume of 150µL of R0.6C/Alhydrogel will be aspired from the vial for the dose of 30µg R0.6C (groups 1A+2A) and a volume of 500µL will aspired for the dose of 100µg R0.6C (groups 3A+4A).
For groups B a volume of 210 µL Matrix-M will be added to each vial containing 800 µL of R0.6C/Alhydrogel and mixed repeated by inversion by hand 10 times. Then 190 µL of R0.6C/Alhydrogel + Matrix-M formulation will be aspired for administration of the 30µg dose (groups 1B+2B). For the 100µg dose groups (groups 3B+4B) 630µL of R0.6C/Alhydrogel + Matrix-M formulation will be aspired for administration. Receipt and disposal of the investigational products will be done according to RUMC SOPs. All vials containing the study product R0.6C/Alhydrogel and Matrix-M, will be stored at 2-8°C, and storage temperatures will be monitored with validated temperature monitoring devices.

7.7 Preparation and labelling of Investigational Medicinal Product

Preparation and labelling of the investigational medicinal products will be done according to the relevant GMP guidelines. After the products are QP-released there will be no manufacturing steps. Dose preparation is described in the Pharmacy Manual.

7.8 Drug accountability

The drug product and adjuvants will be shipped to Radboudumc according to the relevant national and international regulations. The site pharmacist will maintain complete records of all study products received from the sponsor and an accurate record of the inventory, and an accountability record of R0.6C supplies for this study. The site pharmacist will also ensure the security of these documents. Partially used vials will not be used for human administration or for in vitro experimental studies. Unused or open products will be either returned to the sponsor or designee at the end of the vaccination period or destroyed according to the sponsor's instructions.

8. METHODS

8.1 Study endpoints

Primary safety Endpoints:

1) The number of serious adverse events and solicited and unsolicited grade 3 adverse events possibly, probably or definitely related to the vaccine in the period from first R0.6C administration up to 84 days after the last immunization.

Primary efficacy endpoints:

1) The functional transmission reducing activity in the standard membrane feeding assay of volunteer sera collected two weeks after the fourth R0.6C immunization (I4+14), compared to baseline (I1-1) within each of the four dose-adjuvant groups.

Secondary safety endpoints:

1) The number of solicited and unsolicited grade 1 and 2 adverse events possibly, probably or definitely related to the vaccine in the period from first R0.6C administration up to 84 days after the last immunization.

Secondary efficacy endpoints:

1) The TRA at other timepoints (I1+14, I2+14, I3+14, I3+111 [I4-1], and I4+84) compared to baseline (I1-1) in each of the four dose-adjuvant groups.
2) The anti-6C antibody quantity in volunteer sera collected two weeks after fourth R0.6C immunization (I4+14) and at other time points (I1+14, I2+14, I3+14, I3+111 [I4-1], and I4+84) compared to baseline (I1-1) in each of the four dose-adjuvant combinations, as determined by ELISA.

Exploratory Endpoints:

1) The functional TBA in the SMFA of volunteer sera collected at different time points compared to baseline (I1-1) in each of the four dose-adjuvant combinations.
2) The anti-6C antibody decay rate following R0.6C immunization for each of the four dose-adjuvant combinations.
3) The anti R0.6C and anti-R0 antibody quantity in volunteer sera collected at different time points in each of the four dose-adjuvant combinations.
4) The cellular immune responses in volunteers samples collected at different time points in each of the four dose-adjuvant combinations.
5) The difference in TRA between the two dose groups (30 or 100µg R0.6C) and/or between adjuvant groups (alhydrogel and alhydrogel+Matrix-M).
6) The difference in peak anti-6C antibody quantity between the two dose groups (30 or 100µg R0.6C) and/or between adjuvant groups (alhydrogel and alhydrogel+Matrix-M).

8.2 Randomisation, blinding and treatment allocation

This will be an open-label trial to enable appropriate safety evaluation prior to proceeding to the next dose group. Subjects may choose in which of the dose groups (group 1-4) they wish to be enrolled. The time of notification and treatment group will be documented in the electronic case report form (eCRF).
Randomization will be used only for the allocation to one of the two different adjuvant combinations (arms A or B). The volunteers will be allocated to one of the two adjuvant arms per group at random using a Mersenne-Twister random number generator implemented in R. Randomization tables and randomization cards will be generated using the package “blockrand”. Stratification will not be performed. An independent investigator at the Radboudumc, not involved in the clinical trial, is responsible for performing the randomization and for assigning volunteers according to the randomization cards. A second employee, not

involved in the assignment of volunteers, will check to see if randomization is done correctly. The randomization cards are kept in a fireproof clinical trial cabinet at Radboudumc.

8.3 Study procedures

8.3.1 Screening visit

The screening appointment for individual subjects is planned at least 72 hours after the subject has received the information sheet and the informed consent form. The purpose of the screening visit is to provide and clarify study information to the volunteer and to answer any questions the subjects may have, prior to obtaining informed consent and determining whether interested subjects are eligible for participation. The trial physician is responsible for providing the study information and performing the medical screening. A study nurse may draw venous blood, and measure vital parameters.
Upon arrival for the screening visit, the volunteer is asked to fill out a medical questionnaire. Subsequently, and prior to any screening activities, study staff will review the informed consent process with the volunteer. The possibility of withdrawal from the study, at any time and without any declaration of the reason, will be pointed out to the subjects. The subject will be required to pass a (short) written quiz about the study in order to ensure the study subjects has sufficient understanding of what participation entails.
The investigator, or a person designated by the investigator, will fully inform the volunteer of all pertinent aspects of the study and individual consent will be documented by a signature. Subjects may only participate in screening if they have signed the informed consent form. All subjects must consent to an HIV, hepatitis B and hepatitis C serological screening, urine toxicology and for females also a pregnancy test. Subjects who sign the informed consent will undergo screening. If certain elements of the screening procedures already indicate that a subject is ineligible for inclusion, screening procedures do not necessarily all have to be completed. The below activities will occur during the screening:

- Patient history: the answers to the medical questionnaire are discussed with the volunteer and clarified where needed. The subject will be further interviewed to collect demographic data, medical history including details of any chronic or recurrent medical and psychiatric conditions and use of adequate contraception;
- Study information: the study schedule and study rules are discussed with the subject and any questions are answered;
- A physical examination including vital signs, height and weight will be performed;
- Blood specimens will be collected for routine clinical laboratory testing of biochemical and hematological parameters, as well as HIV, hepatitis B and hepatitis C serological screening;
- A urine specimen will be collected for toxicology screening and for females a pregnancy test if the subject is of childbearing potential;
- Consent to inform the general practitioner (and if necessary a medical specialist) of study participation and request relevant information on medical history, will be signed

by the subject and sent after screening.

If physical examination, vital signs or laboratory values are out of the normal range, a repeat measure may be obtained as deemed necessary by the investigators. The medical history, physical examination, and laboratory findings for subjects will be recorded in the source screening data documents. All subjects will be asked to supply a phone number of a partner or roommate who may be contacted in case of emergency. Concomitant medications is recorded at all study visits.
All results of the screening will be reviewed with the subject. Subjects are informed in person or by phone if they have satisfied all the inclusion criteria. If clinically significant abnormalities are identified during screening, subjects will be referred to their primary health provider or appropriate medical center. If identified during the study, subjects may be asked to return to the study site for further evaluation, including clinical evaluation and repeat laboratory testing as warranted.

8.3.2 Medical history

The trial clinician will review the medical history of potential study subjects during the screening visit. The starting point will be a medical questionnaire the subjects have filled in before the screening. Particular attention will be paid to:

- Current or recent (within the previous two weeks) acute respiratory illness with or without fever, including symptoms associated with COVID-19;
- Recent receipt of vaccinations or other immune modulator therapy within the previous six months;
- Hypersensitivity of any kind;
- Clinically relevant history of cardiovascular, renal, gastrointestinal, hematological, dermatological, endocrine, neurological or immunological diseases;
- Known or suspected immunologic function impairment of any kind and/or known HIV infection, hepatitis B or hepatitis C infection;
- Mental illness;
- Tobacco, alcohol, or drug use;
- Medication use in the past 6 months;
- For women, pregnancy and contraceptive use and/or history of surgical sterility.

8.3.3 Physical examination

A complete physical examination will include the examination of general appearance, skin, neck, eyes, throat, lungs, heart, abdomen, back and extremities, and a routine vascular and neurological examination. Height (cm) and body weight (to the nearest 0.1 kilogram [kg] in indoor clothing, but without shoes) will also be measured, at screening only. Body mass index (BMI) will be calculated using the formula: BMI = Body weight (kg) / [Height (m)]2 and converted to an integer. During follow-up visits a focused physical examination will be performed if deemed necessary by the trial physician.

8.3.4 Vital signs

Vital signs including body temperature (degrees Celsius), blood pressure (BP, millimeters of mercury) and pulse measurements (beats per minute) will be recorded at the screening and inclusion visit. Additional measurements will take place at the discretion of the physician. Systolic and diastolic BP will be measured while the subject is sitting, with back supported and both feet placed on the floor, using an automated validated device, with an appropriately sized cuff. In case the cuff sizes available are not large enough for the subject's arm circumference, or in case of doubt of accuracy of the automated device a sphygmomanometer with an appropriately sized cuff may be used.
If vital signs are out-of-range at screening or inclusion, the investigator may obtain two additional readings, so that a total of up to three consecutive assessments are made, with the subject seated quietly for approximately five minutes preceding each repeat assessment. At least the last reading must be within the normal range in order for the subject to qualify. Temperature, blood pressure and pulse are measured as part of the physical examination at screening and inclusion. Additionally, subjects will be given a thermometer to measure their oral temperature on a daily basis for two weeks after each R0.6C administration, which they will collect in their study diaries.

8.3.5 Inclusion visit

Subjects meeting the eligibility criteria during screening (section 5.2 and 5.3) will be invited back for enrolment into the study at the inclusion visit, which will occur prior to the planned first administration. Baseline assessments will be taken on the inclusion day. For each subject, study start (day 0) will be defined as the day of first R0.6C administration. For subjects that do not show up for the inclusion visit, or subjects that do not continue to meet the eligibility criteria, alternate subjects may take their place. At study inclusion, the following activities will occur:

- Patient history will be repeated. Only subjects who still meet the inclusion criteria will be included to receive R0.6C vaccination;
- Blood specimens will be collected for routine clinical laboratory testing of biochemical and hematological parameters;
- A urine specimen will be collected for toxicology screening and for females a pregnancy test if the subject is of childbearing potential;
- Subject will be issued symptom diaries and a thermometer to record any local and systemic symptoms and medication use;
- All subjects will be issued an emergency notification card that details their participation in the study and provides contact phone numbers of the investigators.

8.3.6 Administration of R0.6C TBV candidate

Each enrolled subject will receive a total of four R0.6C vaccinations. The first vaccination will take place on day 0, the second on day 28 (+/- 3 days), the third on day 56 (+/- 3 days) and the last vaccination on day 168 (+/- 7 days). Subjects will be briefly assessed regarding any new medical events since the inclusion visit. The R0.6C vaccination will be prepared at bed-side by study staff (nurse, study physician) or by the pharmacy according to the study arm; arms A will receive R0.6C with Alhydrogel alone, arms B will receive R0.6C with Alhydrogel and Matrix-M. R0.6C will be administered intramuscular in the deltoid muscle on alternating sides. The site of injection will be recorded on the case report form. Subjects will be observed for at least 1 hour after R0.6C administration. A list of solicited local and systemic symptoms will be reviewed after this observation period. If the occurrence of an adverse event or use of medication is confirmed by the study physician, it is recorded in the eCRF. After leaving the hospital, subjects will be asked to examine the site of infusion and record local signs and symptoms for seven days including bruising, erythema, swelling or induration. Subjects will be asked to record any AEs in the memory aid booklet (also see section 8.3.9).

8.3.7 Follow up after R0.6C administration

Follow-up visits will be carried out on an outpatient basis at the study center. Subject will be asked to record local signs and symptoms in their memory aid booklet (also see section 8.3.9). The subject diary will be reviewed at each study visit and used as a base for discussion of possible local and systemic adverse events or medication use. In such cases, it may not be possible to obtain blood samples for non-safety endpoints.
If clinically significant (laboratory) abnormalities are identified during the study, subjects may be asked to return to the study site for further evaluation, including clinical evaluation and repeat laboratory testing as warranted. The trial physician can decide to initiate any additional diagnostics (including safety laboratory evaluations) at all times. For unexpected laboratory abnormalities, the laboratory test will be repeated.

8.3.8 Unscheduled visits

Subjects may need to present to the study center during operating hours for an unscheduled visit should they experience any AE that requires evaluation by the trial clinician. Data for any examinations performed on the subject at an unscheduled visit must be recorded in the eCRF. If an unscheduled visit is performed, the procedures for the next following visit should not be made earlier than scheduled above.

8.3.9 Patient reported outcomes (study diary)

At the inclusion visit, subjects will be issued symptom diaries. They will be asked to record all symptoms and medication use from the day of inclusion until end of study. Study staff will train subjects on use of memory aid booklets for self-assessment of solicited local and general adverse events through 7 days (day of R0.6C administration and subsequent 6 days) post- administration of R0.6C; and for self-assessment of adverse events and concomitant medications until day 84 after the last R0.6C administration.

The subject diary will be reviewed at each study visit and used as a basis for discussion of possible adverse events or medication use If the occurrence of an adverse event or use of medication is confirmed by the study physician, it is recorded in the subject's EPD and eCRF. At the end of the study, the diary will be collected and kept as source data.

8.3.10 Blood sampling and safety laboratory evaluations

During the study, blood samples will be drawn for screening, safety and research purposes. The blood sampling schedule in the flowchart (section 8.3.17) shows the maximum amounts of blood that will be drawn. Following universal precautions, blood will be collected by venipuncture into vacutainer tubes. Blood specimens will be affixed with coded labels that link the specimen to the subject, specimen type, specimen collection date, and time-point. The cumulative blood draw for each subject over the entire course of study participation is maximally 500mL.

Biological safety parameters will be measured at the central laboratory of the RUMC. In the case where a laboratory assessment is outside the reference range, a decision regarding whether the result is of clinical significance or not shall be made by the investigator and shall be based, in part, upon the nature and degree of the observed abnormality. The assessment may first be repeated for confirmation. All abnormalities will be documented in the source documents, including clinical considerations.

Hemoglobin, hematocrit, red blood cell count, white blood cell count with differential (e.g. neutrophils, basophils, eosinophils, monocytes, lymphocytes) and platelet count will be measured at regular time points during the study. Alkaline phosphatase, total bilirubin, gamma-glutamyltransferase (γGT), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), sodium, potassium, creatinine and urea will be measured at regular time points during the study. Glucose, triglycerides and cholesterol will be measured only at screening.

8.3.11 Urine Toxicology analysis

A midstream urine sample (approx. 30ml) will be obtained, in order to avoid contamination with epithelial cells and sediments, to allow proper assessment. A toxicology screening will be performed at screening and at baseline. A positive test for amphetamines and cocaine is a reason for exclusion. A positive test for cannabis on the inclusion day prior to R0.6C administration will also be a reason for exclusion.

8.3.12 Pregnancy Test

A midstream urine sample will be obtained and assessed by commercially available hCG urine tests. This test will be done at screening and inclusion.

8.3.13 Analysis of transmission reducing activity

Samples for SMFA will be collected from all subjects. Sera and/or purified IgG prepared from each serum will be used in the SMFA. Samples will be frozen until SMFA is planned. SMFA assays will be performed at Radboudumc (The Netherlands), using a qualified

standard membrane feeding assays using serum from subjects in cultures with *P. falciparum* and *Anopheles stephensi* mosquitoes.

8.3.14 Immunological assays: ELISAs/ PBMCs collection

Blood samples will be taken for isolation of peripheral blood mononuclear cells (PBMCs) and serum (see chart 8.3.17). PBMCs and sera will be frozen and can then be used by the Radboudumc or its collaborators for exploratory immunological assays to further analyse functionality of the immune response during and after R0.6C vaccinations. An ELISA assay will be used to measure circulating anti-R0.6C antibody titres in the subjects' sera at XX time points as shown in section 8.3.17, (sub)class and functionality (e.g. complement binding) may also be determined. In order to assess (antigen specific) T cell responses, the HLA-type of volunteers may be determined.
Samples that are not used in aforementioned immunological and functional assays will be stored up to 15 years and may be used for future (immunological) research relevant in the field of malaria vaccination.

8.3.15 Case report forms and data collection

All data collected by the investigators is registered in electronic case report forms. The investigator's notes are collected per subject in the coded trial EPD and are considered source data. Since all subjects will be healthy, there is no personal medical file for the study subjects, with exception of the medical file in case of adverse events/reactions resulting in a medical consultation or hospitalization. In this case the medical file will also be considered as source data. The diaries, produced by the study volunteers are also considered source data and will be kept as source documents.

8.3.16 COVID-19 related measures

With regards to the COVID-19 pandemic, the current Radboudumc guidelines will be followed. During the study period subjects will be instructed to call the trial physicians at any time if they experience severe symptoms or symptoms possibly related to COVID-19. SARS-CoV2 testing will be performed in accordance with current Radboudumc-wide guidelines. Follow-up visits not requiring blood collection for safety endpoints may be carried out by telephone, or at home, instead of at the clinical research center for subjects with suspected or confirmed SARS-CoV2 infection, until such time as they are considered no longer (potentially) contagious in accordance with current Radboudumc-wide guidelines. Vaccinations against the novel coronavirus SARS-CoV2 should preferably not take place within 30 days prior to or 14 days after each R0.6C vaccination.

8.3.17 Schedule of trial visits and measurements

Study Day[1]	I-120 to I1 -1	I1-1	I1	I1+1	I1+2	I1+7	I1+14	I2-1	I2	I2+1	I2+2	I2+7	I2+14	I3-1	I3	I3+1	I3+2	I3+7	I3+14	I3+28	I3+56	I4-1	I4	I4+1	I4+2	I4+7	I4+14	I4+28	I4+56	I4+84
Days after first R0.6C administration	N/A	N/A	0	1	2	7	14	27	28	29	30	35	42	55	56	57	58	63	70	84	112	167	168	169	170	175	182	196	224	252
Visit No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Informed consent	●																													
Inclusion/exclusion criteria	●	●																												
Demographic data and medical history	●																													
Physical examination[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]	●[2]
Height and weight measurement	●[2]																													
Blood pressure, temperature, pulse[3]	●	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]	●[3]
Record concomitant medication	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
HIV, HBV, HCV	●																													
Pregnancy test (urine)	●	●																												
Drugs screening (urine)	●	●																												

Study Day[1]	I-120 to I1 -1	I1-1	I1	I1+1	I1+2	I1+7	I1+14	I2-1	I2	I2+1	I2+2	I2+7	I2+14	I3-1	I3	I3+1	I3+2	I3+7	I3+14	I3+28	I3+56	I4-1	I4	I4+1	I4+2	I4+7	I4+14	I4+28	I4+56	I4+84
Distribution of emergency card		●																												
Distribution of diary		●																												
R0.6C administration			●						●						●								●							
Solicited local AEs (days 0–7)			●	●	●	●			●	●	●	●			●	●	●	●					●	●	●	●				
Solicited general AEs (days 0-14)			●	●	●	●	●		●	●	●	●	●		●	●	●	●	●				●	●	●	●	●			
Unsolicited AEs (days 0-84)		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Complete blood count[4]	●	●		●	●	●	●	●		●	●	●	●	●		●	●	●	●	●		●		●	●	●	●	●		●
Biochemistry tests[5]	●	●		●	●	●	●	●		●	●	●	●	●		●	●	●	●	●		●		●	●	●	●	●		●
PBMC isolations and citrate plasma		●																	●								●			
Serology/ELISA		●					●						●						●		●[7]	●					●		●[7]	●
SMFA[8]		●					●						●						●			●					●			●
Safety Report[9]						●[8]																								●[8]
End of study visit																														●
Daily blood volume (ml)	12	63	0	5	5	5	31	5	0	5	5	5	15	5	0	5	5	5	31	5	4	31	0	5	5	5	47	5	4	31
Cumulative blood volume (ml)	12	75	75	80	85	90	121	126	126	131	136	141	156	161	161	166	171	176	207	212	216	247	247	252	257	262	309	314	318	349

1. Study days are represented as relative to the immunisation day. If an immunisation day takes place on a different day (+/-3 days is allowed for immunisation 2 and 3, +/-7 days is allowed for immunisation 4) the study visits related to that immunisation will shift accordingly. Moreover, following any given immunisation, for visits I#+7,I#+14 and I#+28 a relative window of +/-2 days is

allowed, for visits I#+56 and I#+84, a relative window of +/-10 days is allowed.

2. A physical examination including height and weight and vital parameters will be performed at the screening visit. On other visits, measurements may take place at the discretion of the physician.
3. Vital signs including body temperature, blood pressure and pulse will be recorded at the screening and inclusion visit. On other visits, measurements may take place at the discretion of the physician.
4. CBC test includes: hemoglobin, hematocrit, platelets, red blood cell count, MCV, white blood cell count + differentiation
5. Biochemistry test includes: creatinine, urea, sodium, potassium, bilirubin, AF, yGT, AST, ALT and LDH. Additional at screening: serology malaria (2mL), cholesterol, triglyceride and glucose (2mL).
6. For PBMC isolations and citrate plasma collection, timepoints I2+14, I4-1 and I4+84 are optional.
7. For serum samples collected at timepoints I3+56 and I4+56, +/-10 days is allowed.
8. SMFA assays will be performed with serum collected on minimally 3 time points (I1-1, I3+14 and I4+14). SMFA assays for other timepoints may not be performed if the results of I3+14 and I4+14 do not show significant transmission reduction compared to baseline (I1-1).
9. Safety report: A safety report will be prepared by the clinical investigator before initiation of a next group (2, 3 and 4), minimally 7 days after the first R0.6C administration in the preceding group. A safety report of all the safety data is made after the last study visit of group 4.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any penalty or loss of medical benefits. Subjects can also be withdrawn from the study procedures at the discretion of the clinical investigator or the local safety monitor for urgent medical reasons or if exclusion criteria are met. The following reasons may lead to withdrawal of individual subjects:

- Withdrawal of informed consent by volunteer;
- Any serious adverse event;
- Any adverse event that, according to clinical judgment of the investigator, is considered as a definite contraindication to proceeding with the study procedures;
- Immunosuppressant or other immune-modifying drugs administered chronically (i.e., more than 14 days in total) during the study period. Topical steroids are allowed;
- Immunoglobulin and/or any blood products administered during the study period;
- Pregnancy;
- Completely lost to follow-up;
- Ineligibility (arising during the study or retrospectively, having been overlooked at screening);
- If the investigator or safety monitor believes that continuation would be detrimental to the subject's well-being;
- Volunteer non-compliance with study requirements;
- Any other protocol deviation that results in a significant risk to the subject's safety.

For subjects lost to follow-up (i.e. those subjects whose status is unclear because they fail to appear for study visits without stating an intention to withdraw), extensive effort (i.e. documented phone calls and e-mails) will be undertaken to locate or recall the volunteer or at least to determine his or her health status. The investigator should show "due diligence" by documenting in the source documents steps taken to contact the subject. In case of premature withdrawal for any reason, the investigator will exert his/her best effort to:

- Update any ongoing AE/SAEs that remained ongoing at the time of the subject's last visit prior to withdrawal.
- Determine if the subject has had any reaction or AE since the last visit. Where possible, the investigator should visibly or physically assess any reported adverse reaction or AE and document whether it led to the withdrawal.
- Collect blood for biochemical and hematological clinical laboratory parameters.
- Review the subject's memory aid if it is still in use at the time of withdrawal.
- Document the reason for premature withdrawal on the CRF.

8.5 Replacement of individual subjects after withdrawal

If an assigned subject does not present on the day of first R0.6C administration or elects to withdraw the consent on the day of administration, one of the reserve subjects will replace the subject. After subjects have received R0.6C administration they cannot be replaced.

8.6 Follow-up of subjects withdrawn from treatment

A subject may end his or her participation in the study and still be followed up for safety, unless the subject chooses to have complete withdrawal of the consent for further participation in any trial procedures. If a subject chooses to withdraw from the study, the investigator will make a reasonable effort to determine the reason for the subject's withdrawal and complete the study termination eCRF.

8.7 Premature termination of the study

The study may be discontinued by the sponsor:

- On advice of the safety monitor
- On advice of the Safety Monitoring Committee (SMC)
- On advice of the clinical investigator
- On advice of the METC

The investigators, local safety monitor, SMC, METC or Sponsor may decide to put the study on hold based on adverse events, pending discussion with the Sponsor, SMC, METC, local safety monitor or investigators. Following discussion, it may be decided to terminate the study.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded. Abnormal laboratory findings (e.g. clinical chemistry or hematology) or other abnormal assessments that are judged by the investigator to be clinically significant will be recorded as AEs (or SAEs if they meet the definition). The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or any other abnormal assessment is clinically significant. If there are any severe complaints, the volunteer will be evaluated immediately by a qualified clinician using the appropriate clinical assessments according to standard hospital care.

9.2.2 Adverse event data collection and recording

Adverse events will be collected at all follow-up visits, using the memory aid booklets as a basis (see section 8.3.9), and whenever a subject reports signs or symptoms to the trial physician between visits.

After administration with R0.6C the following systemic signs and symptoms will be solicited until 14 days after administration:
Fever, headache, myalgia, fatigue, chills, and rash. The time points that these symptoms are solicited are indicated in section 8.3.17.

After the administration of R0.6C the following local signs and symptoms will be solicited until 7 days after administration:
Pain, itching, swelling, induration and redness at injection site. The time points that these symptoms are solicited are indicated in section 8.3.17.

If known, the trial clinician will record the diagnosis (i.e., disease or syndrome) rather than component signs, symptoms and laboratory values. If the signs and symptoms are considered unrelated to an encountered syndrome or disease they should be recorded as individual AEs. If a primary AE is recorded, events occurring secondary to the primary event should be described in the narrative description of the case (e.g. primary AE = Orthostatic hypotension; secondary event may be fainting, head trauma, etc.). In case of hospitalizations for surgical or diagnostic procedures, the pre-existing condition should be recorded as the SAE, not the procedure itself.
All adverse events/reactions (solicited and unsolicited) will be accurately documented in the case report form by the investigators. For each event/reaction the following details will be recorded:

1. Description of the event/reactions (ICD-10 terminology will be included in the eCRF)
2. Duration (date and time of occurrence and end date and time)
3. Intensity (see explanation directly below)
4. Relationship with the intervention (see section 9.2.3)
5. Action taken, including treatment

The intensity of the symptoms will be ranked as (1) mild, (2) moderate, (3) or severe, according to the following scale:

Mild (grade 1):	awareness of symptoms that are easily tolerated and do not interfere with usual daily activity
Moderate (grade 2):	discomfort that interferes with or limits usual daily activity
Severe (grade 3):	disabling, with subsequent inability to perform usual daily activity, resulting in absence or required bed rest

If an adverse event changes in intensity during the specified reporting period, a new description of the adverse event will be added. Interrupted AEs are registered as one AE if the interruption is <24 hours. When an AE/SAE occurs, it is the responsibility of the investigators to review all documentation (e.g. hospital progress notes, laboratory, and diagnostics reports) related to the event. The investigators will then record all relevant information regarding an AE/SAE on the CRF or SAE Report Form, respectively.

9.2.3 Assessment of causality

The investigators are obliged to assess the relationship between study procedures and the occurrence of each AE/SAE. The investigators will use clinical judgment to determine the relationship. Alternative causes, such as natural history or the underlying diseases, concomitant therapy, other risk factors and the temporal relationship of the event will be considered and investigated. The relationship of the adverse event with the study procedures will be categorized as:

Definitely: administration of the IP is the cause, another etiology causing the adverse event is not known.

Probable: administration of the IP is the most likely cause: however, there are alternative reasonable explanations, even though less likely.

Possible: there is a potential association between the event and administration of the IP, however, there is an alternative etiology that is more likely.

Unlikely: a relationship to the administration of IP is unlikely, however, it cannot be ruled out.

Not related: a relationship to the administration of the IP cannot be reasonably established; another etiology is known to have caused the adverse event or is highly likely to have caused is.

When a regulatory authority requests a binary classification (related vs. unrelated), definitely, probably and possibly related are considered to be “related”, while not related and unlikely related are considered to be “unrelated”. Thus, an intervention-related AE refers to an AE for which there is a possible, probable or definite relationship to the study intervention. The investigator will use clinical judgment to determine the relationship.

9.2.4 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that:

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients’ hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event. The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events (within 24 hours). The sponsor will report all SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.2.5 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered. Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see section 9.2.4);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
   - Summary of Product Characteristics (SPC) for an authorised medicinal product;
   - Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern. The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority. The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States. The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report. As this is an open label study in which the sponsor, investigator and the SMC are not blinded, the code would not have to be broken in the case of a SUSAR.

9.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.
This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
SAEs need to be reported till end of study within the Netherlands, as defined in the protocol. After termination of the trial, the investigator should assure that the subject is referred for medical follow-up, as appropriate.

9.5 Safety Monitoring Committee

An independent SMC composed of three independent individuals will be appointed. The SMC will include a local safety monitor, and two experts nominated by the PI. The SMC will be established for the purpose of monitoring the study and to provide independent, non-binding advice on safety and ethics. The responsibilities and procedures of the SMC members are defined in the SMC Charter.
The advice(s) of the SMC will only be sent to the sponsor of the study. Should the sponsor decide not to fully implement the advice of the DSMB, the sponsor will send the advice to the reviewing METC, including a note to substantiate why (part of) the advice of the SMC will not be followed.

9.5.1 Local safety monitor

For this study, a local safety monitor will be appointed, who will be involved in the review of severe and serious adverse events and volunteer safety. He/she is independent of the sponsor and the investigator. The local safety monitor is notified of all grade 3 adverse events probably or definitely related to R0.6C administration and persisting at grade 3 for >48 hours.

9.5.2 Safety Meetings

The SMC will review safety data at pre-defined time points throughout the study, specifically: prior to commencing the first administration in the subsequent dose group (and ≥7 days of after the first R0.6C-administration of all subjects in the preceding dose-group). A minimum of two SMC meetings must take place: a meeting prior to initiation of the first 100µg R0.6C dose group (group 3), and a meeting after completion of follow up in all groups. The chair of

the SMC will determine for the other time points whether a meeting will be held, or whether a recommendation from all members may be formalized through e-mail. The frequency of these reviews may be adapted upon SMC recommendation if deemed necessary. An ad hoc SMC meeting may be convened at any time or at the request of the PI or local safety monitor to review safety data from subjects and/or groups who meet any of the holding rules as specified in the protocol (section 9.5.5) and SMC charter. If desirable, the SMC can have an additional meeting before the start of the trial, to discuss the protocol, the trial, future meetings and to have the opportunity to clarify any aspects with the sponsor.

9.5.3 Safety Reports

Safety reports will be prepared by the clinical investigator for review by the committee prior to each review. These reports will provide at a minimum the following information:

- Accrual data and subject status data with regard to completion of/discontinuation from the study.
- Summaries of solicited AEs, classified by severity.
- Unsolicited AEs (including SAEs), categorized by ICD-10 coding, severity and relatedness to study vaccine.
- Safety laboratory test results outside of normal institution reference ranges and considered clinically significant, classified by severity grading scale (irrespective of whether assessed as AEs).
- Any new or updated AEs that have met the holding rules.

The SMC will review the safety data within 2 working days. The SMC will summarize their recommendations to the study Sponsor as to whether there are safety concerns and whether the study should continue without change, be modified, or be terminated. If at any time a decision is made to permanently discontinue administration of study R0.6C administrations in all subjects, the Sponsor will notify the CCMO expeditiously.
The advice(s) of the SMC will only be sent to the Sponsor of the study. Should the Sponsor decide not to fully implement the advice of the SMC, the Sponsor will send the SMC advice to the CCMO, including a note to substantiate why (part of) the advice of the SMC will not be followed.

9.5.4 Inter-group progression

Progression to the next dosage group will depend in all cases upon a positive review by the SMC of safety data of the previous dosage group. The data to be reviewed before each dose group includes the safety data (solicited and unsolicited AEs through day 7 post R0.6C

administration) and clinically significant laboratory tests collected at 1, 2 and 7 days following R0.6C administration for all subjects in the specified dose group. The SMC will review the AEs (local, general, and laboratory) and will make recommendations regarding the continuation of the study for each group.

9.5.5 Safety holding rules

The study may be placed on safety hold at any time for the following reasons:

- On advice of the safety monitor;
- On advice of the Principal/Clinical investigators;
- On advice of the SMC;
- On advice of the CCMO;
- If holding rules are met (see below).

If the following holding rules are met, administration of R0.6C will be held for all remaining administrations in that dosage group and subsequent groups:

- One or more participants experience a SAE that is determined to be at least possibly related to the administration of R0.6C
- Two or more subjects experience a grade 3 adverse event (local, clinical systemic or laboratory systemic) possibly, probably or definitely related to R0.6C administration and persisting at grade 3 for >72 hours

If considered necessary by the investigators or SMC, remaining administrations in lower dosage groups may also be put on hold.

The study site member first aware of the event meeting the holding rule will notify the Principal Investigator and the Local Safety Monitor. The PI will alert the appropriate parties. The SMC will be notified within 24 hours. An ad hoc SMC review will be performed. The following considerations must be discussed:

- Relationship of the AE or SAE to the study product
- Relationship of the AE or SAE to R0.6C dose
- Relationship of the AE or SAE to (one of) the adjuvants
- If appropriate, additional screening or laboratory testing is provided to other subjects to identify subjects who may develop similar symptoms
- If any study related SAE is not listed on the current informed consent form (ICF), the PI will revise the ICF and subjects will be asked to provide consent on the new ICF

R0.6C administration to subjects within the affected group and to the next (higher dosage) group may resume only if the local safety monitor, PI, SMC and the sponsor agree it is safe

to resume R0.6C administration. It may be decided to proceed with only R0.6C adjuvated with Alhydrogel (groups A) or only R0.6C adjuvated with Alhydrogel and Matrix-M (groups B). If the CCMO has recommended safety hold, re-initiation of the study will require CCMO concurrence. The CCMO will be informed of a safety hold by the sponsor. Following discussion, it may be decided to terminate the study.

All subjects who have received the study product will be followed for safety until resolution or stabilization (if determined to be chronic sequelae) of their AE(s). If at any time a decision is made to discontinue administration of study product in all subjects, expeditious notification will be provided by the Sponsor to the CCMO within 24 hours. The PI, local safety monitor, SMC or CCMO may stop or suspend the use of this product at any time.

10. STATISTICAL ANALYSIS

All data will be collected and verified prior to analysis. All data will be analysed in accordance with the Statistical Analysis Plan. Final analyses of all data will occur after study completion and final verification of data according to GCP. All data analyses will be conducted using IBM SPSS, R, SAS or Graphpad using the latest version available. Detailed statistical procedures, listings, table shells, and figures will be provided in a SAP prior to analysis. The SAP will be finalized before study close-out and database lock. The following key statistical components will be considered and a detailed description will be documented in the SAP:

- Primary and secondary endpoints and how they will be measured
- Statistical methods and tests that will be used to analyze the endpoints
- Strategy that will be used if the statistical test assumptions are not satisfied
- Indication of whether the comparisons will be using one-tailed or two-tailed *t*-test (with justification of the choice) and the level of significance to be used
- Identification of whether any adjustments to the significance level or the overall P-value will be made to account for any planned or unplanned subgroup analyses or multiple testing
- Specification of potential adjusted analyses and a statement with which covariates or factors will be included
- Planned exploratory analyses and justification of their importance
- Any subgroup effects with biological justification and support from within and outside the study

10.1 Primary study parameter(s)

The number of subjects enrolled, completed, or withdrawn will be summarized. Reasons for withdrawal, when known, will be provided. Demographic data will be summarized by descriptive statistics and will include total number of observations (n), mean, standard deviation (SD) and range for continuous variables, and number and percentages for dichotomous variables. The primary safety, and reactogenicity outcomes will include all subjects who meet the eligibility criteria, received the study product R0.6C, and for whom safety data are available.

Safety endpoints

For the safety analysis, data from all subjects who received at least one dose of R0.6C and for whom safety data are available will be included. All analyses will be descriptive. Data will be presented by dose, overall/dose and overall/subject. Results will be summarized by study group. The percentage of subjects with at least one local AE (solicited or unsolicited), with at least one general AE (solicited or unsolicited) and with any AE during the solicited follow-up period will be tabulated with exact 95% CI (two-sided). No multiplicity adjustment will be implemented in analysis. The same calculations will be performed for AEs rated as grade 3.

The percentage of subjects reporting each individual solicited local and general AE during the solicited follow-up period will be tabulated with exact 95% CI. The same tabulation will be performed for grade 3 AEs and for AEs with relationship to R0.6C administration. The reports of unsolicited AEs will be reviewed by a physician and will be categorized by ICD-10. The percentage of subjects with at least one report of unsolicited AE and reported up to 28 days after R0.6C administration will be tabulated with exact 95% CI. The same tabulation will be performed for grade 3 unsolicited AEs and for unsolicited AEs with a relationship to R0.6C administration. SAEs will be described in detail. Withdrawals due to AEs/SAEs will also be summarized. Vital signs which are outside of the normal range and clinically significant will also be listed in tables. The frequency of signs and symptoms will be compared between groups with the chi- square test or fishers exact test. Serious adverse events (SAEs) occurring at any point during the trial will be summarized and relatedness to vaccine will be assessed.
Any clinically important deviations in routine laboratory test results and/or vital signs as determined by the investigator will be listed. Isolated laboratory abnormalities will be reported as AEs if they are considered clinically relevant by the investigator. Vital signs which are considered clinically relevant by the investigator will be summarized. All adverse events will be listed by participant and will include details of onset time, duration, severity and relationship to the study product.

Efficacy endpoints
SMFA will be performed to determine TRA of R0.6C. Differences will be assessed by comparing mean values between time points using either a two-tailed student's t-test or a non-parametric equivalent. Paired tests will be used if pre-intervention values are compared with post-intervention values. For discrete variables (e.g. the number of positive assays), the chi-squared test or Fisher's exact test will be used (two-tailed).

10.2 Interim analysis

There are no statistical criteria for study termination in this clinical trial. Safety and reactogenicity data will be evaluated after each R0.6C administration before proceeding to the next group. A summary report of AEs will be provided to the SMC after all four groups have completed follow-up.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

This study will be conducted in accordance with the latest Fortaleza revision of the Declaration of Helsinki (2013), the Medical research Involving Human Subjects act (WMO), the ICH Good Clinical Practice, and local regulatory requirements. The investigators are responsible for obtaining all relevant ethical approvals of the protocol and any subsequent amendments in compliance with local law before the start of the study.

11.2 Recruitment and consent

As soon as the study is approved by the CCMO and competent authority, healthy subjects will be recruited to participate in the study. Advertisements will be placed in prominent places on university campuses and other public places as well as on the intranet of the University and social media. Furthermore, a Facebook page (link: http://www.facebook.com/malariavaccin) showing the advertisement text will be used to inform people about the trial. This brief advertisement will indicate a telephone number to call and an e-mail address for contact to request further information. It will furthermore indicate a website (www.malariavaccin.nl) which contains a form. This short questionnaire will be completed using the online form. When seemingly suitable subjects contact investigators via e-mail, telephone or the online form, they will be invited to an information meeting during which the study will be explained to them by the study investigator. Directly after the meeting they will be provided with documents to review at home (the information sheet, the informed consent form, the application form and the insurance text). During and after the meeting there will be time for questions. After this free discussion with the investigator, and any follow-up discussion if necessary, the volunteer will be given sufficient time to consider participation (also see section 8.3.1).

Subjects who are interested in participating will be asked to fill in the application form and will be invited to come for a screening visit. Eligible subjects may only be included in the study after providing written, CCMO- approved informed consent. Informed consent must be obtained before conducting any study-specific procedures (i.e. all of the procedures described in the protocol, including screening procedures). The process of obtaining informed consent should be documented in the subject source documents. During the screening visit, the questionnaire answers will be discussed and inclusion and exclusion criteria will be checked. In addition, a letter for the general practitioner will be signed and sent after screening. Again, the investigator will answer any questions the volunteer has. The possibility of withdrawal from the study, at any time, without penalty and without any

declaration of the reason will be pointed out to the subjects. The investigators will be responsible for providing adequate verbal and written information regarding the objectives and procedures of the study, the potential risks involved and the obligations of the subjects. Subjects will be informed that they will not gain health benefits from this study. Trainees or other students who might be dependent on the investigators or the study group will not be included in the study.

11.3 Benefits and risks assessment

Testing in human subjects remains the only reliable and convincing way to obtain information on the immunological responses that are important for protection against malaria. Explorative studies looking for new and complementary candidate malaria interventions are of paramount importance with the potential of large-scale application in endemic countries. Of course, the compelling need for new methods of malaria interventions needs to be balanced with the potential risks and discomforts for the subjects. Risks for subjects are related to administration with R0.6C TBV candidate. There are no direct benefits to participation in the trial for subjects. Subjects will be advised to take regular malaria chemoprophylaxis when travelling to malaria endemic areas in the future.

11.4 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO. The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

1. € 650.000,-- (i.e. six hundred and fifty thousand Euro) for death or injury for each subject who participates in the research;
2. € 5.000.000,-- (i.e. five million Euro) for death or injury for all subjects who participate in the research;
3. € 7.500.000,-- (i.e. seven million five hundred thousand Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the sponsor as ‘verrichter’ in the meaning of said Act in each year of insurance coverage.

11.5 Incentives

Enrolled subjects will receive up to €1305,- in compensation for their time and for the inconveniences of taking part in this study. These amounts are based on predefined criteria described in our department standard operation procedures (SOP):

- Full day visit (i.e. CHMI, immunization, vaccination) €125 / visit (4 immunizations)
- Brief visit (<1 hour) €25 / visit (25 visits)
- Bonus for study length €20 / month (9 months)

Travel expenses will not be additionally reimbursed, and compensation will not be provided to subjects who are not enrolled i.e. screen failures. Eligible subjects who are enrolled at the inclusion visit as back-ups, will be compensated 50,- Euros for each inclusion visit. If a subject withdraws from the study prior to completion, they will receive reimbursement proportional to number of visits they attended. These compensation amounts are reasonable and in line with Dutch common practice. In case of unexpected medical complications, there will be access to medical treatment with full costs covered by the insurance of Radboudumc.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

A data management plan will be developed prior to start of study describing data management activities from project set-up through data lock and transfer. Designated trial staff will enter the data required by the protocol into the electronic CRF (eCRF). All data should be recorded, handled and stored in a way that allows its accurate reporting, interpretation and verification. An external monitor will review the data entered into the eCRFs by investigational staff for completeness and accuracy and will instruct the site personnel to make any required corrections or additions. Queries are made during each monitoring visit. Designated investigator site staff are required to respond to the queries and confirm or correct the data.

12.2 Monitoring and Quality Assurance

Before study initiation, the protocol and eCRFs together with relevant SOPs will be reviewed by the sponsor, the investigators and their staff. During and after completion of the study, the data monitor will visit the site to check the completeness of records, the accuracy of entries on the eCRFs, the adherence to the protocol and to Good Clinical Practice and the progress of enrolment.

The investigator will maintain source documents for each subject in the study, consisting of case and visit notes containing demographic and medical information, laboratory data, subject's diaries, and the results of any other tests or assessments. All information on eCRFs must be traceable to these source documents in the subject's file. As with all parts of the eCRF, there is an audit trail in place to register every data entry. The investigator will also keep the original informed consent form signed by the subject (a signed copy is given to the subject).

Monitoring

The investigator will give the monitor access to all relevant source documents to confirm their consistency with the eCRF entries. According to the NFU risk classification system, this clinical trial has been classified as high risk. The monitor will perform full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria and documentation of SAEs. The recording of data that will be used for all primary and safety variables will be assessed for minimally 50% of included subjects.

To ensure that the study is conducted in accordance with ICH-GCP and regulatory requirements, monitoring responsibilities will be provided by the Radboud Technology Center Clinical Studies (RTCCS). A site initiation visit will be conducted prior to beginning of the study, and monitoring will be conducted at initiation, during, and at closeout of the study. During the course of the study, monitors will verify compliance to the protocol;

completeness, accuracy, and consistency of the data and study product accountability; and adherence to ICH-GCP and applicable regulations. As needed and when appropriate, the monitors will also provide clarifications and additional training to help the site resolve issues identified during the monitoring visit. As appropriate and informed by risk assessment, remote centralized monitoring activities may be considered in place of or to supplement onsite monitoring. These may include analysis of data quality (e.g., missing or inconsistent data), identification of data trends not easily detected by onsite monitoring, and performance metrics (e.g., screening or withdrawal rates, eligibility violations, timeliness, and accuracy of data submission).
The extent and frequencies of the monitoring visits will be described in a separate monitoring plan developed prior to study initiation. The investigator will be notified in advance of scheduled monitoring visits. The monitors should have access to all trial related sites, subject medical records, study product accountability, and other study-related records needed to conduct monitoring activities. RTCCS will share the findings of the monitoring visit, including any corrective actions, with the site investigator. The site PI and the monitors must cooperate to ensure that any problems detected in the course of these monitoring visits are resolved in a predefined timeframe.
To ensure the quality of clinical data for all subjects, source data verification will be performed on subject data received by RTCCS. During this review, subject data will be checked for consistency, omissions, and any apparent discrepancies. In addition, the data will be reviewed for adherence to the protocol and ICH-GCP. To resolve any questions arising from the source data verification process, data queries and/or site notifications will be sent to the site for resolution as soon as possible and within the period described in the monitoring plan; all queries must be resolved prior to database lock.
Essential documents must be filed in the site study file on an ongoing basis and be available for review by the RTCCS.

12.3 Amendments

Amendments are changes made to the research after a favourable opinion by the CCMO has been given. All amendments will be notified to the CCMO that gave a favourable opinion.

A ‘substantial amendment’ is defined as an amendment to the terms of the CCMO application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the CCMO and to the competent authority. Non-substantial amendments will not be notified to the CCMO and the competent authority, but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the CCMO once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The sponsor will notify the CCMO and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit. The sponsor will notify the CCMO immediately of a temporary halt of the study, including the reason of such an action. In case the study is ended prematurely, the sponsor will notify the CCMO and the competent authority within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the CCMO and the Competent Authority.

12.6 Public disclosure and publication policy

The study is entered by the sponsor into Clinicaltrials.gov registry and registered under NCT04862416. The final report will be prepared by the investigators at the Radboud university medical center. It will be signed by the project leader or the principal investigator. The investigators will make every effort to publish the results in a peer-reviewed journal.

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

a. Level of knowledge about mechanism of action

The R0.6C fusion protein is a chimera consisting of the 6-cysteine C-terminal fragment of PfS48/45 (6C) coupled to the N-terminal region of asexual stage Glutamate Rich Protein GLURP (R0) produced in *Lactococcus lactis*. Immunisation with R0.6C in rodents induces functional antibodies against the 6C subunit. Anti-6C antibodies (Abs) are ingested during the blood meal and can bind male sexual forms in the mosquito gut, preventing their fertilisation of female gametes and thus ookinete and oocyst development.

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

There is no in human experience with the R0.6C/AlOH and R0.6C/AlOH + Matrix-M TBV candidates. However, the GLURP-R0 region, which constitutes 76.7% of R0.6C, forms part of the malaria vaccine candidate, GMZ2, which has been tested in both European and African clinical trials as listed in section 7.3.

To date 994 individuals, of whom the majority are African children below 5 years of age, have been immunized with GMZ2/AlOH. The GMZ2/AlOH formulation has shown excellent safety and tolerability confirming the safety of the expression system and the safety of the GLURP-R0 portion of R0.6C.

The Matrix-M adjuvant technology [17] is a promising technology which has been explored for various infectious diseases and has a good safety profile in humans [18]. To-date Matrix-M has been utilized in more than 25+ clinical trials, including multiple Phase III trials (Table 4, section 7.3). In these studies, Matrix-M has been combined with multiple malaria vaccine candidates, influenza, covid-19, and other disease indications.

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

Immunisation with R0.6C in rodents induces functional antibodies against the 6C subunit, the sera of vaccinated animals were able to induce >99% transmission blocking activity in the standard membrane feeding assay (SMFA) with cultured gametocytes[7]. Anti-6C antibody titres were further increased by immunising with R0.6C adjuvanted with Alhydrogel or Alhydrogel and Matrix-M[8].

d. Selectivity of the mechanism to target tissue in animals and/or human beings

The recombinant protein, R0.6C, is based on the genome of P. falciparum. The amino acid sequence of both R0 (GLURP) and 6C (Pfs48/45) was searched (BLAST) against the genome of homo sapiens (TAXID9606) for an in silico safety assessment of cross-reactivity. In those searches, 6C showed no homology to any human protein, and R0 demonstrated no significant homology to human proteins.

Humans have been exposed to the GLURP-R0 region and the Pfs48/45-6C domain since Plasmodium falciparum was first transmitted to humans. There are no recognized autoimmunity related to antibodies elicited by natural exposure in endemic populations suggesting that anti-R0 and anti-6C antibodies do not cross react with human epitopes. Since R0.6C is strongly recognized by the reduction sensitive mAb45.1, it is highly likely that recombinant Pfs48/45-6C adopts its native fold and primarily elicit antibodies against native epitopes of Pfs48/45. Moreover, the GLURP-R0 region, which constitute 76.7% of R0.6C, forms part of the malaria vaccine candidate, GMZ2, which has been tested in both European and African clinical trials (also see section 7.3). The GMZ2/AlOH formulation has shown excellent safety and tolerability suggesting that the R0 portion of R0.6C elicits antibodies against native epitopes of GLURP and does not elicit cross-reactive antibodies against human protein sequences.

In a rabbit toxicology study of R0.6C-AlOH NZW Rabbits received a total of 4 IM doses of 100 µg R0.6 with or without 50 µg Matrix-M. There were no related mortalities, clinical signs, or effects on body weights or food consumption. Hematology, clinical chemistries, ophthalmology, gross pathology and organ weights were unaffected at any dose. Histopathological evaluation of a panel of tissues revealed no effects associated with treatment with R0.6C-AlOH with or without Matrix-M other than histopathology changes with marked focal inflammatory response at sight of injection. Partial recovery was recorded when comparing the site dosed 3 days prior to necropsy with the site dosed 46 days prior to necropsy. All animals in the two test article immunized groups (immunized with R0.6C-AlOH or R0.6C-AlOH + Matrix-M) showed significant and consistent antibody responses (≥ 409.600 fold dilution) at the terminal bleed (day 46 for main animals and day 71 for recovery animals).

e. Analysis of potential effect

The R0.6C dose administered in the rabbit toxicity study (100 µg/rabbit) translates to a human equivalent dose of 774 µg for a 60 kg human, providing, a safety margin of >7x higher than the highest dose to be administered in this first in human trial (100 µg).

f. Pharmacokinetic considerations

Biodistribution studies of R0.6C have not been conducted because the vaccine does not include whole/live parasite material and is not capable of replication or productive infection. The route of administration (IM) is well understood. Consistent with International Council for Harmonisation (ICH) guidelines, formal pharmacokinetic studies were not conducted.
The antibodies induced by the R0.6C TBV candidate will be evaluated in human subjects by analysis from human sera collected at several time points after R0.6C administration. See sections 8.1 and the schedule 8.3.17 for details.

g. Study population

Included subjects are healthy young adult subjects, who have been extensively screened for any evidence of co-morbidity including but not limited to: immune deficiency, hypersensitivity

and cardiovascular risk factors. Female subjects of child-bearing potential are screened for pregnancy by urine test and are required to use contraception throughout the study period.

h. Interaction with other products

R0.6C is not known to interact or be incompatible with any other products.

i. Predictability of effect

The SMFA will be used to evaluate the transmission reducing abilities of the R0.6C TBV candidate. SMFA is the most widely used assay to determine the TRA and an accepted surrogate of TRA for public health interventions [5]. Laboratory reared Anopheles mosquitoes are fed through membrane feeders with in vitro cultured gametocytes to which serum or purified anti-R0.6C antibodies in various concentrations are added. Subsequently, the oocyst density in mosquitoes are compared between the experimental and control group by means of microscopy of the mosquito gut, parasite DNA detection or immune-assays [5, 9]. The SMFA can also be used to determine the transmission blocking activity (TBA) by comparing the number of infected mosquitoes between the experimental and control group.
Because the mode of action of TBVs is so explicit, there is a highly informative endpoint in the SMFA that is a clear indicator of later public health impact. For other vaccines, there is no such clear in vitro assay. The TRA measured in the SMFA assay around which there is confidence that blocking activity is present was set by consensus at 80% reduction in oocyst intensity, however even TBVs with a TRA of less than 80% could eliminate plasmodium at low transmission levels over consecutive transmission seasons[10].

Additionally, anti-6C, anti-R0 and anti-R0.6C antibody quantities will be measured by ELISA from subject sera collected at pre-specified time points (also see section 8.1 and 8.3.17).

j. Can effects be managed?

Potential mild-moderate local or systemic adverse reactions to immunization will be managed symptomatically (e.g. paracetamol, ibuprofen, antihistamines). In the event of an anaphylactic reaction, this will be managed according to hospital-wide emergency protocols.

13.2 Synthesis

Please see section 7.4 for a summary of known and potential risks and benefits that includes mitigation strategies.

14. REFERENCES

1. Ashley, E.A., et al., *Spread of artemisinin resistance in Plasmodium falciparum malaria.* N Engl J Med, 2014. **371**(5): p. 411-23.
2. The mal, E.R.A.C.G.o.V., *A Research Agenda for Malaria Eradication: Vaccines.* PLOS Medicine, 2011. **8**(1): p. e1000398.
3. *World malaria report 2019*. 2019, Geneva: World Health Organisation.
4. Gallup, J.L. and J.D. Sachs, *The economic burden of malaria.* Am J Trop Med Hyg, 2001. **64**(1-2 Suppl): p. 85-96.
5. Sauerwein, R.W. and T. Bousema, *Transmission blocking malaria vaccines: Assays and candidates in clinical development.* Vaccine, 2015. **33**(52): p. 7476-82.
6. Targett, G.A. and B.M. Greenwood, *Malaria vaccines and their potential role in the elimination of malaria.* Malaria Journal, 2008. **7**(1): p. S10.
7. Singh, S.K., et al., *Construct design, production, and characterization of Plasmodium falciparum 48/45 R0.6C subunit protein produced in Lactococcus lactis as candidate vaccine.* Microb Cell Fact, 2017. **16**(1): p. 97.
8. Singh, S.K., et al., *A Reproducible and Scalable Process for Manufacturing a Pfs48/45 Based Plasmodium falciparum Transmission-Blocking Vaccine.* Frontiers in Immunology, 2021. **11**(3369).
9. Bousema, T. and C. Drakeley, *Epidemiology and infectivity of Plasmodium falciparum and Plasmodium vivax gametocytes in relation to malaria control and elimination.* Clin Microbiol Rev, 2011. **24**(2): p. 377-410.
10. Blagborough, A.M., et al., *Transmission-blocking interventions eliminate malaria from laboratory populations.* Nature Communications, 2013. **4**(1): p. 1812.
11. Rabinovich, R.N., et al., *malERA: An updated research agenda for malaria elimination and eradication.* PLOS Medicine, 2017. **14**(11): p. e1002456.
12. Griffin, J.T., et al., *Reducing Plasmodium falciparum Malaria Transmission in Africa: A Model-Based Evaluation of Intervention Strategies.* PLOS Medicine, 2010. **7**(8): p. e1000324.
13. Singh, S.K., et al., *A Plasmodium falciparum 48/45 single epitope R0.6C subunit protein elicits high levels of transmission blocking antibodies.* Vaccine, 2015. **33**(16): p. 1981-6.
14. Esen, M., et al., *Safety and immunogenicity of GMZ2 - a MSP3-GLURP fusion protein malaria vaccine candidate.* Vaccine, 2009. **27**(49): p. 6862-8.
15. Belard, S., et al., *A randomized controlled phase Ib trial of the malaria vaccine candidate GMZ2 in African children.* PLoS One, 2011. **6**(7): p. e22525.
16. Sirima, S.B., et al., *A phase 2b randomized, controlled trial of the efficacy of the GMZ2 malaria vaccine in African children.* Vaccine, 2016. **34**(38): p. 4536-42.
17. Bengtsson, K.L., et al., *Matrix-M adjuvant: enhancing immune responses by 'setting the stage' for the antigen.* Expert Rev Vaccines, 2013. **12**(8): p. 821-3.
18. Shinde, V., et al., *Improved Titers against Influenza Drift Variants with a Nanoparticle Vaccine.* N Engl J Med, 2018. **378**(24): p. 2346-2348.
19. Talaat, K.R., et al., *Safety and Immunogenicity of Pfs25-EPA/Alhydrogel®, a Transmission Blocking Vaccine against Plasmodium falciparum: An Open Label Study in Malaria Naïve Adults.* PLOS ONE, 2016. **11**(10): p. e0163144.
20. Hu, J., et al., *Safety and immunogenicity of a malaria vaccine, Plasmodium falciparum AMA-1/MSP-1 chimeric protein formulated in montanide ISA 720 in healthy adults.* PLoS One, 2008. **3**(4): p. e1952.
21. Ellis, R.D., et al., *Phase 1 trial of the Plasmodium falciparum blood stage vaccine MSP1(42)-C1/Alhydrogel with and without CPG 7909 in malaria naïve adults.* PLoS One, 2010. **5**(1): p. e8787.