Life Sciences, a division of SGS Belgium NV

Medicines for Malaria Venture

REVISED CLINICAL STUDY PROTOCOL

A single centre, open label, pilot phase Ib study to investigate blood stage malaria infection after Direct Venous Inoculation of cryopreserved *Plasmodium falciparum* (NF54 strain) Sporozoites (*Pf*SPZ-DVI) in malaria naïve healthy adult volunteers

Product	Riamet® (artemether-lumefantrine)
Protocol Number	MMV_*Pf*SPZ-DVI Blood Stage_19_01
EudraCT Number	2019-004317-14
Clinical Phase	Ib
Clinical Indication	Acute Uncomplicated *Plasmodium falciparum* Malaria in Adults
Issue Date (Version)	24-Jun-2020 (Final 3.0) (Final Approved Protocol Amendment 1 dated 27-Jan-2020 [Final 2.0])

Sponsor	Medicines for Malaria Venture (MMV)
Sponsor Representative	Farouk Chughlay, MD Phone: +41 22 555 0355 Mobile: +41 79 123 4289 Fax: +41 22 555 0369

This study will be conducted in compliance with this protocol, the ICH Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and with the applicable regulatory requirement(s).

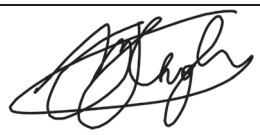
SIGNATURES

Signature of Sponsor Representative

Title: **A single centre, open label, pilot phase Ib study to investigate blood stage malaria infection after Direct Venous Inoculation of cryopreserved *Plasmodium falciparum* (NF54 strain) Sporozoites (*Pf*SPZ-DVI) in malaria naïve healthy adult volunteers**

Name: Farouk Chughlay, MD

'This Revised Clinical Study Protocol has been reviewed and approved by the Sponsor to ensure compliance with Good Clinical Practice.'

Signature: 

Date: 24 June 2020

Signature of Investigator

Title: **A single centre, open label, pilot phase Ib study to investigate blood stage malaria infection after Direct Venous Inoculation of cryopreserved *Plasmodium falciparum* (NF54 strain) Sporozoites (*Pf*SPZ-DVI) in malaria naïve healthy adult volunteers**

Name:	Pieter-Jan Berghmans, MD
Affiliation:	SGS Life Sciences, Clinical Pharmacology Unit
Address:	Lange Beeldekensstraat 267, 2060 Antwerpen, Belgium

'I have read this Revised Clinical Study Protocol and agree that it contains all information necessary for proper conduct of the study. I will carry out the study as outlined herein and will complete the study within the designated time.'

Signature: 

Date: 25 JUN 2020

PROTOCOL HISTORY

Protocol History[a]
Medicines for Malaria Venture (MMV) – MMV_PfSPZ-DVI Blood Stage_19_01

Document	Issue Date	Amendment Type	Comments
Final Approved Protocol [Final 1.0]	17-Dec-2019	Not applicable	-
Amendment 1 [Final 2.0]	27-Jan-2020	Substantial	Revised Clinical Study Protocol 2.0
Amendment 2 [Final 3.0]	24-Jun-2020	Substantial	Revised Clinical Study Protocol 3.0

[a] This overview only lists general amendments to the protocol. Site- and country-specific amendments to the protocol are not included.

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PROTOCOL AMENDMENT 2: SUMMARY OF CHANGES

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union.

Overall Reason for Amending the Protocol: This amendment was created to implement the Sponsor's safety measures in the protocol with regard to the coronavirus disease 2019 pandemic.

Changes are summarized below together with a rationale for each change.

1. Change in Exclusion Criteria

Rationale:

Per Sponsor's decision, the study exclusion criteria have been updated to exclude any subject who tests positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): since participants could experience flu-like symptoms following Direct Venous Inoculation of cryopreserved *Plasmodium falciparum*, for the safety of the participants, it is important to screen for SARS-CoV-2 infection prior to inoculation. Also before second confinement to the study site, participants should be tested for SARS-CoV-2 infection, and must be withdrawn from the study if the test result is positive.
Testing before first confinement to the study site (from the participants who already completed the other screening tests and are still eligible to be enrolled in the study) and before second confinement to the study site will be performed by taking a nasopharyngeal swab to detect presence of SARS-CoV-2 ribonucleic acid, using a real-time reverse transcription polymerase chain reaction (rRT-PCR) test.

Applicable Section(s)	Description of Changes
Synopsis	An additional exclusion criterion 24 was added to exclude any subject with SARS-CoV-2 infection.
Time and Events Schedule	The schedule was updated to reflect the additional visits which can take place up to Day -2 (before the first confinement to the study site) and on Day 7-9 (before the second confinement), on which the nasopharyngeal swabs will be taken.
Section 4.2	An additional exclusion criterion 24 was added to exclude any subject with SARS-CoV-2 infection.
Section 7.1.1	The text on the screening of participants was updated to reflect the need for SARS-CoV-2 testing during screening.
Section 7.1.2	The text was updated to reflect the need for SARS-CoV-2 testing before the second confinement to the study site.
Section 8.1	The text was updated to reflect the need for participant withdrawal from the study if he or she tests positive for SARS-CoV-2 before confinement to the study site.

2. Minor Corrections

Rationale:

Minor correction

Applicable Section(s)	**Description of Changes**
Synopsis	The text on the relationship of adverse events to malaria was updated. The text on coagulation testing was updated to align with the text on the criterion for modified Hy's law.
List of Abbreviations and Definitions of Terms	The list of abbreviations was updated.
Time and Events Schedule	The top rows of the flowchart were updated to clarify that Day -1 is not an actual part of the 'Challenge, treatment and follow-up' period. The footnotes on coagulation testing were updated to align with the text on the criterion for modified Hy's law. The schedule was updated to include extra International normalized ratio (INR) tests which will be performed post-baseline at the same time points as the post-baseline haematology and liver biochemistry tests.
Section 1.6.1	The text on total blood volume sampling was updated.
Section 5.1	The text on the relationship of adverse events to malaria was updated.
Section 7.1.1 and Section 7.1.2	The text on Day -1 was moved from Section 7.1.2 to Section 7.1.1 to clarify that Day -1 is not part of the treatment period.
Section 7.3.3	The text on coagulation testing was updated to align with the text on the criterion for modified Hy's law.
Section 7.6	The text on total blood volume sampling was updated.
Section 8.1	The text on the relationship of adverse events to the *Pf*SPZ-DVI Challenge agent and/or the antimalarial treatment was updated.
Section 10.1	Minor formatting corrections.
Section 10.3	The text on the relationship of adverse events to the *Pf*SPZ-DVI Challenge agent and/or the antimalarial treatment was changed: this relationship is now classified as Related or Not related. The term 'suspected' was removed from the category.
Section 10.7	The text was updated to clarify that Adverse Events of Special Interest must also be reported on the Serious Adverse Event Form.
Section 10.8	Induced abortion was removed from the list of abnormal pregnancy outcomes that are considered serious adverse events.

PROTOCOL AMENDMENT 1: SUMMARY OF CHANGES

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union

Overall Reason for Amending the Protocol: This amendment was created to make adaptations following review of the protocol by the Belgian Federal Agency for Medicines and Health Products (FAMHP).

Changes are summarized below together with a rationale for each change.

1. Change in Exclusion Criteria

Rationale:

The study exclusion criteria have been updated based on the Agency's recommendations to exclude any subject with hepatic disorders and in line with the Food and Drug Administration (FDA) Guidance pertaining to Drug-Induced Liver Injury (FDA, 2009[1]).

1. The hepatitis blood test panel at study screening has been significantly expanded to ensure a comprehensive assessment of the most common causes of infectious hepatitis disorders in Phase 1 populations prior to study inclusion. Given that infection with hepatitis D virus only occurs in people who are already infected with the hepatitis B virus (HBV), testing for hepatitis D virus will be performed exclusively in those participants who screen positive for HBV.

2. An exclusion criterion has been added to ensure that study participants with documented hepatic disorders other than common causes of hepatitis are not included (including but not limited to viral hepatitis, auto-immune hepatitis, non-alcoholic steatohepatitis [NASH], alpha-1-antitrypsin deficiency, alcoholic liver disease, primary biliary cholangitis [PBC], primary sclerosing cholangitis [PSC], hemochromatosis, Wilson disease or suspected hepatocellular carcinoma [HCC]).

Applicable Section(s)	Description of Changes
Synopsis	Exclusion criterion 6 was updated to expand the hepatitis blood test panel. An additional exclusion criterion 7 was added to exclude any subject with hepatic disease. Serological testing in the safety assessments was updated to reflect the expanded hepatitis blood test panel.
Time and Events Schedule	Footnote related to serological testing was updated to reflect the expanded hepatitis blood test panel.

[1] FDA Guidance for Industry Drug-Induced Liver Injury: Premarketing Clinical Evaluation, U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research (CBER), July 2009

Applicable Section(s)	Description of Changes
Section 4.2	Exclusion criterion 6 was updated to expand the hepatitis blood test panel. An additional exclusion criterion 7 was added to exclude any subject with hepatic disease.
Section 7.3.3	Serological testing was updated to reflect the expanded hepatitis blood test panel.

2. Change in Inclusion Criteria

Rationale:

The study inclusion criteria have been updated based on the Agency's recommendation that liver transaminases at screening should not exceed the upper limit of normal (ULN) (>1x ULN, instead of >1.25x ULN).

Applicable Section(s)	Description of Changes
Synopsis Section 4.1	Inclusion criterion 4 has been updated to reflect that transaminases should not exceed >1x ULN.

3. Addition of Clinically Acceptable Ranges for Clinically Important Study Inclusion Laboratory Tests

Rationale:

Based on the Agency's recommendation, the Clinically Acceptable Ranges for Clinically Important Study Inclusion Laboratory Tests (including total and conjugated bilirubin) have been added in Attachment 4 of the protocol to guide inclusion/exclusion of study participants.

Applicable Section(s)	Description of Changes
Section 4.1 Section 4.2	A reference to Attachment 4 was added.
Attachment 4	Clinically Acceptable Ranges for Clinically Important Study Inclusion Laboratory Tests have been added.

4. Change in Composition of Safety Review Team (SRT)

Rationale:

Based on the Agency's recommendation, and in line with the CHMP Guideline on data monitoring committees, an independent Early Phase and Clinical Pharmacology MD expert experienced in malaria volunteer infections studies was included as the chair of the SRT, in addition to the Medical Monitor, the Medical/Project Director, the Principal Investigator (PI) and an independent infectious disease/malaria expert.

Applicable Section(s)	Description of Changes
Synopsis Section 3.4	Addition of an independent Early Phase and Clinical Pharmacology MD expert experienced in malaria volunteer infection studies as SRT chair to the corrected SRT composition.

5. Change in Toxicity Criteria

Rationale:

Based on the Agency's recommendation, and in line with the European Medicines Agency (EMA) Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products, the toxicity criteria taken into account to progress from Cohort 1 to Cohort 2 were updated. The criterion on Riamet®-related AEs was changed from "two or more Riamet®-related severe (grade 3 or higher), same organ class AEs" to "two or more Riamet®-related severe (grade 3 or higher) AEs, independent of within or not within the same system organ class".

Applicable Section(s)	Description of Changes
Section 3.4.1	The toxicity criterion on Riamet®-related AEs was updated.

6. Minor Corrections

Rationale:

Minor corrections

Applicable Section(s)	**Description of Changes**
Study Administrative Structure and Investigators Section 12.5	Clinical Monitoring details were added.
Section 3.4 Section 3.4.1	The composition of the SRT was moved from Section 3.4.1 to the beginning of Section 3.4.
Synopsis Section 3.4 Section 5.1	'Malaria expert' or 'infectious diseases physician with expertise in malaria' was replaced by 'independent infectious disease/malaria expert'.
Synopsis Time and Events Schedule Section 7.3.2 Section 7.3.4	Temperature measurements were included into the vital signs part of the protocol because: - temperature measurement is required to assess the malaria clinical score - fever; but - the temperature also needs to be viewable as a data point for the medics.
Attachment 1	Timing of grading malaria clinical score was adapted.

PROTOCOL SYNOPSIS

Study Title	**A single centre, open label, pilot phase Ib study to investigate blood stage malaria infection after Direct Venous Inoculation of cryopreserved *Plasmodium falciparum* (NF54 strain) Sporozoites (*Pf*SPZ-DVI) in malaria naïve healthy adult volunteers**		
Product	Riamet® (artemether-lumefantrine)	**Clinical Phase**	Ib
Protocol Number	MMV_*Pf*SPZ-DVI Blood Stage_19_01	**Indication**	Acute Uncomplicated *Plasmodium falciparum* Malaria in Adults
EudraCT Number	2019-004317-14		

Sponsor	Medicines for Malaria Venture (MMV)
Sponsor Representative	Farouk Chughlay, MD
Clinical Centre	SGS Life Sciences, Clinical Pharmacology Unit, Lange Beeldekensstraat 267, 2060 Antwerpen, Belgium

Objectives and Endpoints:

OBJECTIVES	ENDPOINTS
Primary	
• To evaluate the safety and tolerability associated with blood-stage malaria infection in naïve healthy participants after inoculation of *Plasmodium falciparum* sporozoites through direct venous inoculation (*Pf*SPZ-DVI Challenge).	• The incidence and severity of observed (i.e., malaria signs and symptoms graded by the malaria clinical score) or self-reported adverse events (AEs) considered *Pf*SPZ-DVI Challenge inoculum-related until end of study (EOS) visit. • The change in malaria clinical score from *Pf*SPZ-DVI Challenge until parasite clearance (defined as a quantitative polymerase chain reaction [qPCR] value of 0 parasites per mL blood after initiating antimalarial therapy). • Changes from baseline in haematology, clinical chemistry and urinalysis parameters, vital signs and electrocardiogram (ECG) parameters.
• To characterise key stages in the blood-stage parasite growth profile in malaria infection in naïve healthy participants after *Pf*SPZ-DVI Challenge.	• The parasite growth profiles of *Plasmodium falciparum* (*P. falciparum*) NF54 after infection via injection of sporozoites by direct venous inoculation (DVI) will be characterised by: o time to first PCR positivity (i.e., time elapsed between *Pf*SPZ-DVI Challenge and first qPCR outcome equal to or greater than 250 parasites per mL blood, with a maximum of 28 days in the absence of parasitaemia); o parasitaemia at first PCR positivity; o time to parasitaemia of ≥5000 parasites per mL blood (Cohorts 1 and 2); o parasitaemia at the time parasitaemia ≥5000 parasites per mL blood (Cohorts 1 and 2); o time to parasitaemia of ≥10000 parasites per mL blood (Cohort 2); o parasitaemia at the time parasitaemia ≥10000 parasites per mL blood (Cohort 2);

	○ time to first dose of treatment with artemether-lumefantrine (Riamet®) (Cohorts 1 and 2); and ○ parasitaemia at first dose of treatment with Riamet® (Cohorts 1 and 2). • The number and proportion of participants with presence of positive PCR and parasitaemia of ≥5000 or ≥10000 parasites per mL blood between inoculation with *Pf*SPZ-DVI Challenge and Day 28 (per cohort).
Secondary	
• To determine the safety and tolerability of Riamet® in blood-stage malaria infection in naïve healthy participants after *Pf*SPZ-DVI Challenge.	• The incidence, severity and relationship to Riamet® of observed or self-reported, treatment-emergent adverse events (TEAEs) until EOS visit.
• To characterise the blood-stage parasite profile in malaria infection in naïve healthy participants after *Pf*SPZ-DVI Challenge.	• The blood-stage parasite profile of *P. falciparum* NF54 after infection via injection of sporozoites by DVI will be characterised by: ○ parasite growth rate expressed as the parasite multiplication rate (PMR) standardised to 48 h (PMR_{48}) (Cohort 1, Cohort 2 and overall); ○ parasite growth rate expressed as the PMR per life cycle (if not 48 h) (PMR_{LC}) (Cohort 1, Cohort 2 and overall); and ○ predicted time to reach parasitaemia threshold of first positive PCR, ≥5000 parasites per mL blood, ≥10000 parasites per mL blood for Cohorts 1 and 2.
• To characterise the blood-stage parasite clearance profile of Riamet® in malaria infection in naïve healthy participants after *Pf*SPZ-DVI Challenge.	• The effect of Riamet® on clearance of *P. falciparum* blood-stage parasites will be evaluated using: ○ time to parasite clearance, i.e., time between drug administration and first PCR below the limit of quantification (Cohort 1, Cohort 2 and overall); ○ $\log_{10}$ parasite reduction ratio per 48 h ($\log_{10}PRR_{48}$), i.e., ratio of the parasite density at a specific time point to the parasite density 48 h later and expressed in $\log_{10}$ (Cohort 1, Cohort 2 and overall); ○ maximum $\log_{10}PRR_{48}$ ($\log_{10}PRR_{48,max}$) (Cohort 1, Cohort 2 and overall); and ○ parasite clearance half-life ($PC_{½}$), i.e., time taken for the parasite density to be reduced by 50% after the first dose administration of Riamet® (Cohort 1, Cohort 2 and overall).
Exploratory	
• To characterise the individual variability of blood-stage parasite profile.	• The individual variability of blood-stage parasite profile will be characterised by: ○ the inter-individual variability of the parasite growth rate; and ○ predicted time to reach parasitaemia threshold of first positive PCR, ≥5000 parasites per mL blood, ≥10000 parasites per mL blood including their 95% confidence interval (CI).

Overview of Study Design:

This is a single-centre, open-label, Phase Ib study designed to assess if intravenous bolus injection of approximately 3200 *P. falciparum* (NF54 strain) sporozoites can be safely administered to achieve blood-stage parasitaemia with a kinetics/PCR profile that will allow for the future characterisation of antimalarial blood-stage activity of new chemical entities in a relatively small number of participants during early drug development.

Up to 16 healthy, malaria-naïve males and females, aged 18-55 years, will be enrolled in a maximum of 2 cohorts (up to 8 participants per cohort; a participant may be enrolled in one cohort only). Enrolment into the cohorts will proceed sequentially, with two target levels of parasitaemia previously achieved in healthy participants enrolled in malaria Volunteer Infection Studies (VIS) at other study sites, i.e., 5000 parasites/mL blood in Cohort 1 and 10000 parasites/mL blood in Cohort 2.

Each participant will be admitted to the clinical unit in the morning of Day -1 and inoculated with approximately 3200 *P. falciparum* sporozoites (NF54 strain) by DVI on Day 1. Participants will be discharged 2 h post inoculation on Day 1 and will be monitored daily via phone call from Day 2 until Day 6 to solicit any AEs. Participants will come to the clinical unit daily from Day 7 until Day 9 and together with the malaria clinical score, the presence of parasites will be assessed once daily by a specific qPCR targeting the *var*ATS (the acidic terminal segment in *Plasmodium falciparum var* genes) multigenic family; this to accurately describe parasite growth even in case PCR positivity, i.e., a qPCR outcome ≥250 parasites per mL blood, is confirmed this early (very low probability and with low densities). Participants will be confined to the clinical unit from Day 10 in the morning. qPCR will be performed and malaria clinical score assessed twice daily and participants will be administered registered antimalarial therapy, i.e., Riamet®, when the following criteria are met:

1. Cohort 1: ≥5000 parasites/mL blood or earlier if a participant has a malaria clinical score >6 or at Investigator's discretion.
2. Cohort 2: ≥10000 parasites/mL blood or earlier if a participant has a malaria clinical score >6 or at Investigator's discretion.

The registered 3-day antimalarial therapy regimen will be further administered and monitored. qPCR assessments of parasitaemia will be carried out at multiple time points (2, 6, 8, 12, 16, 24, 36, 48 and 72 h) following initiation of Riamet® and malaria clinical score will be assessed twice daily during confinement in the clinical unit. Safety and tolerability will be monitored during the whole study duration, specific assessments will be done at periodic pre-specified time points from Day 10 and for at least 72 h after initiating antimalarial therapy, i.e., during confinement in the clinical unit (see below).

Of note, all participants must consent to receiving antimalarial therapy, i.e., the registered 3-day Riamet® regimen approved for treatment of uncomplicated malaria. Even in the case of withdrawal from the study, all participants administered the *Pf*SPZ-DVI Challenge are to receive antimalarial therapy as soon as possible, and to have all appropriate visits and assessments as required. If an intolerance or contraindication to Riamet® develops, Malarone® will be administered.

Upon parasite clearance (defined as a qPCR value of 0 parasites per mL blood after initiating antimalarial therapy) and at least 72 h after initiating antimalarial therapy (estimated to occur on or before Day 19 and on or before Day 22 for Cohort 1 and Cohort 2, respectively), and if clinically well, participants will be discharged from the clinical unit and will be followed up for safety assessments, clinical evaluation and malaria qPCR in the clinical unit at the EOS visit on Day 28.

All participants who received antimalarial therapy will be asked non-leading questions to determine the occurrence of any AEs throughout the study and at the EOS visit.

All participants inoculated with *Pf*SPZ-DVI Challenge will commence antimalarial therapy no later than Day 24 for both cohorts, regardless if they reach pre-defined cohort-specific PCR parasitaemia/malaria clinical score thresholds (i.e., 5000 parasites/mL blood for Cohort 1 and 10000 parasites/mL blood for Cohort 2 and/or a malaria clinical score ≥6 for Cohorts 1 and 2). Participants who start antimalarial therapy on Day 24 will only be discharged from confinement at the end of the EOS visit on Day 28.

Antimalarial therapy may be initiated whenever deemed necessary by the Investigators, e.g., if there is a concern regarding the safety of a study participant. Therapy may be amended according to the treating physician if the participant does not respond to treatment or the condition worsens.

A sentinel strategy will be employed in Cohorts 1 and 2. Each cohort will consist of 2 subgroups of participants, to be enrolled sequentially: Subgroup 1 will be composed of 2 participants; Subgroup 2 will be composed of 6 participants. Participants in Subgroup 2 will not be treated until the last participant in Subgroup 1 has completed antimalarial therapy and only upon decision of the Principal Investigator (PI), medical director and medical monitor after review of available safety and tolerability data (safety assessments, AEs and malaria clinical scores) and parasitaemia data, including clearance of parasitaemia, of Subgroup 1.

Review of available Cohort 1 safety/tolerability and parasitaemia data up to the last dose of Riamet® for all inoculated participants in Cohort 1 will be performed by a Safety Review Team (SRT) before enrolment of Cohort 2. For progression from Cohort 1 to Cohort 2, the SRT members will include at least an independent Early Phase and Clinical Pharmacology MD expert experienced in malaria volunteer infection studies (SRT chair), the Medical Monitor, the Medical/Project Director, the PI and an independent infectious disease/malaria expert (or their delegates).

Study Population:

Approximately 16 participants are planned to be enrolled sequentially in 2 cohorts of up to 8 participants per cohort. A participant may be enrolled in one cohort only.

Eligibility Criteria:

Inclusion Criteria:

1. Informed Consent Form signed voluntarily before any study-related procedure is performed, indicating that the participant understands the purpose of and procedures required for the study and is willing to participate in the study, including administration of registered antimalarial therapy;
2. Male or female, between 18 and 55 years old (extremes included) at screening;
3. Body weight of at least 50 kg and a body mass index (BMI) of 19.0 to 30.0 kg/m^2 (extremes included);
4. Good general health without clinically relevant medical illness, physical exam findings including vital signs, and laboratory abnormalities (e.g., without liver transaminases >1x the upper limit of normal [ULN] and as defined in the protocol) as determined by the Investigator;
5. Willing to adhere to the prohibitions and restrictions specified in this protocol, including willingness to stay confined to the inpatient unit for the required duration and willingness to avoid travelling outside of Benelux during the study period;
6. Female participants should fulfil one of the following criteria:
   a. At least 1 year postmenopausal (amenorrhea >12 months and follicle stimulating hormone [FSH] >30 mIU/mL) prior to screening;
   b. Surgically sterile (bilateral oophorectomy, hysterectomy or bilateral salpingectomy);
   c. Will use contraceptives as outlined in inclusion criterion 7;
7. Female participants of childbearing potential (excluding females with female partners) must agree to the use of a highly effective method of birth control from the screening visit until 40 days after the EOS visit at Day 28 (covering a full menstrual cycle of 30 days starting after 5 half-lives of last dose of Riamet®);

   Note: Highly effective birth control methods include: combined (oestrogen- and progestogen-containing) oral/intravaginal/transdermal hormonal contraception associated with inhibition of ovulation, progestogen-only oral/injectable/implantable hormonal contraception associated with inhibition of ovulation, intrauterine device, intrauterine hormone-releasing system, bilateral tubal occlusion, vasectomised partner or sexual abstinence from heterosexual intercourse.

8. Female participant has a negative pregnancy test at screening and upon admission in the clinical unit;

   Note: Pregnancy testing will consist of serum β-human chorionic gonadotropin (β-HCG) tests at screening and at the EOS visit and a urine β-HCG tests on Day -1, in all women.

9. Different ways of being reachable 24/7 (e.g., by mobile phone, regular phone or electronic mail) during the whole study period.

Exclusion Criteria:

1. Nursing (lactating) women;
2. Participation in any other clinical drug or vaccine study within 30 days (or 5 half-lives for drugs) preceding the day of *Pf*SPZ-DVI Challenge (whichever is longer), or plans to participate in other investigational drug or vaccine research during the study period;
3. Participants who took standard vaccinations within 3 months before the start of the study or are planning to take standard vaccinations during the study period up to 8 weeks after *Pf*SPZ-DVI Challenge;
4. Blood product donation to any blood bank during the 8 weeks (whole blood) or 4 weeks (plasma and platelets) prior to admission in the clinical unit on Day -1;
5. Mean ECG outside normal range and deemed clinically relevant by the Investigator. Examples of clinically significant ECG abnormalities for this study include:
   - PR-interval >220 ms;
   - QRS-complex >120 ms;
   - Absolute QT greater than >500 ms;
   - QT interval corrected according to Bazett's formula (QTcB) or QT interval corrected according to Fridericia's formula (QTcF >450 ms for male participants, >470 ms for female participants;
   - Pathologic Q wave;
   - Significant ST-T wave changes;
   - Left or right ventricular hypertrophy;
   - Non-sinus rhythm except isolated premature atrial contractions and ventricular extrasystole <2 per 10 s ECG lead;
   - Incomplete left bundle branch block, or complete or intermittent right or left bundle branch block;
   - Second or third degree A-V heart block.
6. Seropositive human immunodeficiency virus (HIV), hepatitis A immunoglobulin M (IgM) antibody, hepatitis B virus (HBV) (hepatitis B surface antigen [HBsAg]), hepatitis C virus (HCV) (antibody), hepatitis D antibody, hepatitis E IgM antibody, cytomegalovirus (CMV) IgM antibody or Epstein Barr Virus (EBV) IgM antibody;
7. Previous or current diagnosis of hepatitis including but not limited to viral hepatitis, auto-immune hepatitis, non-alcoholic steatohepatitis (NASH), alpha-1-antitrypsin deficiency, alcoholic liver disease, primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), hemochromatosis, Wilson disease or suspected hepatocellular carcinoma (HCC).
8. History or presence of diagnosed food or known drug allergies (including but not limited to allergy to any of the antimalarial medications to be used in the study), or history of anaphylaxis or other severe allergic reactions;

   Note: Participants with seasonal allergies/hay fever, house dust mite or allergy to animals that are untreated and asymptomatic at the time of dosing can be enrolled in the study.
9. History of convulsion or severe head trauma, excluding fever convulsion under 5 years of age;

   Note: A medical history of a single febrile convulsion during childhood is not an exclusion criterion.
10. History of serious psychiatric condition that may affect participation in the study or preclude compliance with the protocol, including but not limited to past or present psychoses, disorders requiring lithium, a history of attempted or planned suicide, more than one previous episode of major depression, any previous single episode of major depression lasting for or requiring treatment for more than 6 months, or any episode of major depression during the 5 years preceding screening;

    Note: The Beck Depression Inventory will be used as an objective tool for the assessment of depression at screening. In addition to the conditions listed above, participants with a score of 20 or more on the Beck Depression Inventory and/or a response of 1, 2 or 3 for item 9 of this inventory (related to suicidal ideation) will not be eligible for participation. Participants with a Beck score of 17 to 19 may be enrolled at the discretion of the Investigator if they do not have a history of the psychiatric conditions mentioned in this criterion and their mental state is not

considered to pose additional risk to the health of the volunteer or to the execution of the study and interpretation of the data gathered.

11. A medical, occupational or family problem as a result of alcohol or illicit drug abuse during the past 12 months or current alcohol or illicit drug abuse or addiction (positive alcohol breath test or positive drug screen for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine or opiates at screening or upon check-in at the clinical unit);

    Note: Excessive use of alcohol is defined as an intake of >21 units per week for males and >14 units per week for females where one alcohol unit is defined as 10 mL or 8 g of pure alcohol. A single unit is equal to one 25-mL (single) measure of whisky (alcohol by volume [ABV] 40%), or a third of a pint of beer (190 mL; ABV 5-6%) or half a standard (175 mL) glass of wine (ABV 12%).

12. Participants are non-smokers or ex-smokers for more than 90 days prior to screening, or smoke no more than 5 cigarettes per day. If users of nicotine products (i.e., spray, patch, e-cigarette, etc.), they should use the equivalent of no more than 5 cigarettes per day. Participants must agree to abstain from smoking while in the unit;
13. Use of any prescription drugs, herbal supplements (e.g., St John's Wort) or over-the-counter medication within 7 days or 5 half-lives (whichever is longer) prior to the *Pf*SPZ-DVI Challenge, or an anticipated requirement for the use of these during the course of the study;

    Note: If necessary, the incidental use of non-steroidal anti-inflammatory drugs (NSAIDs) and paracetamol (2 g/day, 10 g/week) may be acceptable at the Investigator's discretion and will be documented in the eSource system. The use of nutritional supplements during this time that are not believed to have the potential to affect participant safety nor the overall results of the study, may be permitted on a case-by-case basis by the Investigator.

14. Any surgical or medical condition possibly affecting drug absorption (e.g., cholecystectomy, gastrectomy, bowel disease), distribution, metabolism or excretion;
15. Personnel (e.g., Investigator, sub-investigator, research assistant, pharmacist, study coordinator or anyone mentioned in the delegation log) directly involved in the conduct of the study;
16. Any condition that in the opinion of the Investigator would jeopardise the safety or rights of a person participating in the study or would render the person unable to comply with the protocol;
17. Personal history of malaria;
18. Volunteer has travelled to or lived in a malaria-endemic area within 6 months prior to planned study enrolment;
19. Plans to travel to malaria-endemic region during the study period up to last follow-up visit;
20. Previous participation in any malaria vaccine or Controlled Human Malaria Infection (CHMI) study/VIS;
21. Falling in moderate or higher risk category for a fatal or non-fatal cardiovascular event within 5 years (> 5%) determined by a validated risk estimation system, e.g., SCORE;
22. Use of systemic antibiotics with known antimalarial activity within 5 half-lives of *Pf*SPZ-DVI Challenge (e.g., trimethoprim-sulfamethoxazole, doxycycline, tetracycline, clindamycin, erythromycin, fluoroquinolones or azithromycin) or an anticipated requirement for the use of these during the study period;
23. Receipt of blood or blood-derived products (including immunoglobulin) within 3 months prior to screening. Receipt of packed red blood cells (RBCs) given for an emergent indication in an otherwise healthy person, and not required as ongoing treatment is not exclusionary (for example packed RBCs emergently given during an elective surgery).
24. Participants who test positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

    Note: In case of an out-of-range clinical laboratory test (as defined in the protocol), vital sign or ECG value that will determine a participant's eligibility, or in case of a positive drug screen, a retest or expert evaluation can be requested. Results of any retest must be available prior to inoculation. The result of the retest will be considered for participant eligibility at the Investigator's discretion. Participants can be rescreened at the discretion of the Investigator.

***Pf*SPZ Challenge, Riamet® and Malarone®; Dose, Mode of Administration:**

Manufacturing, packaging, and labelling of *Pf*SPZ-DVI Challenge inoculum will be done under the responsibility of the biotechnology company Sanaria (USA).

The clinical unit will be responsible for acquiring the registered antimalarial drugs artemether lumefantrine (Riamet®) and atovaquone-proguanil (Malarone®):

- Participants will be prescribed with Riamet® according to the pre-defined cohort-specific PCR parasitaemia/malaria clinical score thresholds (i.e., 5000 parasites/mL blood for Cohort 1 and 10000 parasites/mL blood for Cohort 2 and/or a malaria clinical score ≥6 for Cohorts 1 and 2) to ensure parasite clearance prior to the end-of-study evaluation. In addition, participants will also be administered with Riamet®:
  - o if they experience ≥1 inoculum-related serious adverse event (SAE), irrespective of severity; or
  - o if they experience ≥1 Common Terminology Criteria for Adverse Events (CTCAE) grade ≥3 (severe) AE deemed related to malaria and not self-resolved or relieved with concomitant medications; or
  - o if the PI or delegate considers it necessary for participant safety. In this situation, the PI or delegate will consult with the independent infectious disease/malaria expert (Prof. Jean-Pierre Van geertruyden). However, antimalarial medication may be administered prior to consultation if immediate treatment is deemed necessary for participant safety.
- If an intolerance or contraindication to Riamet® develops, Malarone® will be administered.

All participants inoculated with *Pf*SPZ-DVI Challenge will commence antimalarial therapy no later than Day 24 for both cohorts, regardless if they reach pre-defined cohort-specific PCR parasitaemia/malaria clinical score thresholds (i.e., 5000 parasites/mL blood for Cohort 1 and 10000 parasites/mL blood for Cohort 2 and/or a malaria clinical score ≥6 for Cohorts 1 and 2).

All participants must consent to receiving antimalarial therapy. Even in the case of withdrawal from the study, all participants administered the *Pf*SPZ-DVI Challenge are to receive antimalarial therapy as soon as possible, and to have all appropriate visits and assessments as required.

The *Pf*SPZ-DVI Challenge agent consists of a strain of *P. falciparum* NF54 sporozoites used for CHMI/VIS studies that is known to be sensitive to the registered antimalarial therapy treatments described above.

Intervention Name	*Pf*SPZ-DVI Challenge	artemether-lumefantrine (Riamet®)	atovaquone-proguanil (Malarone®); only to be used if an intolerance or contraindication to Riamet® develops
Intervention Type	inoculum	drug	
Dose Formulation	cryovial	tablet	
Unit Dose Strength(s)	15000 or 50000 aseptic, cryopreserved *P. falciparum* sporozoites	20 mg artemether and 120 mg lumefantrine	250 mg atovaquone and 100 mg proguanil hydrochloride
Dosage Level	3200 *P. falciparum* sporozoites once	6 doses of 4 tablets over a period of 3 days at approximately 0, 8, 24, 36, 48 and 60 h	4 tablets as a single daily dose for 3 consecutive days
Route of Administration	intravenously by DVI	oral	
Meals in Relation to Dosing	Not applicable	The first dose of antimalarial drug should be taken as soon as possible according to the pre-defined cohort-specific PCR parasitaemia/malaria clinical score thresholds (i.e., 5000 parasites/mL blood for Cohort 1 and 10000 parasites/mL blood for Cohort 2 and/or a malaria clinical score ≥6 for Cohorts 1 and 2) or on reaching Day 24. Antimalarial drug administration should be immediately followed by a meal or drinks rich in fat (e.g., milk).	
Use	challenge agent	antimalarial medication	

Study/Treatment Duration:

It is estimated that each participant will be in the study for a maximum of 56 days, including a screening period of up to 28 days and a study period of 28 days, including *Pf*SPZ-DVI Challenge on Day 1 and a follow-up period of 27 days afterwards.

Assessments:

Pharmacodynamics

Parasitaemia

A blood sample will be collected via direct venepuncture from each participant at the scheduled time points where parasitological assessments are scheduled. The assessment of malaria parasitology by qPCR will be as follows: *var*ATS multigenic family targeted qPCR assay of parasite load will be performed in accordance with the centre of excellence for tropical medicine (ITM) standard operating procedure and the Laboratory Manual. Given the high sensitivity of qPCR, this method will be used to confirm parasite clearance after definitive antimalarial therapy for all participants. The results of the PCR at the ITM will be available in approximately 6 h from arrival of samples at the ITM. A participant will be considered cured following completion of the course of antimalarial therapy and after qPCR indicates 0 parasites per mL blood.

Safety

Adverse Events

Adverse events will be monitored continuously from informed consent until the last study-related activity. At regular intervals during the study, participants will be asked non-leading questions to determine the occurrence of any AEs. All AEs reported spontaneously during the course of the study will be recorded as well.

Close monitoring of expected signs and symptoms associated with malaria infection form part of the safety evaluation of the study and will be used as part of the decision criteria to administer registered antimalarial

therapy. These events will be classified as AEs, and may also be further sub-classified as inoculum-related AEs:

An inoculum-related AE is a sign or symptom associated with malaria infection, i.e., confirmed by a protocol-defined PCR positivity (defined for the purpose of this study as a qPCR outcome ≥250 parasites per mL blood) at

- the onset of the event; or
- if the event started within 1 day of parasite clearance (defined as a qPCR value of 0 parasites per mL blood after initiating antimalarial therapy) or laboratory-defined PCR positivity (a qPCR outcome of 50-249 parasites per mL blood).

If the presence of *P. falciparum* malaria is confirmed (see above), then these AEs will be further sub-classified as inoculum-related AEs, following the guidelines on AE reporting, i.e., followed-up until resolution with the same causality assessment, and reported as such in the final clinical study report.

If the PCR results for *P. falciparum* are between 0 and <250 parasites per mL blood at the time of the onset of the event and the day before, usual AE/SAE reporting procedures and criteria will apply, but the event will not be classified as an inoculum-related AE.

Final classification of signs and symptoms as AE and inoculum-related AE, or as AE only, will occur after PCR results are available.

Malaria Clinical Score

The malaria clinical score, which assesses malaria signs and symptoms at the scheduled time points, will be part of the close monitoring for known/identified risks related to *Pf*SPZ-DVI Challenge/malaria. The malaria clinical score has been widely used in the conduct of induced blood-stage malaria (IBSM) CHMI/VIS studies in Australia and also implemented in the recently conducted *Pf*SPZ-DVI Chemoprotection Challenge study with P218 (MMV_P218_17_01 study [EudraCT number: 2018-003004-39]) at the SGS Clinical Pharmacology Unit in Antwerp, Belgium. This quantitative tool will be used as part of the decision criteria to administer registered antimalarial therapy along with the level of parasitaemia.

The following 14 signs/symptoms frequently associated with malaria will be graded using a 4-point scale (absent: 0; mild: 1; moderate: 2; severe: 3) and summed to generate a total malaria clinical score (maximum score possible is 42): headache, myalgia (muscle ache), arthralgia (joint ache), fatigue/lethargy, malaise (general discomfort/uneasiness), chills/shivering/rigors, sweating/hot spells, anorexia, nausea, vomiting, abdominal discomfort, fever, tachycardia and hypotension. To determine severity of the 14 signs/symptoms we use the CTCAE grading scale grade 1 - 5. Mild (1) equates to CTCAE grade 1, Moderate (2) equates to CTCAE grade 2 and Severe (3) equates to CTCAE grade 3 or above. Individual scores for each symptom as well as the total score will be recorded.

For the grading of fever, the vital sign parameter body temperature (sublingual) will be assessed (see below).

Clinical Laboratory Tests

Blood samples will be collected by venepuncture or via indwelling cannula at the scheduled time points. Biochemistry and haematology testing will be performed on these samples, as well as viral serology testing (hepatitis A IgM antibody, HbsAg, anti-HCV antibody, hepatitis D antibody [only in subjects positive for HbsAg], hepatitis E IgM antibody, CMV IgM antibody, EBV IgM antibody and HIV) on the sample from screening. In all female participants, also serum β-HCG assessments at screening and at the EOS visit and a urine β-HCG assessment on Day-1 will be performed. FSH will be measured at screening in all women.

All blood samples for safety assessments should be taken in a fasted state (after overnight fast for at least 8 h for unbiased glucose determination), except for the ones taken during antimalarial therapy for troponin T measurements, and except for the one on Day of first PCR ≥5000 parasites/mL in Cohort 1 or ≥10000 parasites/mL in Cohort 2 and prior to initiating antimalarial therapy, where a non-fasted state is allowed.

Standard laboratory tests will be performed by ZNA Middelheim.

The following biochemistry and haematology tests will be performed on the safety blood samples:

- Liver biochemistry: albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamylaminotransferase (GGT), total and direct bilirubin and total serum proteins;
- Biochemistry other than liver: sodium, potassium, chloride, bicarbonate, urate, inorganic phosphate, creatinine, estimated glomerular filtration rate (eGFR), glucose, lactate dehydrogenase (LDH), blood urea nitrogen (BUN) and creatine phosphokinase (CPK);
- High sensitive troponin T and;
- C-reactive protein (CRP);
- Haematology: haemoglobin, haematocrit, RBC count, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), white blood cell (WBC) count, platelet count, reticulocytes, neutrophils, eosinophils, basophils, lymphocytes and monocytes;
- Coagulation: prothrombin time (PT) and activated partial thromboplastin time (aPTT) (at screening and on Day -1 only), and international normalized ratio (INR).

A midstream urine sample will be collected for urinalysis by dipstick for glucose, protein, nitrite, pH and occult blood at the scheduled time points for biochemistry other than liver. Microscopic examination for WBC, RBC and casts will be performed.

A urine drug screen (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine and opiates) and an alcohol breath test will be performed at the scheduled time points.

The Investigator must review the laboratory report, document this review and record any change occurring during the study he/she considers to be clinically relevant in the eSource system. Laboratory values outside the normal range will be flagged and their clinical relevance will be assessed by the Investigator.

Vital Signs

Vital sign parameters will be assessed after at least 10 min in supine position at the scheduled time points. The vital sign parameters that will be assessed are supine systolic and diastolic blood pressure (SBP and DBP, respectively) and pulse rate. Body temperature (sublingual) will also be assessed, and this whenever the malaria clinical score will be evaluated (see above).Orthostatic changes to BP and pulse rate will also be assessed at screening: participants will be requested to stand after completion of the supine measurements and blood pressure and pulse rate will be recorded after at least 2 min in the standing position.

These parameters will be measured using a completely automated device consisting of an inflatable cuff and an oscillatory detection system. All values will be registered on a built-in recorder so that measurements are observer-independent.

Any change from baseline in vital sign values occurring during the study that is considered to be clinically relevant by the Investigator should be recorded in the eSource system.

Electrocardiogram

Twelve-lead ECG recordings will be recorded in triplicate after at least 10 min in supine position at the scheduled time points.

Paper speed will be 25 mm/s, so that the different ECG intervals can be measured manually.

The interpretations of the ECGs will be performed by the Investigator or his/her designee at the clinical unit. Any change from baseline ECG occurring during the study that is considered to be clinically relevant by the Investigator should be recorded in the eSource system.

Physical Examination

Physical examination will be performed at the scheduled time points.

Height is to be measured barefoot and at screening only. Body weight is to be measured at the scheduled time points. To obtain the actual body weight, participants must be weighed lightly clothed.

Any change in physical examination occurring during the study that is considered to be clinically relevant by the Investigator should be recorded in the eSource system.

Full physical examination will be conducted at screening, on Day -1, within ±24 h after first PCR ≥250 parasites/mL blood and at the EOS visit on Day 28. Targeted, symptom-driven physical examination

will be performed in case malaria clinical score >6 on Day 10 (upon confinement to the clinical unit), within 24 h after first PCR ≥5000 (Cohort 1) or ≥10000 (Cohort 2) parasites/mL blood and at pre-discharge from clinical unit; and will be focused on changes since the previous examination, but will always include at least: chest/respiratory, general appearance, heart/cardiovascular, abdomen, neurological and skin/mucous membranes examination.

Beck Depression Inventory

The Beck Depression Inventory is performed at screening only.

The questionnaire is scored by the participant. The inventory completed by the participant will be reviewed/checked for completeness and the total score calculated by the study personnel.

Parasite Transcriptomics

Different environmental conditions that occur during a human infection (such as the presence of drugs) can select for parasites with different expression patterns. We aim to evaluate the effect of in-host factors on parasite transcriptional/epigenetic responses during experimental infections.

To this aim, blood samples will be collected via direct venepuncture at the scheduled time points. Sample aliquots will be stored in Trizol for RNA preservation and cryopreserved for parasite culture in case higher parasite densities are needed to increase starting material for transcriptomic analysis of parasite isolates.

Protocols for transcriptomic analysis of parasite isolates have been validated and are currently in use at the Malariology Unit at the ITM.

Statistical Methods:

All statistical analysis will be performed by SGS Life Sciences, under the supervision and responsibility of the Sponsor, using SAS® (SAS Institute Inc., Cary, NC, USA; version 9.4 or higher) software for statistical computations.

All statistical methods shall be detailed in a Statistical Analysis Plan (SAP) that will be finalised before database lock.

The final analysis will be performed once all participants have completed the follow-up visit or have discontinued earlier.

Data from all participants enrolled and inoculated with *Pf*SPZ-DVI Challenge will be included in the data analysis. The following analysis populations will be defined:

- Inoculation Set: all participants who were inoculated with *Pf*SPZ-DVI Challenge;
- Pharmacodynamic (PD) Analysis Set: all inoculated participants with at least one available PD data who received all Riamet® doses and who experienced no protocol deviations with relevant impact on PD data;
- Safety Analysis Set: all inoculated participants who received any treatment.

Unless specified otherwise, the Inoculation Set will be used for analysis of demographics, the PD analysis set will be used for pharmacodynamic statistical analysis and the Safety Analysis Set will be used for safety/tolerability analysis.

Sample Size

Up to 16 participants will be enrolled in 2 cohorts of 8 participants per cohort. In agreement with the Sponsor, additional participants may be recruited in each cohort, to replace discontinuations for non-safety reasons and achieve the required cohort size.

This is an exploratory study focusing on the methodology of malaria inoculation in healthy participants, thus no formal sample size calculation is performed.

Initial Characteristics Data of the Participant Sample

For all participants who receive *Pf*SPZ-DVI Challenge, descriptive statistics will be provided for demographic (e.g., age, height, weight, BMI, race, gender) and other initial participant characteristics (alcohol and drug screening tests, pregnancy test, orthostatic changes to blood pressure and pulse rate, serology, medical and social history, concomitant diseases, Beck Depression Inventory). Standard descriptive statistics for continuous variables are the number of participants (N), mean, standard deviation (SD), median, minimum and maximum values. The standard descriptive statistics for categorical variables are the number of participants in the category and the proportion expressed as a percentage.

Prior and concomitant medications will be coded using the WHO_DRUG Dictionary.

Pharmacodynamic Data

Parasitaemia

Primary endpoints:

To characterise key stages in the parasite growth:

- first PCR positivity;
- threshold of 5000 parasites/mL for Cohort 1 and threshold of 10000 parasites/mL for Cohort 2; and
- parasitaemia levels at drug administration.

This will be done by calculating:

- Descriptive statistics (geometric mean, SD, 95% CI, range) of time to first PCR positivity. In the absence of positive PCR, the duration will be set to a maximum of 28 days;
- Descriptive statistics (N, geometric mean, SD, 95% CI, range) of parasitaemia at first PCR positivity;
- Descriptive statistics (geometric mean, SD, 95% CI, range) of time to parasitaemia of ≥5000 parasites per mL blood (Cohorts 1 and 2);
- Descriptive statistics (geometric mean, SD, 95% CI, range) of parasitaemia at the time parasitaemia ≥5000 parasites per mL blood (Cohorts 1 and 2);
- Descriptive statistics (geometric mean, SD, 95% CI, range) of time to parasitaemia of ≥10000 parasites per mL blood (Cohort 2);
- Descriptive statistics (geometric mean, SD, 95% CI, range) of parasitaemia at the time parasitaemia ≥10000 parasites per mL blood (Cohort 2);
- Descriptive statistics per cohort (geometric mean, SD, 95% CI, range) of time to first dose of treatment with Riamet® (Cohorts 1 and 2); and
- Descriptive statistics per cohort (geometric mean, SD, 95% CI, range) of parasitaemia at first dose of treatment with Riamet® (Cohorts 1 and 2).

The number and proportion of participants with presence of positive PCR and parasitaemia of ≥5000 or ≥10000 parasites per mL blood between inoculation with *Pf*SPZ-DVI Challenge and Day 28 will be summarised per cohort. Corresponding two-sided 90% Exact Clopper-Pearson confidence limits will be presented as well.

Secondary endpoints:

To characterise the blood-stage parasite profile, a model will be fitted to the measured parasitaemia data prior to Riamet® administration to be able to predict the growth of parasites as a function of time. This model may be a log-linear model or, if the parasitaemia profile shows a cyclic behaviour due to synchronicity of the parasites and their sequestration during the late stages of their lifecycle, it may include a sinus function. The latter will allow the estimation of the period of the observed waves in addition to the parasite growth. The following parameters will be calculated from the individual fits of the data:

- Descriptive statistics per cohort and overall (geometric mean, SD, 95% CI, range) of parasite growth rate expressed as the PMR_{48};
- If cycles are observed and if their estimated period is not 48 h, descriptive statistics per cohort and overall (geometric mean, SD, 95% CI, range) of parasite growth rate expressed as the PMR_{LC}; and
- Descriptive statistics (geometric mean, SD, 95% CI, range) of predicted time to reach parasitaemia threshold of first positive PCR, ≥5000 parasites per mL blood, ≥10000 parasites per mL blood for Cohorts 1 and 2.

To characterise the blood-stage parasite clearance profile of Riamet® in malaria infection in naïve healthy participants after *Pf*SPZ-DVI Challenge; by calculating:

- Descriptive statistics per cohort and overall (geometric mean, SD, 95% CI, range) of time to parasite clearance, i.e., time between drug administration and first PCR below the limit of quantification;
- Descriptive statistics per cohort and overall (geometric mean, SD, 95% CI, range) of $\log_{10}PRR_{48}$;
- Descriptive statistics per cohort and overall (geometric mean, SD, 95% CI, range) of $\log_{10}PRR_{48,max}$; and
- Descriptive statistics per cohort and overall (geometric mean, SD, 95% CI range) of $PC_{½}$.

The parasite reduction ratios (PRRs) and the clearance half-life will be estimated from a log-linear model that will be fitted to the parasitaemia data observed from the administration of the treatment onwards. The PRR_{48}, $PRR_{48,max}$ and $PC_{½}$ will be calculated from the individual parameters and summarised by cohort.

Exploratory endpoints:

To characterise the individual variability of blood-stage parasite profile:

- Obtain the inter-individual variability of the parasite growth rate; and
- 5^{th}, 50^{th} and 95^{th} percentiles of predicted time to reach parasitaemia threshold of first positive PCR, ≥5000 parasites per mL blood, ≥10000 parasites per mL blood including their 95% CI.

Both these estimates will be based on the mixed model on parasite growth data described above.

Safety

Safety parameters will be tabulated and analysed descriptively.

Adverse Events

The original terms entered in the eSource system by Investigators to identify AEs will be fully described and coded according to the Medical Dictionary for Regulatory Activities (MedDRA).

The reported AEs will be allocated to phases based on their start date. All AEs will be listed. All AEs with onset after *Pf*SPZ-DVI Challenge will be summarised by cohort. Summaries will be made per MedDRA primary system organ class, MedDRA preferred term, severity, with the number and percentage of participants and the number of events. Similar summaries will be prepared for AEs considered to be related to Riamet® and AEs considered to be related to the *Pf*SPZ-DVI Challenge, for serious AEs and AEs of special interest.

Special attention will be paid to those participants who died, discontinued the investigational products due to an AE or experienced a severe or serious AE. Summaries, listings and narratives may be provided, as appropriate.

Malaria Clinical Score

For the malaria clinical score that will be administered by the PI or his/her trained delegate, actual values and changes from baseline will be evaluated by means of descriptive statistics. Additionally, expected signs and symptoms will be summarised by score.

Clinical Laboratory Tests

Each continuous biochemistry and haematology laboratory test will be evaluated by means of descriptive statistics on the actual values, at each assessment time point and by cohort. Changes from baseline will also be summarised using descriptive statistics by assessment time point and by cohort.

Relative changes in clinical laboratory test values compared to values at baseline will be evaluated in accordance with the normal ranges of the clinical laboratory (below, within or above normal range). The percentage of participants with clinical laboratory test abnormalities will be summarised by cohort.

The number and percentage of participants with liver enzyme elevations after inoculation with the *Pf*SPZ-DVI Challenge as defined below will be summarised:

- ALT or AST >3 x ULN;
- ALT or AST >5 x ULN;
- ALT or AST >8 x ULN;
- ALT or AST >3 x ULN and bilirubin >2 x ULN at the same time point, together with a conjugated bilirubin fraction> 35% (Potential Hy's law cases).

A listing of participants with any clinical laboratory test result outside the reference ranges will be provided.

Vital Signs

Vital signs parameters will be assessed after at least 10 min in supine position at the scheduled time points. Pulse rate, SBP and DBP will be evaluated by means of descriptive statistics (actual values and changes from baseline).

The percentage of participants with vital signs abnormalities will be summarised by cohort in a cross-tabulation of post-baseline versus baseline abnormalities to the normal ranges.

Electrocardiogram

Twelve-lead ECG recordings will be performed in triplicate after participants remained in a supine position for at least 10 min.

All ECG data automatically measured by ECG devices (PR, QRS, QT, QTcB, QTcF and HR) and overall ECG evaluation will be listed. The ECG data, along with changes from baseline will be summarised by means of descriptive statistics at each assessment time point and by cohort.

The percentage of participants with ECG abnormalities will be summarised by cohort in a cross-tabulation of post-baseline versus baseline abnormalities to the normal ranges. This cross-tabulation will include categorical assessment on actual values and changes from baseline of QTcB and QTcF prolongation.

Physical Examination

Abnormal findings in physical examination will be listed.

TIME AND EVENTS SCHEDULE

Cohort 1

Assessments			Challenge, treatment and follow-up								
Study Day/Event	Screening Visit Day -28 to Day -2	Day -1[a]	Day 1		Day 2-6	Day 7-9	Day 10: Start of Confinement in Clinical Unit	Day of First PCR ≥250 Parasites/mL Until Day of First PCR ≥5000 Parasites/mL	Day of First PCR ≥5000 Parasites/mL Until ≥72 h After Initiating Antimalarial Therapy	Day of Discharge From Confinement: Day of Parasite Clearance (0 Parasites/mL) AND ≥72 h After Initiating Antimalarial Therapy	EOS Visit Day 28[v]
Time point (h) in relation to *Pf*SPZ-DVI Challenge			0[b]	2						Estimated On or Before Day 19	
Ambulatory visit	X					X					X
Ambulatory visit (SARS-CoV-2 test)	X					X					
Confinement in clinical unit[c]		X	X				X	X	X	(X)	
Discharge from clinical unit				X						X	(X)
Telephonic Monitoring for AEs					X						
Eligibility criteria	X	X									
Informed consent[d]	X										
Demographics	X										
Medical and social history	X										
Beck Depression Inventory	X										
Alcohol & drug screen[e]	X	X									
Height & weight[f]	X	X									X
Physical examination[g]	X	X					X	X	X	X	X
Vital signs[h]	X	X	X	X			X	X	X	X	X
12-lead ECG[i]	X								X		
Viral serology[j]	X										
Pregnancy test[k]	X	X									X

Assessments	**Screening Visit Day -28 to Day -2**		**Challenge, treatment and follow-up**								
Study Day/Event		**Day -1[a]**	**Day 1**		**Day 2-6**	**Day 7-9**	**Day 10: Start of Confinement in Clinical Unit**	**Day of First PCR ≥250 Parasites/mL Until Day of First PCR ≥5000 Parasites/mL**	**Day of First PCR ≥5000 Parasites/mL Until ≥72 h After Initiating Antimalarial Therapy**	**Day of Discharge From Confinement: Day of Parasite Clearance (0 Parasites/mL) AND ≥72 h After Initiating Antimalarial Therapy**	**EOS Visit Day 28[v]**
Time point (h) in relation to *Pf*SPZ-DVI Challenge			**0[b]**	**2**						**Estimated On or Before Day 19**	
DVI of *Pf*SPZ Challenge			X								
Riamet®[l]									X[l]		
Malaria Clinical Score[m]			X			X	X	X	X	X	X
Haematology & liver biochemistry[n]	X	X				X	X	X	X	X	X
Biochemistry other than liver[o]	X	X					X		X		X
CRP analysis[p]		X				X	X	X	X	X	X
Coagulation parameters[q]	X	X				X	X	X	X	X	X
rRT-PCR for SARS-CoV-2	X[r]					X[s]					
qPCR for parasites[t]			X			X	X	X	X	X	X
Parasite Transcriptomics[u]						X	X	X	X	X	X
Previous medications	X										
Concomitant medications	X----------										----------X
AEs	X----------										----------X
SAE reporting	X----------										----------X

β-HCG: β-human chorionic gonadotropin, AE: adverse event, aPTT: activated partial thromboplastin time, CMV: cytomegalovirus, CRP: C-reactive protein, DVI: direct venous inoculation, EBV: Epstein Barr Virus, ECG: electrocardiogram, EOS: end of study, FSH: follicle stimulating hormone, HbsAg: hepatitis B surface antigen , HCV: hepatitis C virus, HIV: human immunodeficiency virus, IgM: immunoglobulin M, INR: international normalized ratio, *Pf*SPZ: *Plasmodium falciparum* sporozoites, PT: prothrombin time, qPCR: quantitative polymerase chain reaction, rRT-PCR: real-time reverse transcription polymerase chain reaction, SAE: serious adverse event, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

a. Assessments begin as of participant confinement in the clinical unit and must be completed and safety assessment outcomes must be available before *Pf*SPZ-DVI Challenge on Day 1.
b. On Day 1, assessments begin as of 3 h before challenge (except for urinalysis assessments, morning void allowed). The assessments indicated must be completed and safety assessment outcomes must be available before *Pf*SPZ-DVI Challenge inoculation.
c. Confinement to the clinical unit will occur as follows:
   - Participants will be admitted to the clinical unit in the morning of Day -1 and discharged from confinement 2 h post *Pf*SPZ-DVI Challenge on Day 1.
   - Participants will be admitted to the clinical unit in the morning of Day 10 and discharged on Day of discharge from confinement (Day of parasite clearance AND ≥72 h after initiating antimalarial therapy), estimated to occur on or before Day 19. Of note, participants who commence antimalarial therapy on Day 24 (see l) will only be discharged from confinement at the end of the EOS visit on Day 28.
d. No study-related procedure is to be performed before voluntarily signing of the informed consent form.
e. Alcohol breath test and urine dipstick screening for drug abuse.
f. Height to be measured at screening only; body weight to be measured at screening, at admission on Day -1 and at the EOS visit.
g. Physical examination will be conducted:
   - once at screening and once on Day -1;
   - once on Day 10 (upon admission to clinical unit for confinement);
   - once within 24 h after first PCR ≥250 parasites/mL;
   - once within 24 h after first PCR ≥5000 parasites/mL;
   - once at pre-discharge from clinical unit on Day of discharge from confinement (Day of parasite clearance AND ≥72 h after initiating antimalarial therapy); and
   - once at the EOS.

   Full physical examination will be conducted at screening, on Day -1, within ±24 h after first PCR ≥250 parasites/mL and at the EOS visit on Day 28. Targeted (symptom-driven) physical examination will be performed on the other time points in case malaria clinical score >6.
h. Vital signs (blood pressure and pulse) will be measured after remaining in a supine position for at least 10 min. All measurements will be performed as follows:
   - once at screening and once on Day -1;
   - on Day 1 (pre and post *Pf*SPZ-DVI Challenge);
   - twice daily from Day 10 (admission to clinical unit for confinement) onwards until Day of discharge from confinement (Day of parasite clearance AND ≥72 h after initiating antimalarial therapy); and
   - once at the EOS.

   Body temperature (sublingual) will also be assessed, and this whenever the malaria clinical score will be evaluated (see m).
   At screening, orthostatic changes to blood pressure and pulse rate will also be assessed: participants will be requested to stand after completion of the supine measurements and blood pressure and pulse rate will be recorded after at least 2 min in the standing position.
i. 12-lead ECGs recordings will be performed in triplicate after participants have remained in a supine position for at least 10 min. All recordings will be performed as follows:
   - once at screening; and
   - once on Days 2 and 3 of antimalarial therapy.
j. Serological testing for HIV, hepatitis A IgM antibody, HbsAg, anti-HCV antibody, hepatitis D antibody (only in subjects positive for HbsAg), hepatitis E IgM antibody, CMV IgM antibody and EBV IgM antibody, to determine eligibility for the study.
k. Pregnancy testing consists of serum β-HCG assessments at screening and at the EOS visit and a urine β-HCG assessment on Day-1.
l. Every participant will be prescribed a 3-day oral course of Riamet® (20 mg artemether and 120 mg lumefantrine tablets) (see Section 5.1). The first dose should be taken as soon as possible when a PCR threshold of 5000 parasites/mL is achieved or earlier if a participant has a malaria clinical score >6 or at Investigator's discretion. Of note, all participants inoculated with *Pf*SPZ-DVI Challenge will commence antimalarial therapy no later than Day 24, regardless if they reach the pre-defined cohort-specific PCR parasitaemia/malaria clinical score thresholds (i.e., 5000 parasites/mL blood and/or a malaria clinical score ≥6) (see Section 4.3).
   If an intolerance or contraindication to Riamet® develops since inclusion, Malarone® will be administered (see Section 5.1).

m. Malaria clinical score for malaria signs and symptoms and the vital sign parameter body temperature (sublingual) (see h) are assessed:
- on Day 1 (pre-*Pf*SPZ-DVI Challenge);
- once daily during ambulatory visits on Day 7, 8 and 9;
- twice daily from Day 10 (admission to clinical unit for confinement) onwards until Day of discharge from confinement (Day of parasite clearance AND ≥72 h after initiating antimalarial therapy); and
- once at the EOS.

n. Haematology and liver biochemistry laboratory tests will be performed after overnight fast for at least 8 h (see Section 4.3):
- once at screening and once on Day -1;
- once during ambulatory visit on Day 7;
- once on Day 10 (upon admission to clinical unit for confinement);
- once within 24 h after first PCR ≥250 parasites/mL;
- once on Day of first PCR ≥5000 parasites/mL AND prior to initiating antimalarial therapy (non-fasted state allowed);
- once at pre-discharge from clinical unit on Day of discharge from confinement (Day of parasite clearance AND ≥72 h after initiating antimalarial therapy); and
- once at the EOS.

  Troponin T will be measured at screening (fasted state), at baseline prior to inoculation on Day -1 (fasted state), and on Days 1, 2 and 3 of antimalarial therapy (non-fasted state). FSH will be measured at screening in all women.

o. Biochemistry laboratory tests other than liver (including urinalysis; morning void allowed) will be performed after overnight fast for at least 8 h (see Section 4.3):
- once at screening and once on Day -1;
- once on Day 10 (upon admission to clinical unit for confinement);
- once on Day of first PCR ≥5000 parasites/mL AND prior to initiating antimalarial therapy (non-fasted state allowed); and
- once at the EOS.

p. CRP laboratory tests will be performed at the following time points:
- on Day -1;
- once during ambulatory visit on Day 7;
- once on Day 10 (upon admission to clinical unit for confinement);
- once within 24 h after first PCR ≥250 parasites/mL;
- once on Day of first PCR ≥5000 parasites/mL AND prior to initiating antimalarial therapy;
- on Days 2 and 3 of antimalarial therapy;
- once at pre-discharge from clinical unit on Day of discharge from confinement (Day of parasite clearance AND ≥72 h after initiating antimalarial therapy); and
- once at the EOS.

q. INR, PT and aPTT laboratory tests will be performed once at screening and once on Day -1. On the other time points thereafter, only the INR test will be performed.

r. A nasopharyngeal swab will be taken at an ambulatory visit during screening up to Day -2 (preferably as close as possible to the day of admission, based on test capacity and according to national laws) from participants who are still eligible to be enrolled in the study after completing all other screening tests. An rRT-PCR test will be performed on these swabs to screen for infection with SARS-CoV-2.

s. A nasopharyngeal swab will be taken prior to confinement to the study site on Day 10, based on test capacity and according to national laws.

t. Blood samples for the assessment of parasitaemia by qPCR will be drawn at the following time points:
- on Day 1 (pre-*Pf*SPZ-DVI Challenge);
- once daily during ambulatory visits on Day 7, 8 and 9;
- twice daily from Day 10 (admission to clinical unit for confinement) onwards until Day of first PCR ≥5000 parasites/mL AND prior to initiating antimalarial therapy;
- at multiple time points (2, 6, 8, 12, 16, 24, 36, 48 and 72 h) following initiation of Riamet®;
- once on Day of discharge from confinement (Day of parasite clearance AND ≥72 h after initiating antimalarial therapy); and
- once at the EOS.

All qPCR blood samples will be drawn for immediate analysis, except for the ones drawn at the multiple time points during Riamet® therapy.

u. Blood samples for parasite transcriptomics will be drawn at the following time points:
   - once daily during ambulatory visits on Day 7, 8 and 9;
   - once daily from Day 10 (admission to clinical unit for confinement) onwards until Day of discharge from confinement (Day of parasite clearance AND ≥72 h after initiating antimalarial therapy); and
   - once at the EOS.
v. All participants will be asked non-leading questions to determine the occurrence of any AEs at the EOS visit.

Of note, for participants who commence antimalarial therapy on Day 24 (l), the Day of discharge from confinement will coincide with the EOS. In this case, applicable assessments will not be performed in duplicate.

Cohort 2

Assessments			Challenge, treatment and follow-up								
Study Day/Event	Screening Visit Day -28 to Day -2	Day -1[a]	Day 1		Day 2-6	Day 7-9	Day 10: Start of Confinement in Clinical Unit	Day of First PCR ≥250 Parasites/mL Until Day of First PCR ≥10000 Parasites/mL	Day of First PCR ≥10000 Parasites/mL Until ≥72 h After Initiating Antimalarial Therapy	Day of Discharge From Confinement: Day of Parasite Clearance (0 Parasites/mL) AND ≥72 h After Initiating Antimalarial Therapy	EOS Visit Day 28[v]
Time point (h) in relation to *Pf*SPZ-DVI Challenge			0[b]	2						Estimated On or Before Day 22	
Ambulatory visit	X					X					X
Ambulatory visit (SARS-CoV-2 test)	X					X					
Confinement in clinical unit[c]		X	X				X	X	X	(X)	
Discharge from clinical unit				X						X	(X)
Telephonic Monitoring for AEs					X						
Eligibility criteria	X	X									
Informed consent[d]	X										
Demographics	X										
Medical and social history	X										
Beck Depression Inventory	X										
Alcohol & drug screen[e]	X	X									
Height & weight[f]	X	X									X
Physical examination[g]	X	X					X	X	X	X	X
Vital signs[h]	X	X	X	X			X	X	X	X	X
12-lead ECG[i]	X								X		
Viral serology[j]	X										
Pregnancy test[k]	X	X									X
DVI of *Pf*SPZ Challenge			X								
Riamet®[l]									X[l]		

Assessments			**Challenge, treatment and follow-up**								
Study Day/Event	**Screening Visit Day -28 to Day -2**	**Day -1[a]**	**Day 1**		**Day 2-6**	**Day 7-9**	**Day 10: Start of Confinement in Clinical Unit**	**Day of First PCR ≥250 Parasites/mL Until Day of First PCR ≥10000 Parasites/mL**	**Day of First PCR ≥10000 Parasites/mL Until ≥72 h After Initiating Antimalarial Therapy**	**Day of Discharge From Confinement: Day of Parasite Clearance (0 Parasites/mL) AND ≥72 h After Initiating Antimalarial Therapy**	**EOS Visit Day 28[v]**
Time point (h) in relation to *Pf*SPZ-DVI Challenge			**0[b]**	**2**						**Estimated On or Before Day 22**	
Malaria Clinical Score[m]			X			X	X	X	X	X	X
Haematology & liver biochemistry[n]	X	X				X	X	X	X	X	X
Biochemistry other than liver[o]	X	X					X		X		X
CRP analysis[p]		X				X	X	X	X	X	X
Coagulation parameters[q]	X	X				X	X	X	X	X	X
rRT-PCR for SARS-CoV-2	X[r]					X[s]					
qPCR for parasites[t]			X			X	X	X	X	X	X
Parasite Transcriptomics[u]						X	X	X	X	X	X
Previous medications	X										
Concomitant medications	X----------										----------X
AEs	X----------										----------X
SAE reporting	X----------										----------X

β-HCG: β-human chorionic gonadotropin, AE: adverse event, aPTT: activated partial thromboplastin time, CMV: cytomegalovirus, CRP: C-reactive protein, DVI: direct venous inoculation, EBV: Epstein Barr Virus, ECG: electrocardiogram, EOS: end of study, FSH: follicle stimulating hormone, HbsAg: hepatitis B surface antigen , HCV: hepatitis C virus, HIV: human immunodeficiency virus, IgM: immunoglobulin M, INR: international normalized ratio, *Pf*SPZ: *Plasmodium falciparum* sporozoites, PT: prothrombin time, qPCR: quantitative polymerase chain reaction, rRT-PCR: real-time reverse transcription polymerase chain reaction, SAE: serious adverse event, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

a. Assessments begin as of participant confinement in the clinical unit and must be completed and safety assessment outcomes must be available before *Pf*SPZ-DVI Challenge on Day 1.

b. On Day 1, assessments begin as of 3 h before challenge (except for urinalysis assessments, morning void allowed). The assessments indicated must be completed and safety assessment outcomes must be available before *Pf*SPZ-DVI Challenge inoculation.

c. Confinement to the clinical unit will occur as follows:
   - Participants will be admitted to the clinical unit in the morning of Day -1 and discharged from confinement 2 h post *Pf*SPZ-DVI Challenge on Day 1.
   - Participants will be admitted to the clinical unit in the morning of Day 10 and discharged on Day of discharge from confinement (Day of parasite clearance AND ≥72 h after initiating antimalarial therapy), estimated to occur on or before Day 22. Of note, participants who commence antimalarial therapy on Day 24 (see l) will only be discharged from confinement at the end of the EOS visit on Day 28.

d. No study-related procedure is to be performed before voluntarily signing of the informed consent form.
e. Alcohol breath test and urine dipstick screening for drug abuse.
f. Height to be measured at screening only; body weight to be measured at screening, at admission on Day -1 and at the EOS visit.
g. Physical examination will be conducted:
   - once at screening and once on Day -1;
   - once on Day 10 (upon admission to clinical unit for confinement);
   - once within 24 h after first PCR ≥250 parasites/mL;
   - once within 24 h after first PCR ≥10000 parasites/mL;
   - once at pre-discharge from clinical unit on Day of discharge from confinement (Day of parasite clearance AND ≥72 h after initiating antimalarial therapy); and
   - once at the EOS.

   Full physical examination will be conducted at screening, on Day -1, within ±24 h after first PCR ≥250 parasites/mL and at the EOS visit on Day 28. Targeted (symptom-driven) physical examination will be performed on the other time points in case malaria clinical score >6.
h. Vital signs (blood pressure and pulse) will be measured after remaining in a supine position for at least 10 min. All measurements will be performed as follows:
   - once at screening and once on Day -1;
   - on Day 1 (pre and post *Pf*SPZ-DVI Challenge);
   - twice daily from Day 10 (admission to clinical unit for confinement) onwards until Day of discharge from confinement (Day of parasite clearance AND ≥72 h after initiating antimalarial therapy); and
   - once at the EOS.

   Body temperature (sublingual) will also be assessed, and this whenever the malaria clinical score will be evaluated (see m).
   At screening, orthostatic changes to blood pressure and pulse rate will also be assessed: participants will be requested to stand after completion of the supine measurements and blood pressure and pulse rate will be recorded after at least 2 min in the standing position.
i. 12-lead ECGs recordings will be performed in triplicate after participants have remained in a supine position for at least 10 min. All recordings will be performed as follows:
   - once at screening; and
   - once on Days 2 and 3 of antimalarial therapy.

j. Serological testing for HIV, hepatitis A IgM antibody, HbsAg, anti-HCV antibody, hepatitis D antibody (only in subjects positive for HbsAg), hepatitis E IgM antibody, CMV IgM antibody and EBV IgM antibody, to determine eligibility for the study.
k. Pregnancy testing consists of serum β-HCG assessments at screening and at the EOS visit and a urine β-HCG assessment on Day-1.
l. Every participant will be prescribed a 3-day oral course of Riamet® (20 mg artemether and 120 mg lumefantrine tablets) (see Section 5.1). The first dose should be taken as soon as possible when a PCR threshold of 10000 parasites/mL is achieved or earlier if a participant has a malaria clinical score >6 or at Investigator's discretion. Of note, all participants inoculated with *Pf*SPZ-DVI Challenge will commence antimalarial therapy no later than Day 24, regardless if they reach the pre-defined cohort-specific PCR parasitaemia/malaria clinical score thresholds (i.e., 10000 parasites/mL blood and/or a malaria clinical score ≥6) (see Section 4.3).
   If an intolerance or contraindication to Riamet® develops since inclusion, Malarone® will be administered (see Section 5.1).
m. Malaria clinical score for malaria signs and symptoms and the vital sign parameter body temperature (sublingual) (see h) are assessed:
   - on Day 1 (pre-*Pf*SPZ-DVI Challenge);
   - once daily during ambulatory visits on Day 7, 8 and 9;
   - twice daily from Day 10 (admission to clinical unit for confinement) onwards until Day of discharge from confinement (Day of parasite clearance AND ≥72 h after initiating antimalarial therapy); and
   - once at the EOS.

n. Haematology and liver biochemistry laboratory tests will be performed after overnight fast for at least 8 h (see Section 4.3):
- once at screening and once on Day -1;
- once during ambulatory visit on Day 7;
- once on Day 10 (upon admission to clinical unit for confinement);
- once within 24 h after first PCR ≥250 parasites/mL;
- once on Day of first PCR ≥10000 parasites/mL AND prior to initiating antimalarial therapy (non-fasted state allowed);
- once at pre-discharge from clinical unit on Day of discharge from confinement (Day of parasite clearance AND ≥72 h after initiating antimalarial therapy); and
- once at the EOS.

Troponin T will be measured at screening (fasted state), at baseline prior to inoculation on Day -1 (fasted state), and on Days 1, 2 and 3 of antimalarial therapy (non-fasted state).
FSH will be measured at screening in all women.

o. Biochemistry laboratory tests other than liver (including urinalysis; morning void allowed) will be performed after overnight fast for at least 8 h (see Section 4.3):
- once at screening and once on Day -1;
- once on Day 10 (upon admission to clinical unit for confinement);
- once on Day of first PCR ≥10000 parasites/mL AND prior to initiating antimalarial therapy (non-fasted state allowed); and
- once at the EOS.

p. CRP laboratory tests will be performed at the following time points:
- on Day -1;
- once during ambulatory visit on Day 7;
- once on Day 10 (upon admission to clinical unit for confinement);
- once within 24 h after first PCR ≥250 parasites/mL;
- once on Day of first PCR ≥10000 parasites/mL AND prior to initiating antimalarial therapy;
- on Days 2 and 3 of antimalarial therapy;
- once at pre-discharge from clinical unit on Day of discharge from confinement (Day of parasite clearance AND ≥72 h after initiating antimalarial therapy); and
- once at the EOS.

q. INR, PT and aPTT laboratory tests will be performed once at screening and once on Day -1. On the other time points thereafter, only the INR test will be performed.

r. A nasopharyngeal swab will be taken at an ambulatory visit during screening up to Day -2 (preferably as close as possible to the day of admission, based on test capacity and according to national laws) from participants who are still eligible to be enrolled in the study after completing all other screening tests. An rRT-PCR test will be performed on these swabs to screen for infection with SARS-CoV-2.

s. A nasopharyngeal swab will be taken prior to confinement to the study site on Day 10, based on test capacity and according to national laws.

t. Blood samples for the assessment of parasitaemia by qPCR will be drawn at the following time points:
- on Day 1 (pre-*Pf*SPZ-DVI Challenge);
- once daily during ambulatory visits on Day 7, 8 and 9;
- twice daily from Day 10 (admission to clinical unit for confinement) onwards until Day of first PCR ≥10000 parasites/mL AND prior to initiating antimalarial therapy;
- at multiple time points (2, 6, 8, 12, 16, 24, 36, 48 and 72 h) following initiation of Riamet®;
- once on Day of discharge from confinement (Day of parasite clearance AND ≥72 h after initiating antimalarial therapy); and
- once at the EOS.

All qPCR blood samples will be drawn for immediate analysis, except for the ones drawn at the multiple time points during Riamet® therapy.

u. Blood samples for parasite transcriptomics will be drawn at the following time points:
- once daily during ambulatory visits on Day 7, 8 and 9;
- once daily from Day 10 (admission to clinical unit for confinement) onwards until Day of discharge from confinement (Day of parasite clearance AND ≥72 h after initiating antimalarial therapy); and
- once at the EOS.

v. All participants will be asked non-leading questions to determine the occurrence of any AEs at the EOS visit.

Of note, for participants who commence antimalarial therapy on Day 24 (l), the Day of discharge from confinement will coincide with the EOS. In this case, applicable assessments will not be performed in duplicate.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

List of Abbreviations

ABV	Alcohol by volume
AE	Adverse event
AESI	Adverse event of special interest
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
aPTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
β-HCG	β-human chorionic gonadotropin
BMI	Body mass index
BSC	Biosafety cabinet
CHMI	Controlled Human Malaria Infection
CI	Confidence interval
CMV	Cytomegalovirus
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
DBP	Diastolic blood pressure
DVI	Direct venous inoculation
EBV	Epstein Barr Virus
ECG	Electrocardiogram
EOS	End of study
FSH	Follicle stimulating hormone
GCP	Good Clinical Practice
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HSA	Human serum albumin
IBSM	Induced blood-stage malaria
ICF	Informed Consent Form
ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IgM	Immunoglobulin M
IMP	Investigational medicinal product
INR	International normalized ratio
IRB	Institutional Review Board
ITM	Centre of excellence for tropical medicine
$Log_{10}PRR_{48}$	Log_{10} parasite reduction ratio per 48 h
$Log_{10}PRR_{48,max}$	Maximum log_{10} parasite reduction ratio per 48 h
LPLV	Last Patient Last Visit
MedDRA	Medical Dictionary for Regulatory Activities
NCE	New chemical entities
NSAID	Non-steroidal anti-inflammatory drug
PBS	Phosphate buffered saline

$PC_{½}$	Parasite clearance half-life
PCR	Polymerase chain reaction
PD	Pharmacodynamics
Pf	*P. falciparum*
P. falciparum	*Plasmodium falciparum*
*Pf*SPZ	*P. falciparum* sporozoites
*Pf*SPZ-DVI Challenge	Inoculation of *P. falciparum* sporozoites through direct venous inoculation
PI	Principal Investigator
PMR	Parasite multiplication rate
PMR_{48}	Parasite multiplication rate standardised to 48 h
PMR_{LC}	Parasite multiplication rate per life cycle (if not 48 h)
PRR	Parasite reduction ratio
PT	Prothrombin time
qPCR	Quantitative polymerase chain reaction
QTc	Corrected QT interval
QTcB	QT interval corrected according to Bazett's formula
QTcF	QT interval corrected according to Fridericia's formula
RBC	Red blood cell
rRT-PCR	Real-time reverse transcription polymerase chain reaction
SAE	Serious adverse event
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SBP	Systolic blood pressure
SD	Standard deviation
SmPC	Summary of Product Characteristics
SPZ	Sporozoites
SRT	Safety Review Team
TBS	Thick Blood Smear
ULN	Upper Limit of Normal
*var*ATS	The acidic terminal segment in *Plasmodium falciparum var* genes
VIS	Volunteer Infection Study
WBC	White blood cell
WHO	World Health Organization

Definitions of Terms

BMI	Weight in kilogram divided by the square of height in meters
QTcB	QT interval corrected according to Bazett's formula: QTcB (ms)=QT (ms)/$RR^{1/2}$ where RR=(60/heart rate)*1000
QTcF	QT interval corrected according to Fridericia's formula: QTcF (ms)=QT (ms)/$RR^{1/3}$ where RR=(60/heart rate)*1000
Laboratory-defined PCR positivity	qPCR outcome 50-249 parasites per mL blood
Protocol-defined PCR positivity	qPCR outcome ≥250 parasites per mL blood
PCR negative	Applies to all qPCR assessments undertaken prior to initiating antimalarial therapy, with a value of 1-49 parasites per mL blood
Parasite clearance	Defined as a qPCR value of 0 parasites per mL blood after initiating antimalarial therapy
Time to first PCR positivity	Time elapsed between *Pf*SPZ-DVI Challenge and first qPCR outcome equal to or greater than 250 parasites per mL blood, with a maximum of 28 days in the absence of parasitaemia
Time to parasitaemia of ≥5000 parasites per mL blood	Time elapsed between *Pf*SPZ-DVI Challenge and first qPCR equal to or greater than 5000 parasites per mL blood, with a maximum of 28 days in the absence of parasitaemia
Time to parasitaemia of ≥10000 parasites per mL blood	Time elapsed between *Pf*SPZ-DVI Challenge and first qPCR equal to or greater than 10000 parasites per mL blood, with a maximum of 28 days in the absence of parasitaemia
Parasite growth rate	Expressed as the PMR_{48} or as the PMR_{LC}
Time to parasite clearance	Time between drug administration and first PCR below the limit of quantification
$Log_{10}PRR_{48}$	Ratio of the parasite density at a specific time point to the parasite density 48 h later and expressed in log_{10}
$Log_{10}PRR_{48,max}$	Maximum $log_{10}PRR_{48}$
$PC_{½}$	Time taken for the parasite density to be reduced by 50% after the first dose administration of Riamet®

STUDY ADMINISTRATIVE STRUCTURE AND INVESTIGATORS

SPONSOR	Medicines for Malaria Venture (MMV) ICC – Building G 20, route de Pré-Bois P O Box 1826 1215 Geneva 15 Switzerland
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1. INTRODUCTION

1.1 MALARIA BACKGROUND INFORMATION

Malaria is an infectious disease that threatens half of the world's population and is caused mainly by two protozoan parasites: *Plasmodium falciparum* (*P. falciparum*) and *Plasmodium vivax*. The World Health Organization (WHO) reported 219 million cases of the disease in 2017. In the same year, malaria caused 435000 deaths; 93% of the mortality was in Africa, with children <5 years of age attributing 61.1% of deaths [29].

Around the world, effective disease control programs relying on artemisinin-containing combination therapies (ACT) such as Coartem®/Riamet® have contributed to a global reduction in the mortality rate of *P. falciparum* malaria. However, recent reports suggest that decades of continuous use of artemisinins as monotherapies may have fostered drug resistance against artemisinin-derivatives, the last widely effective antimalarial drugs [28].

Malaria is a serious and life-threatening disease and due to increasing resistance of the current antimalarial therapies, new antimalarial therapies are required for both treatment and prevention of the disease to address a growing unmet medical need.

1.2 MALARIA CHMI/VIS MODELS

Volunteer Infection Studies (VIS) in malaria (previously referred to as Controlled Human Malaria Infection (CHMI) studies) are a valuable tool in antimalarial drug development, providing an opportunity to rapidly assess drug efficacy and obtain pharmacokinetic and pharmacodynamic (PD) data in healthy, non-immune volunteers and earlier entry into patients. VIS entail deliberate infection with malaria parasites, either by inoculation of sporozoites through mosquito bite or direct injection of sporozoites (i.e., inoculation of *P. falciparum* sporozoites through direct venous inoculation, *Pf*SPZ-DVI Challenge) or by direct venous injection of parasitised erythrocytes (i.e., Induced Blood-Stage Malaria, IBSM). VIS has been applied in malaria vaccine and drug development, where it is used to evaluate products in well-controlled early-phase proof-of-concept clinical studies, thus facilitating progression of only the most promising candidates for further evaluation in areas where malaria is endemic.

1.3 *PLASMODIUM FALCIPARUM* SPOROZOITES CHALLENGE MODEL (*PF*SPZ CHALLENGE)

Recent advances to support vaccine and drug development have included cryopreservation of sporozoites (*Pf*SPZ-DVI Challenge model). *Pf*SPZ-DVI Challenge consists of aseptic, purified, live, fully infectious, cryopreserved (to maintain potency for an extended period) *Plasmodium falciparum* (*Pf*) sporozoites (SPZ) (*Pf*SPZ) obtained from the salivary glands of *Anopheles stephensi* mosquitoes reared under aseptic conditions and infected with *Pf*SPZ of either the NF54 strain or 7G8 clone. This advance will help to accelerate malaria vaccine and drug development by making the reagents for VIS more widely accessible and enabling a more rigorous evaluation with multiple parasite strains and species.

The *Pf*SPZ-DVI Challenge agent was developed to be used to infect Phase I volunteers in VIS to assess the protective efficacy or efficacy of antimalarial drugs and vaccines, and

the effects of naturally acquired immunity and innate resistance. By facilitating the development of liver stage infection, *Pf*SPZ-DVI Challenge can be utilized in the assessment of causal prophylactic activity of drugs, in contrast with IBSM model which results in blood-stage infection only, and consequently, for only assessing drug activity against the blood stage of malaria. As is the case with IBSM, the *Pf*SPZ-DVI Challenge model, which has been extensively used for testing vaccine and drug prevention strategies, has the potential to also be utilised in the assessment of blood-stage activity of new antimalarial drugs, and thus increasing the existing MMV and MMV partners' capability for conducting blood-stage malaria VIS at various study centres during early drug development of new chemical entities (NCEs).

Conducting VIS in antimalarial vaccine and drug studies with *Pf*SPZ-DVI Challenge as compared to *P. falciparum* sporozoite-infected mosquitoes or parasitised red blood cells (RBCs) (IBSM model) provides the following potential advantages:

1. Capacity to conduct clinical studies at multiple sites that do not have access to *Pf*SPZ-infected mosquitoes or a Master Cell Bank with parasitised erythrocytes;
2. Increased capacity to conduct VIS critical to the development of NCEs/drugs for acute treatment of blood-stage malaria;
3. Capacity to determine and control the specific numbers of *Pf*SPZ with which volunteers are inoculated;
4. Capacity to eliminate the potential impact of batch to batch variation on infectivity of sporozoites when performing parallel clinical studies at multiple sites or sequential clinical studies at the same site
5. Capacity to conduct VIS in volunteers without the study schedule being contingent upon a time-consuming and financially expensive process for producing infected mosquitoes that must be used within a one to two-week window. Benefits include the ability to perform repeated low-dose VIS for time-course studies with vaccines and drugs, to stagger the VIS for different groups, to "catch-up" volunteers that miss the scheduled VIS day, and to use adaptive study designs where the date and nature of VIS can be made dependent on outcome variables measured during the study;
6. Potentially significantly reduced costs.

*Pf*SPZ-DVI Challenge has been safely used in multiple clinical studies in the United States, Europe and Africa via the intradermal (ID), intravenous (IV) and intramuscular (IM) routes [1,2,7,8,10,11,18,19,23,24,25,26]. As of 17 April 2018, 913 volunteers have received 1439 doses of *Pf*SPZ Challenge (NF54) and 34 volunteers have received 34 doses of PfSPZ Challenge (7G8). Extensive safety and efficacy data are available [9]. In addition, *Pf*SPZ-DVI Challenge has been safely administered to volunteers in combination with antimalarial drugs and to assess the effects of naturally acquired immunity and innate resistance (e.g., sickle cell trait). Currently, the validated inoculation method for drug and vaccine evaluation consists of direct venous inoculation (DVI) with an inoculum size of 3200 sporozoites which has been shown to induce subclinical malaria in 100% of inoculated healthy volunteers [18].

For more detailed information on the *Pf*SPZ-DVI Challenge (NF54 strain) used in this study, refer to the current IB [9].

1.4 ANTIMALARIAL THERAPY

The registered antimalarial therapy Riamet® (tablet containing 20 mg artemether and 120 mg lumefantrine) has been approved for the treatment of acute uncomplicated *P. falciparum* malaria infection in adults, children and newborns weighing ≥5 kg. For adult patients, the treatment regimen consists of 6 doses of 4 tablets administered over a period of 60 h (1 intake at diagnosis and the 5 following doses at 8, 24, 36, 48 and 60 h after the initial dose). This Riamet® therapeutic regimen was tested in the IBSM model in Australia [15] and will also be used in this study.

In adults, the most frequently reported adverse reactions are decreased appetite, sleep disorders, headache, dizziness, palpitations, vomiting, abdominal pain, nausea, arthralgia and myalgia (reported in ≥1/10 patients [very common]) and insomnia, paresthesia, clonus electrocardiogram (ECG) QT prolonged, diarrhoea, rash and pruritus (reported in ≥1/100 to <1/10 patients [common]).

For more information on Riamet®, refer to the Summary of Product Characteristics (SmPC) [22].

1.5 OVERALL RATIONALE FOR THE STUDY

The intent of this study is to evaluate the feasibility of *Pf*SPZ-DVI Challenge in the assessment of NCEs and drugs for the acute treatment of blood-stage malaria. Unlike malaria chemoprotection studies where study participants are treated early with rescue medication, the intention of utilising the *Pf*SPZ-DVI Challenge for the assessment of NCEs/drugs for the acute treatment of blood-stage malaria, is to safely and reliably generate blood-stage malaria with appropriate/acceptable parasitaemia densities in healthy participants to generate key data supporting further dose selection for patient studies in the field. To our knowledge the *Pf*SPZ-DVI Challenge model has not been used for assessing antimalarial blood-stage activity in an early-drug development context, and a study in healthy participants is needed to determine its safety/tolerability and feasibility in assessing antimalarial blood-stage activity.

Existing evidence in support of this methodology in achieving blood-stage parasitaemia in healthy participants, whilst demonstrating acceptable safety and tolerability is based on published literature [7,18,10,2].

1.6 RISK BENEFIT ANALYSIS

1.6.1 *Potential Risks*

Participants receiving the *Pf*SPZ-DVI Challenge will be exposed to potential risks, including the development of malaria symptoms as well as adverse effects of the registered antimalarial therapy (Riamet® or Malarone®; see SmPCs [22] and [12]) to be prescribed as curative therapy for parasite clearance prior to the end of the study.

The VIS *Pf*SPZ-DVI Challenge model with the DVI technique is a well-validated method for obtaining informative data on the clinical activity of novel antimalarial compounds. It has replaced the traditional method of CHMI by the bite of mosquitos to measure vaccine and drug efficacy. In a series of clinical studies, inoculation of *Pf*SPZ-DVI Challenge was found to be safe and well tolerated with the optimal dose established as 3200 *Pf*SPZ administered by DVI [18].

Overall, the PCR (Polymerase chain reaction) monitoring used in these studies ensure early treatment preventing high levels or prolonged duration of parasitaemia that would put the participant at undue risk. Severe/complicated malaria has never been reported in a VIS. Mild malaria symptoms are expected in some study participants and include headache, myalgia, fever, chills, sweats, nausea, vomiting and diarrhoea. Rapid diagnosis by quantitative PCR (qPCR) and subsequent treatment by Riamet® in the study is expected to quickly attenuate the illness so that the infection does not place the participant at undue risk.

Risks to participants in this Phase Ib study will be minimised in the following ways:

- Sentinel strategy for both Cohort 1 and 2 (please refer to Section 3.1);
- Progression to consecutive study Cohort 2 only after evaluation of data from previous Cohort 1 and approval from Safety Review Team (SRT) (please refer to Section 3.4);
- Adherence to the inclusion/exclusion criteria: only participants considered suitable according to these criteria and who are not at any perceived risk will be included;
- The dose of *Pf*SPZ-DVI Challenge and route of administration has previously been shown to be safe and well-tolerated in healthy human participants [2,7,10,18];
- Routine monitoring to ensure the safety and well-being of the study participants will include the following:
    - Admission to the clinical unit in the morning of Day -1, i.e. before *Pf*SPZ-DVI Challenge, until 2 h post *Pf*SPZ-DVI Challenge and again from Day 10 in the morning until parasite clearance (defined as a qPCR value of 0 parasites per mL blood after initiating antimalarial therapy) and at least 72 h after initiating antimalarial therapy or until Day 28 when antimalarial therapy initiated on Day 24;
    - Vital signs (blood pressure, heart rate);
    - 12-lead ECGs;
    - Safety laboratory assessments of biochemistry and haematological blood parameters performed at scheduled time points and repeated, if necessary (including repeat of any alanine aminotransferase [ALT] or aspartate aminotransferase [AST] >2x ULN), to ensure appropriate follow-up of any clinically relevant abnormality;
    - Daily parasitaemia assessments by qPCR during daily visits to the clinical unit from Day 7 until Day 9 and twice daily from Day 10 post inoculation until parasite clearance and at the EOS visit at Day 28;
    - Objective/directed evaluation of Malaria signs and symptoms through a clinical score.
- All participants will be prescribed curative therapy for malaria for radical clearance during the study;
- The total volume of blood drawn per the Time and Events Schedule from each participant will not exceed the maximum allowable volume of approximately 460 mL;
- In addition to the Principal Investigator (PI) or his representative, the site has access to an expert medical malariologist to help in making clinical decisions as

necessary, including in the rare event that hospitalisation is required, which will be done at the Antwerp University Hospital (UZA). Serious adverse event (SAEs) will be treated according to their nature at a specialist inpatient facility.

1.6.2 *Potential Benefits*

Benefits of the study are society-based and related to contributing to the future development of new antimalarial therapies. Taking into consideration the proposed risk-management plan as described, the risk to participants participating in this study is considered to be minimal and acceptable.

1.6.3 *Conclusion*

No benefit is expected for individual participants participating in this study. Benefits of the study are society-based and related to possible future antimalarial therapies that would be developed using this VIS model. Taking into consideration the proposed risk-management plan as described, the risk to participants participating in this study is considered to be minimal and acceptable.

2. OBJECTIVES AND ENDPOINTS

Table 1: Objectives and Endpoints

OBJECTIVES	ENDPOINTS For definitions of terms, see List of Abbreviations and Definitions of Terms
Primary	
• To evaluate the safety and tolerability associated with blood-stage malaria infection in naïve healthy participants after *Pf*SPZ-DVI Challenge.	• The incidence and severity of observed (i.e., malaria signs and symptoms graded by the malaria clinical score; see Attachment 1) or self-reported adverse events (AEs) considered *Pf*SPZ-DVI Challenge inoculum-related until end of study (EOS) visit.
	• The change in malaria clinical score from *Pf*SPZ-DVI Challenge until parasite clearance.
	• Changes from baseline in haematology, clinical chemistry and urinalysis parameters, vital signs and ECG parameters.

OBJECTIVES	**ENDPOINTS** For definitions of terms, see List of Abbreviations and Definitions of Terms
Primary	
• To characterise key stages in the blood-stage parasite growth profile in malaria infection in naïve healthy participants after *Pf*SPZ-DVI Challenge.	• The parasite growth profiles of *P. falciparum* NF54 after infection via injection of sporozoites by DVI will be characterised by: o time to first PCR positivity (for the purpose of this study, 'PCR positivity' is used for the 'protocol-defined PCR positivity', unless mentioned otherwise); o parasitaemia at first PCR positivity; o time to parasitaemia of ≥5000 parasites per mL blood (Cohorts 1 and 2); o parasitaemia at the time parasitaemia ≥5000 parasites per mL blood (Cohorts 1 and 2); o time to parasitaemia of ≥10000 parasites per mL blood (Cohort 2); o parasitaemia at the time parasitaemia ≥10000 parasites per mL blood (Cohort 2); o time to first dose of treatment with artemether-lumefantrine (Riamet®) (Cohorts 1 and 2); and o parasitaemia at first dose of treatment with Riamet® (Cohorts 1 and 2). • The number and proportion of participants with presence of positive PCR and parasitaemia of ≥5000 or ≥10000 parasites per mL blood between inoculation with *Pf*SPZ-DVI Challenge and Day 28 (per cohort).

OBJECTIVES	**ENDPOINTS** For definitions of terms, see List of Abbreviations and Definitions of Terms
Secondary	
• To determine the safety and tolerability of Riamet® in blood-stage malaria infection in naïve healthy participants after *Pf*SPZ-DVI Challenge.	• The incidence, severity and relationship to Riamet® of observed or self-reported, treatment-emergent adverse events (TEAEs) until EOS visit.
• To characterise the blood-stage parasite profile in malaria infection in naïve healthy participants after *Pf*SPZ-DVI Challenge.	• The blood-stage parasite profile of *P. falciparum* NF54 after infection via injection of sporozoites by DVI will be characterised by: o parasite growth rate expressed as the parasite multiplication rate (PMR) standardised to 48 h (PMR_{48}) (Cohort 1, Cohort 2 and overall); o parasite growth rate expressed as the PMR per life cycle (if not 48 h) (PMR_{LC}) (Cohort 1, Cohort 2 and overall); and o predicted time to reach parasitaemia threshold of first positive PCR, ≥5000 parasites per mL blood, ≥10000 parasites per mL blood for Cohorts 1 and 2.
• To characterise the blood-stage parasite clearance profile of Riamet® in malaria infection in naïve healthy participants after *Pf*SPZ-DVI Challenge.	• The effect of Riamet® on clearance of *P. falciparum* blood-stage parasites will be evaluated using: o time to parasite clearance (Cohort 1, Cohort 2 and overall); o $\log_{10}$ parasite reduction ratio per 48 h ($\log_{10}PRR_{48}$) (Cohort 1, Cohort 2 and overall); o maximum $\log_{10}PRR_{48}$ ($\log_{10}PRR_{48,max}$) (Cohort 1, Cohort 2 and overall); and o parasite clearance half-life ($PC_{½}$) (Cohort 1, Cohort 2 and overall).

OBJECTIVES	**ENDPOINTS** For definitions of terms, see List of Abbreviations and Definitions of Terms
Exploratory	
• To characterise the individual variability of blood-stage parasite profile.	• The individual variability of blood-stage parasite profile will be characterised by: o the inter-individual variability of the parasite growth rate; and o predicted time to reach parasitaemia threshold of first positive PCR, ≥5000 parasites per mL blood, ≥10000 parasites per mL blood including their 95% confidence interval (CI).

HYPOTHESIS: This study is exploratory in nature and therefore no hypothesis will be tested.

3. STUDY DESIGN

3.1 OVERVIEW OF STUDY DESIGN

This is a single-centre, open-label, Phase Ib study designed to assess if intravenous bolus injection of approximately 3200 *P. falciparum* (NF54 strain) sporozoites can be safely administered to achieve blood-stage parasitaemia with a kinetics/PCR profile that will allow for the future characterisation of antimalarial blood-stage activity of NCEs in a relatively small number of participants during early drug development.

Up to 16 healthy, malaria-naïve males and females, aged 18-55 years, will be enrolled in a maximum of 2 cohorts (up to 8 participants per cohort; a participant may be enrolled in one cohort only). Enrolment into the cohorts will proceed sequentially, with two target levels of parasitaemia previously achieved in healthy participants enrolled in malaria VIS at other study sites (see Section 3.2), i.e., 5000 parasites/mL blood in Cohort 1 and 10000 parasites/mL blood in Cohort 2.

Each participant will be admitted to the clinical unit in the morning of Day -1 and inoculated with approximately 3200 *P. falciparum* sporozoites (NF54 strain) by DVI on Day 1. Participants will be discharged 2 h post inoculation on Day 1 and will be monitored daily via phone call from Day 2 until Day 6 to solicit any AEs. Participants will come to the clinical unit daily from Day 7 until Day 9 and together with the malaria clinical score (see Attachment 1), the presence of parasites will be assessed once daily by a specific qPCR targeting the *var*ATS (the acidic terminal segment in *Plasmodium falciparum var* genes) multigenic family; this to accurately describe parasite growth even in case PCR positivity, i.e., a qPCR outcome ≥250 parasites per mL blood, is confirmed this early (very low probability and with low densities). Participants will be confined to the clinical unit from Day 10 in the morning. qPCR will be performed and malaria clinical score assessed twice daily and participants will be administered registered antimalarial therapy, i.e., Riamet® (see Section 5.1), when the following criteria are met:

1. Cohort 1: ≥5000 parasites/mL blood or earlier if a participant has a malaria clinical score >6 or at Investigator's discretion.
2. Cohort 2: ≥10000 parasites/mL blood or earlier if a participant has a malaria clinical score >6 or at Investigator's discretion.

The registered 3-day antimalarial therapy regimen will be further administered and monitored. qPCR assessments of parasitaemia will be carried out at multiple time points (2, 6, 8, 12, 16, 24, 36, 48 and 72 h) following initiation of Riamet® and malaria clinical score will be assessed twice daily during confinement in the clinical unit. Safety and tolerability will be monitored during the whole study duration, specific assessments will be done at periodic pre-specified time points from Day 10 and for at least 72 h after initiating antimalarial therapy, i.e., during confinement in the clinical unit (see below).

Of note, all participants must consent to receiving antimalarial therapy, i.e., the registered 3-day Riamet® regimen approved for treatment of uncomplicated malaria. Even in the case of withdrawal from the study, all participants administered the *Pf*SPZ-DVI Challenge are to receive antimalarial therapy as soon as possible, and to have all appropriate visits and assessments as required. If an intolerance or contraindication to Riamet® develops, Malarone® will be administered (see Section 5.1).

Upon parasite clearance (defined as a qPCR value of 0 parasites per mL blood after initiating antimalarial therapy) and at least 72 h after initiating antimalarial therapy (estimated to occur on or before Day 19 and on or before Day 22 for Cohort 1 and Cohort 2, respectively), and if clinically well, participants will be discharged from the clinical unit and will be followed up for safety assessments, clinical evaluation and malaria qPCR in the clinical unit at the EOS visit on Day 28.

All participants who received antimalarial therapy will be asked non-leading questions to determine the occurrence of any AEs throughout the study and at the EOS visit.

All participants inoculated with *Pf*SPZ-DVI Challenge will commence antimalarial therapy no later than Day 24 for both cohorts, regardless if they reach pre-defined cohort-specific PCR parasitaemia/malaria clinical score thresholds (i.e., 5000 parasites/mL blood for Cohort 1 and 10000 parasites/mL blood for Cohort 2 and/or a malaria clinical score ≥6 for Cohorts 1 and 2). Participants who start antimalarial therapy on Day 24 will only be discharged from confinement at the end of the EOS visit on Day 28.

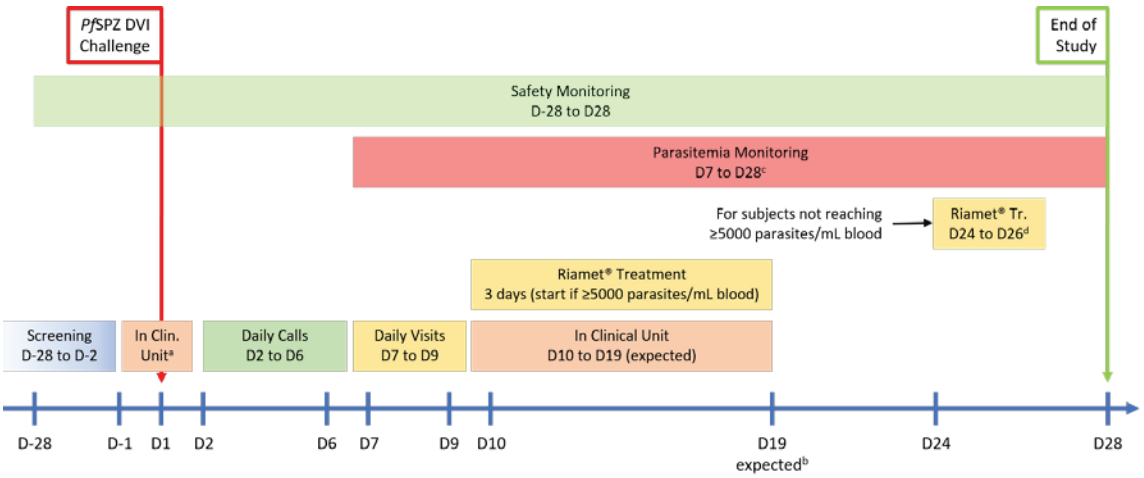
Antimalarial therapy may be initiated whenever deemed necessary by the Investigators, e.g., if there is a concern regarding the safety of a study participant (see Section 5.1). Therapy may be amended according to the treating physician if the participant does not respond to treatment or the condition worsens.

A sentinel strategy will be employed in Cohorts 1 and 2. Each cohort will consist of 2 subgroups of participants, to be enrolled sequentially: Subgroup 1 will be composed of 2 participants; Subgroup 2 will be composed of 6 participants. Participants in Subgroup 2 will not be treated until the last participant in Subgroup 1 has completed antimalarial therapy and only upon decision of the PI, medical director and medical monitor after review of available safety and tolerability data (safety assessments, AEs and malaria clinical scores) and parasitaemia data, including clearance of parasitaemia, of Subgroup 1.

Review of available Cohort 1 safety/tolerability and parasitaemia data up to the last dose of Riamet® for all inoculated participants in Cohort 1 will be performed by an SRT before enrolment of Cohort 2 (see Section 3.4).

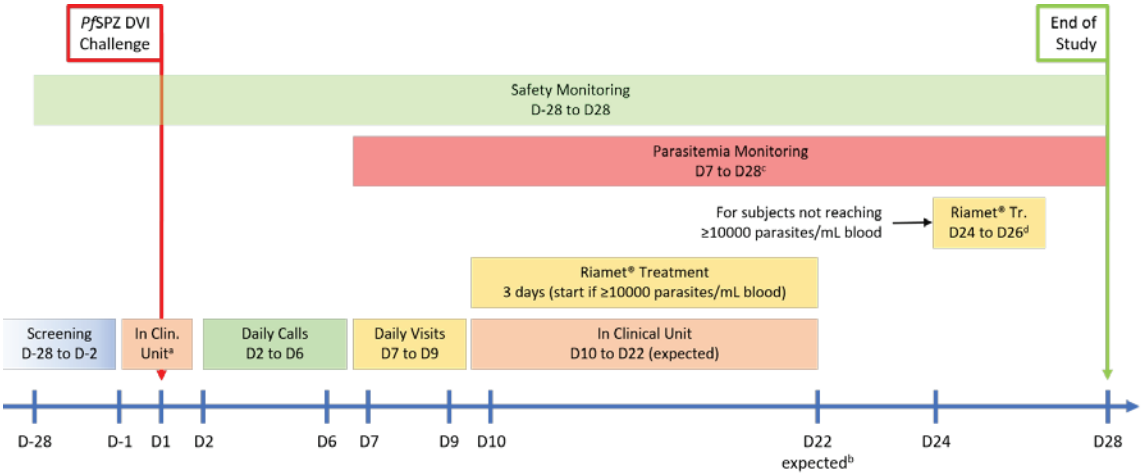
A schematic overview of the study design is shown in Figure 1 for Cohort 1 and in Figure 2 for Cohort 2. The assessments to be performed are summarised per visit in the Time and Events Schedule for Cohort 1 and Cohort 2.

Figure 1: Schematic Overview of the Study for Cohort 1



a. Confinement in clinical unit from Day -1 until 2 h *Pf*SPZ DVI challenge.
b. Upon parasite clearance (defined as a qPCR value of 0 parasites per mL blood after initiating antimalarial therapy) and at least 72 h after initiating antimalarial therapy (estimated to occur on or before Day 19), and if clinically well, subjects will be discharged from the clinical unit.
c. Twice daily during confinement in clinical unit and at all timepoints specified in Time and Events schedule (eg, during Riamet® treatment and EOS).
d. Participants who start antimalarial treatment on Day 24 will remain confined in the Clinical Unit until EOS.

Figure 2: Schematic Overview of the Study for Cohort 2



a. Confinement in clinical unit from Day -1 until 2 h *Pf*SPZ DVI challenge.
b. Upon parasite clearance (defined as a qPCR value of 0 parasites per mL blood after initiating antimalarial therapy) and at least 72 h after initiating antimalarial therapy (estimated to occur on or before Day 22), and if clinically well, subjects will be discharged from the clinical unit.
c. Twice daily during confinement in clinical unit and at all timepoints specified in Time and Events schedule (eg, during Riamet® treatment and EOS).
d. Participants who start antimalarial treatment on Day 24 will remain confined in the Clinical Unit until EOS.

3.2 DISCUSSION OF STUDY DESIGN AND DOSE SELECTION

Dose Selection

Currently, the validated inoculation method for drug and vaccine evaluation consists of direct venous inoculation (DVI) with an inoculum size of 3200 sporozoites which has been shown to induce subclinical malaria in 100% of inoculated healthy volunteers [18].

Based on published data in Phase I participants inoculated with malaria, the registered regimen of artemether-lumefantrine (Riamet®) will clear asexual parasitaemia in less than 48 h [15].

Target PCR-Based Parasitaemia Thresholds Informing the Initiation of Antimalarial Therapy

The proposed PCR-based parasitaemia thresholds to initiate antimalarial therapy have been selected to maximise participant safety whilst ensuring study objectives are met through efficient conduct of the study as follows:

1. Cohort 1: ≥5000 parasites/mL blood or earlier if a participant has a malaria clinical score >6 or at Investigator's discretion.
2. Cohort 2: ≥10000 parasites/mL blood or earlier if a participant has a malaria clinical score >6 or at Investigator's discretion.

The aim of the proposed clinical study is the evaluation of the feasibility of *Pf*SPZ-DVI Challenge to safely generate adequate levels of asexual blood-stage malaria parasitaemia for the evaluation of NCEs/drugs for acute treatment of blood-stage malaria. There is available evidence from published Phase I validation studies supporting the feasibility of this model for this purpose:

1. A Phase I *Pf*SPZ-DVI Challenge study [7,18] was conducted in healthy volunteers, including inoculation of 3200 *Pf*SPZ-DVI Challenge in 6 participants resulting in:
   - Infectivity rate of 100%;
   - Geometric mean time to Thick Blood Smear (TBS)-positive asexual parasitaemia of 11.4 days (11.0, 10.4, 12.3, 10.9, 11.9, 12.2 days);
   - Geometric mean time to qPCR-positive asexual parasitaemia of 8.3 days (6.9, 9.1, 8.0, 8.0, 9.0, 8.9 days);
   - Geometric mean parasite density at time of TBS-positivity (parasites/mL blood) of 6600 (5000, 1300, 10000, 8000, 12000, 14000 parasites/mL blood); and
   - *Pf*SPZ-DVI Challenge was well tolerated with AEs occurring at the time of parasitaemia detection. AEs were mostly grade 1 (51/85) or grade 2 (28/85) in severity, with only a few grade 3 AEs reported (6/85). No SAEs occurred.
2. A Phase I study was conducted which assessed standardised VIS by DVI of 3200 *Pf*SPZ in healthy, adult, lifelong malaria–exposed volunteers [10]. The study included 25 participants in total, of which 5 were healthy and non-immune/malaria-naïve. Findings in these 5 participants are as follows:
   - Infectivity rate of 100% confirmed by TBS and qPCR;
   - Geometric mean time to TBS-positive parasitemia of 12.6 days (range:12-14 days);
   - Geometric mean time to qPCR-positive parasitemia of 7.9 days (range: 7-9 days);
   - Geometric mean parasite density at first parasitemia of 5000 parasites/mL blood (range: 2000-9000 mL); and
   - *Pf*SPZ-DVI Challenge was well tolerated and no serious AE occurred during the study. In the 5/5 healthy, malaria-naïve participants, majority of AEs were grade 1 (27/50) in severity. A total of 13/50 AEs were grade 2 and a further 10/50 AEs were grade 3 in severity.

3. In addition, the chemoprotection MMV_P218_17_01 study conducted in Belgium (EudraCT number: 2018-003004-39) [2] data indicate:
   - An infectivity rate of 100%, with asexual parasitaemia detected in 6 out of the 6 inoculated participants administered placebo. Although the study protocol required administration of rescue treatment at the threshold of 250 parasites/mL blood, 2/6 participants achieved parasite densities of 1583 and 4432 parasites/mL blood at the time of rescue treatment administration;
   - In Cohort 1 (2 x 1000 mg doses of P218 administered 48 h apart, no sporozoite inoculation): No SAEs or severe AEs were reported. A total of 6 AEs occurred in 3/8 participants, all 6 were grade 1 AEs (mild). Only 1/6 AEs (diarrhoea, grade 1) was assessed as being related/suspected to be due to P218 with the remaining 5/6 deemed not related to P218. In addition, no clinically significant findings were noted on vital signs, ECG (no clinically concerning prolongation in QT interval corrected according to Fridericia's formula [QTcF] [5]) and laboratory investigations. These findings supported the Safety Review Committees' decision after Cohort 1, to proceed with Cohort 2;
   - In Cohort 2 (sporozoite inoculation followed by 2 x 1000 mg doses of P218 administered 48 h apart): No SAEs or severe AEs were reported. A total of 26 AEs occurred in 11/12 participants, with 21/26 grade 1 AEs (mild) and 5/26 grade 2 AEs (moderate). A total of 2/26 AEs in 1 participant (2 headache events, grade 1) was assessed as being related to P218 with the remaining 24/26 AEs deemed not related to P218. A total of 4/26 AEs in 2 participants (fatigue, malaise, flu-like symptoms and transaminase elevation) was assessed as being related to *Pf*SPZ-DVI Challenge. In addition, no clinically significant findings were noted on vital signs, ECG (no clinically concerning QTc prolongation) and laboratory investigations. These findings supported the Safety Review Committees' decision after Cohort 2, to proceed with Cohort 3;
   - In Cohort 3 (sporozoite inoculation followed by 2 x 100 mg doses of P218 administered 48 h apart): No SAEs or severe AEs were reported. A total of 7 AEs occurred in 4/12 participants, all were grade 1 AEs (mild). All AEs were considered not related to P218. A total of 2/7 AEs in two participants (both flu-like symptoms) was assessed as being related to *Pf*SPZ-DVI Challenge. In addition, no clinically significant findings were noted on vital signs, ECG (no clinically concerning QTc prolongation) and laboratory investigations; and
   - Of note was the occurrence of a transient, asymptomatic transaminase elevation (ALT) >3x the upper limit of normal (ULN) (with increases ≤3x ULN in AST and no effect on bilirubin) in the participant who achieved a peak parasitaemia of 4432 parasites/mL. ALT/AST values were back to normal range before study end without specific intervention.

Further indirect evidence in support of the safety rationale for the proposed PCR-thresholds is derived from completed MMV-sponsored IBSM studies conducted in Australia with the NCEs DSM265 [3] and MMV390048 [14]. The level of parasitaemia

to be observed in our proposed pilot study for both Cohorts 1 (5000 parasites/mL) and 2 (10000 parasites/mL) is expected to be covered by the range of parasitaemia documented in these Australian Phase I IBSM studies:

1. The IBSM study with DSM265 [3] comprised of 8 healthy participants who developed asexual blood stage parasitaemia following inoculation with *P. falciparum* parasitised erythrocytes (IBSM model). Among the 7 participants who became parasitaemic, the geometric mean parasitaemia upon initial dosing with 400 mg DSM265 was 7,851 parasites/mL blood (95% CI, 2,245 to 27,462 parasites/mL blood). No SAEs were reported. A total of 88 AEs which were mostly mild in severity (87.5%) were reported, and the majority were deemed related to malaria parasite infection (77.3%). Eleven AEs were moderate in severity, while none were classified as severe. The severity of the malaria symptoms and signs experienced by the participants during the study was generally mild, as assessed by use of a malaria clinical scoring tool. At the time the first dose of DSM265 was administered, 4 of the 7 participants who were parasitaemic had no malaria symptoms or signs (clinical score, 0), while the remaining 3 participants each had a score of 3. The peak malaria clinical score recorded during the study was 8; this was recorded for a single participant approximately 36 h after administration of the first dose of DSM265.
2. The IBSM study with MMV390048 [13] comprised of 15 healthy volunteers, who developed asexual blood stage parasitaemia following inoculation with *P. falciparum* parasitised erythrocytes (IBSM model). As the protocol was calling for the full cohort to be inoculated on the same study day, asexual blood stage parasitaemia densities prior to receiving any investigational medicinal product (IMP) was variable between participants and ranged from 1508 parasites/mL blood to 95851 parasites/mL blood. Additionally, the malaria clinical score associated with the timing of this parasite density in each of these 15 participants, included a score of 0/42 (absence of malaria symptoms/signs) in 10/15 participants, a score of 1/42 in 2/15 participants, a score of 2/42 in 2/15 participants and a score of 3/42 in 1/15 participants.
3. Due to the mechanism of action of the test drug and evaluation of doses associated with suboptimal efficacy, peak asexual blood stage parasitaemia densities for these participants was observed after administration of the drug for the majority of the participants and ranged from 7946 parasites/mL to 281387 parasites/mL. Additionally, the malaria clinical score associated with the timing of this parasite density in each of these 15 participants, included a score of 1/42 in 1/15 participants, 2/42 in 6/15 participants, 4/42 in 2/15 participants, 6/42 in 3/15 participants, 7/42 in 1/15 participants and 8/42 in 2/15 participants.
4. A total of 202 AEs occurred with 176 of these being assessed as inoculum-related. Most common AEs reflecting manifestations of blood stage malaria infection included: headache (57 cases in 15 participants), myalgia (25 cases in 12 participants), malaise (18 cases in 9 participants), reduction in lymphocyte count (11 cases in 8 participants), abdominal discomfort (7 cases in 6 participants), fatigue (10 cases in 5 participants) and arthralgia (7 cases in 5 participants). Most AEs were of mild severity (145/202). There were 52 AEs of moderate severity, which were predominantly signs and symptoms associated with malaria infection. There were 5 severe AEs recorded in 3 participants and all

considered to be inoculum related (1 participant with severe headache, 1 participant with transient asymptomatic elevated ALT; elevated AST; and decreased lymphocyte count, 1 participant with decreased lymphocyte count). No SAEs were reported.

A review of data from 5 completed IBSM-*Pf* studies that collected both parasitaemia and malaria clinical score on first antimalarial treatment day, showed that only a small percentage of participants experienced malaria sign/symptoms. The majority had a low malaria clinical score of below 3 out of a maximum score of 42 (data on file at MMV and QIMR). For the total of 54 participants who were inoculated in these studies, the range of parasitaemia at first antimalarial treatment day was 634 to 107981 parasites/mL. At treatment initiation, 17 of 54 participants (31.5%) were symptomatic. Of those 17 participants, 16 (94.1%) had a malaria clinical score ranging from 1-3 and the remaining participant had a score of 7. The parasitaemia associated with the clinical score of 7 was 31666 parasites/mL whereas the highest parasitaemia observed in this analysis (107981 parasites/mL) was observed in an asymptomatic participant [3,4,6,13,14].

Lastly, it should be noted that from a general safety standpoint, the VIS studies in healthy volunteers achieve blood-stage parasitaemia levels that are approximately 1000-fold below those observed in patients with acute malaria infection.

Period for In-House Stays in the Clinical Unit

The period of in-house stays proposed for the current study is aimed at maximising participant safety and convenience, and ensuring efficient clinical study conduct. Participants will be discharged 2 h after inoculation. Experience from previous VIS Phase Ib studies with the DVI technique has shown that inoculation is not expected to induce inoculum-related AEs or laboratory tests changes (due to incubation period) [2,20,27]. Potential tolerability issue is limited to a possible haematoma or vasovagal reaction in relation with venepuncture and can be covered by the observation following injection.

In line with experience from previous VIS IBSM Phase Ib studies conducted by MMV at a Phase I Unit in Australia and the chemoprotection MMV_P218_17_01 study conducted in Belgium (EudraCT number: 2018-003004-39), safety will be assessed for both cohorts by daily monitoring via phone call from Day 2 until Day 6 and safety, parasitaemia and malaria signs and symptoms by daily visits to the clinical unit from Day 7 until Day 9, until start of second confinement in the unit on Day 10:

1. Experience with the MMV_P218_17_01 study:
    - Placebo participants developed protocol-defined PCR positivity ($\geq$ 250 parasites/mL blood) on Day 10 (1/6) or Day 12 (5/6), aligned with a geometric mean time to positive parasitaemia of 10.615 (9.918; 11.360) days.
    - Malaria signs and symptoms were reported in 4 (66.7%) participants overall and there was a delay between detection of positive parasitaemia and malaria sign or symptom onset, with symptoms occurring after PCR positivity and starting on Day 12 in 1 out of the 4 participants and on Day 13 for each of the remaining 3 participants.

- For each of the 4/6 participants, a maximum individual symptom score of one (mild) was observed and no moderate or severe sign/symptom was reported.
- Total malaria clinical scores for the 4 participants were 2/42, 2/42, 3/42 and 5/42.

2. Although occurring earlier than Day 10, previous studies [7,10,18] defined PCR positivity with a limit of detection between 20 and 30 parasites per mL blood, which is lower than our threshold of 250 parasites per mL blood.

Participants will be confined and closely monitored in the unit starting from Day 10 until the cohort-specific qPCR parasitaemia and/or malaria clinical scores thresholds for initiation of registered antimalarial therapy (Riamet®/Malarone®) are reached.

At the time cohort-specific qPCR thresholds (i.e. 5000 parasites/mL blood for Cohort 1 and 10000 parasites/mL blood for Cohort 2) and/or malaria clinical score (6/42 or more) are met, participants will be administered the registered antimalarial therapy. Participants will continue to be monitored at the clinical until complete parasite clearance and for a minimum of 72 h from initiating antimalarial therapy. Then, if clinically well, participants will be discharged and return for an ambulatory EOS visit.

All participants inoculated with PfSPZ-DVI Challenge will commence antimalarial therapy no later than Day 24 for both cohorts, regardless if they reach pre-defined cohort-specific PCR parasitaemia/malaria clinical score thresholds (i.e., 5000 parasites/mL blood for Cohort 1 and 10000 parasites/mL blood for Cohort 2 and/or a malaria clinical score ≥6 for Cohorts 1 and 2). Participants who start antimalarial therapy on Day 24 will only be discharged from confinement at the end of the EOS visit on Day 28.

The Applicant believes there are no further specific measures needed in this proposed study due to the use of the registered 3-day regimen of Riamet®/Malarone®.

3.3 END OF STUDY DEFINITION

A participant will be considered to have completed the study if he or she has completed all phases of the study including the required EOS visit.

The end of the study is defined as the date of the last EOS visit of the last participant in the study.

3.4 SAFETY REVIEW COMMITTEE (SRT)

The study will be overseen by an SRT.

The SRT members will include at least an independent Early Phase and Clinical Pharmacology MD expert experienced in malaria volunteer infection studies (SRT chair), the Medical Monitor, the Medical/Project Director, the PI and an independent infectious disease/malaria expert (or their delegates). The MMV Head of Experimental Medicine and Clinical Pharmacology or further internal or external experts such as a clinical lead and/or a statistician may be consulted by the SRT as necessary.

The SRT will review safety/tolerability, parasitaemia and malaria signs and symptoms data during the study. SRT meetings will be scheduled at one prespecified time (see Section 3.4.1) and ad-hoc if deemed necessary. The required data for SRT review will be

detailed in a separate study specific SRT Charter and will be sent to the SRT at least 24 h prior to an SRT meeting.

The decision of the SRT will be taken in consensus between the members of the SRT. If consensus cannot be reached, then the most cautious approach will proceed. The SRT decision and decision-making will be documented in the SRT meeting minutes, which will be provided to the attendees after the meeting.

Safety will be continuously monitored by the Investigators, the Sponsor's medical monitor, and study personnel.

Criteria for discontinuation of dosing in individual participants are listed in Section 8.

The SRT will not review the data from the sentinel groups in Cohort 1 and Cohort 2. Progression from Subgroup 1 to Subgroup 2 in both Cohorts 1 and 2 will occur upon decision of the PI, medical director and medical monitor only (see Section 3.1).

3.4.1 *Toxicity Criteria and Progression From Cohort 1 to Cohort 2*

An SRT meeting is scheduled to occur at least a week after the conclusion of malaria therapy of Cohort 1, prior to commencing Cohort 2. The SRT will review available Cohort 1 safety/tolerability and parasitaemia data up to the last dose of Riamet® for all inoculated participants in Cohort 1 before enrolment of Cohort 2 and determine whether progression to Cohort 2 is indicated. If no safety concerns are identified during SRT review, progression to Cohort 2 will take place. Initiation of Cohort 2 will be put on hold and further review will be conducted by the SRT, if any of the following toxicity criteria are observed in Cohort 1:

- *Pf*SPZ-DVI Challenge-related or Riamet®-related SAE; or
- any other critical *Pf*SPZ-DVI Challenge-related finding that may place participants at risk within the same cohort or in the next cohort; or
- two or more Riamet®-related severe (grade 3 or higher) AEs, independent of within or not within the same system organ class.

If after data review the SRT confirms that any of the above has been met, including a relationship with the *Pf*SPZ and/or Riamet®, progression to Cohort 2 with higher target levels of parasitaemia might not be conducted.

3.4.2 *Final SRT Review at Study End*

The SRT will also meet at the end of the study when data from Cohort 2 are available for a final assessment in order to review the study results and share feedback on possible safety signals/events.

Throughout the study, the SRT can be convened at the request of any member should they have cause for concern regarding participant safety in relation to the challenge agent, where no other cause can be attributed. In the event that it is not possible to quickly convene the SRT for a review of the data, or not all the data are available, the study will be interrupted or temporarily halted, at the discretion of the PI or Sponsor, at any time.

4. SELECTION OF STUDY POPULATION

Screening for eligible participants will be performed within approximately 4 weeks prior to first confinement and up to Day -2.

Approximately 16 participants are planned to be enrolled sequentially in 2 cohorts of up to 8 participants per cohort. A participant may be enrolled in one cohort only.

For details on the sample size calculation, please refer to Section 9.2.

4.1 INCLUSION CRITERIA

Participants meeting all of the following criteria are eligible to participate in this study:

1. Informed Consent Form signed voluntarily before any study-related procedure is performed, indicating that the participant understands the purpose of and procedures required for the study and is willing to participate in the study, including administration of registered antimalarial therapy;
2. Male or female, between 18 and 55 years old (extremes included) at screening;
3. Body weight of at least 50 kg and a body mass index (BMI) of 19.0 to 30.0 kg/m^2 (extremes included);
4. Good general health without clinically relevant medical illness, physical exam findings including vital signs, and laboratory abnormalities (e.g., without liver transaminases >1x ULN and according to the clinically acceptable ranges for study inclusion laboratory tests in Attachment 4) as determined by the Investigator;
5. Willing to adhere to the prohibitions and restrictions specified in this protocol (see Section 4.3), including willingness to stay confined to the inpatient unit for the required duration and willingness to avoid travelling outside of Benelux during the study period;
6. Female participants should fulfil one of the following criteria:
   a. At least 1 year postmenopausal (amenorrhea >12 months and follicle-stimulating hormone [FSH] >30 mIU/mL) prior to screening;
   b. Surgically sterile (bilateral oophorectomy, hysterectomy or bilateral salpingectomy);
   c. Will use contraceptives as outlined in inclusion criterion 7;
7. Female participants of childbearing potential (excluding females with female partners) must agree to the use of a highly effective method of birth control from the screening visit until 40 days after the EOS visit at Day 28 (covering a full menstrual cycle of 30 days starting after 5 half-lives of last dose of Riamet®);

   Note: Highly effective birth control methods include: combined (oestrogen- and progestogen-containing) oral/intravaginal/transdermal hormonal contraception associated with inhibition of ovulation, progestogen-only oral/injectable/implantable hormonal contraception associated with inhibition of ovulation, intrauterine device, intrauterine hormone-releasing system, bilateral tubal occlusion, vasectomised partner or sexual abstinence from heterosexual intercourse.

8. Female participant has a negative pregnancy test at screening and upon admission in the clinical unit;

   Note: Pregnancy testing will consist of serum β-human chorionic gonadotropin (β-HCG) tests at screening and at the EOS visit and a urine β-HCG tests on Day -1, in all women.

9. Different ways of being reachable 24/7 (e.g., by mobile phone, regular phone or electronic mail) during the whole study period.

4.2 EXCLUSION CRITERIA

Participants meeting any of the following criteria are excluded from participation in this study:

1. Nursing (lactating) women;
2. Participation in any other clinical drug or vaccine study within 30 days (or 5 half-lives for drugs) preceding the day of *Pf*SPZ-DVI Challenge (whichever is longer), or plans to participate in other investigational drug or vaccine research during the study period;
3. Participants who took standard vaccinations within 3 months before the start of the study or are planning to take standard vaccinations during the study period up to 8 weeks after *Pf*SPZ-DVI Challenge;
4. Blood product donation to any blood bank during the 8 weeks (whole blood) or 4 weeks (plasma and platelets) prior to admission in the clinical unit on Day -1;
5. Mean ECG outside normal range and deemed clinically relevant by the Investigator. Examples of clinically significant ECG abnormalities for this study include:
   - PR-interval >220 ms;
   - QRS-complex >120 ms;
   - Absolute QT greater than >500 ms;
   - QT interval corrected according to Bazett's formula (QTcB) or QTcF >450 ms for male participants, >470 ms for female participants;
   - Pathologic Q wave;
   - Significant ST-T wave changes;
   - Left or right ventricular hypertrophy;
   - Non-sinus rhythm except isolated premature atrial contractions and ventricular extrasystole <2 per 10 s ECG lead;
   - Incomplete left bundle branch block, or complete or intermittent right or left bundle branch block;
   - Second or third degree A-V heart block.
6. Seropositive human immunodeficiency virus (HIV), hepatitis A immunoglobulin M (IgM) antibody, hepatitis B virus (HBV) (hepatitis B surface antigen [HBsAg]), hepatitis C virus (HCV) (antibody), hepatitis D antibody, hepatitis E IgM antibody, cytomegalovirus (CMV) IgM antibody or Epstein Barr Virus (EBV) IgM antibody;

7. Previous or current diagnosis of hepatitis including but not limited to viral hepatitis, auto-immune hepatitis, non-alcoholic steatohepatitis (NASH), alpha-1-antitrypsin deficiency, alcoholic liver disease, primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), hemochromatosis, Wilson disease or suspected hepatocellular carcinoma (HCC).
8. History or presence of diagnosed food or known drug allergies (including but not limited to allergy to any of the antimalarial medications to be used in the study, see Section 5.1), or history of anaphylaxis or other severe allergic reactions;

   Note: Participants with seasonal allergies/hay fever, house dust mite or allergy to animals that are untreated and asymptomatic at the time of dosing can be enrolled in the study.
9. History of convulsion or severe head trauma, excluding fever convulsion under 5 years of age;

   Note: A medical history of a single febrile convulsion during childhood is not an exclusion criterion.
10. History of serious psychiatric condition that may affect participation in the study or preclude compliance with the protocol, including but not limited to past or present psychoses, disorders requiring lithium, a history of attempted or planned suicide, more than one previous episode of major depression, any previous single episode of major depression lasting for or requiring treatment for more than 6 months, or any episode of major depression during the 5 years preceding screening;

    Note: The Beck Depression Inventory (Attachment 2) will be used as an objective tool for the assessment of depression at screening. In addition to the conditions listed above, participants with a score of 20 or more on the Beck Depression Inventory and/or a response of 1, 2 or 3 for item 9 of this inventory (related to suicidal ideation) will not be eligible for participation. Participants with a Beck score of 17 to 19 may be enrolled at the discretion of the Investigator if they do not have a history of the psychiatric conditions mentioned in this criterion and their mental state is not considered to pose additional risk to the health of the volunteer or to the execution of the study and interpretation of the data gathered.
11. A medical, occupational or family problem as a result of alcohol or illicit drug abuse during the past 12 months or current alcohol or illicit drug abuse or addiction (positive alcohol breath test or positive drug screen for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine or opiates at screening or upon check-in at the clinical unit);

    Note: Excessive use of alcohol is defined as an intake of >21 units per week for males and >14 units per week for females where one alcohol unit is defined as 10 mL or 8 g of pure alcohol. A single unit is equal to one 25-mL (single) measure of whisky (alcohol by volume [ABV] 40%), or a third of a pint of beer (190 mL; ABV 5-6%) or half a standard (175 mL) glass of wine (ABV 12%).
12. Participants are non-smokers or ex-smokers for more than 90 days prior to screening, or smoke no more than 5 cigarettes per day. If users of nicotine products (i.e., spray, patch, e-cigarette, etc.), they should use the equivalent of no more than 5 cigarettes per day. Participants must agree to abstain from smoking while in the unit;

13. Use of any prescription drugs, herbal supplements (e.g., St John's Wort) or over-the-counter medication within 7 days or 5 half-lives (whichever is longer) prior to the *Pf*SPZ-DVI Challenge, or an anticipated requirement for the use of these during the course of the study (see Section 6.2);

    Note: If necessary, the incidental use of non-steroidal anti-inflammatory drugs (NSAIDs) and paracetamol (2 g/day, 10 g/week) may be acceptable at the Investigator's discretion and will be documented in the eSource system. The use of nutritional supplements during this time that are not believed to have the potential to affect participant safety nor the overall results of the study, may be permitted on a case-by-case basis by the Investigator.

14. Any surgical or medical condition possibly affecting drug absorption (e.g., cholecystectomy, gastrectomy, bowel disease), distribution, metabolism or excretion;
15. Personnel (e.g., Investigator, sub-investigator, research assistant, pharmacist, study coordinator or anyone mentioned in the delegation log) directly involved in the conduct of the study;
16. Any condition that in the opinion of the Investigator would jeopardise the safety or rights of a person participating in the study or would render the person unable to comply with the protocol;
17. Personal history of malaria;
18. Volunteer has travelled to or lived in a malaria-endemic area within 6 months prior to planned study enrolment;
19. Plans to travel to malaria-endemic region during the study period up to last follow-up visit;
20. Previous participation in any malaria vaccine or CHMI study/VIS;
21. Falling in moderate or higher risk category for a fatal or non-fatal cardiovascular event within 5 years (> 5%) determined by a validated risk estimation system, e.g., SCORE [21];
22. Use of systemic antibiotics with known antimalarial activity within 5 half-lives of *Pf*SPZ-DVI Challenge (e.g., trimethoprim-sulfamethoxazole, doxycycline, tetracycline, clindamycin, erythromycin, fluoroquinolones or azithromycin) or an anticipated requirement for the use of these during the study period (see Section 6.2);
23. Receipt of blood or blood-derived products (including immunoglobulin) within 3 months prior to screening. Receipt of packed RBCs given for an emergent indication in an otherwise healthy person, and not required as ongoing treatment is not exclusionary (for example packed RBCs emergently given during an elective surgery).
24. Participants who test positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

    Note: In case of an out-of-range clinical laboratory test (according to the clinically acceptable ranges for study inclusion laboratory tests in Attachment 4), vital sign or ECG value that will determine a participant's eligibility, or in case of a positive drug screen, a retest or expert evaluation can be requested. Results of any retest must be available prior to inoculation. The result of the retest will be considered for participant eligibility at the Investigator's discretion. Participants can be rescreened at the discretion of the Investigator.

4.3 LIFESTYLE CONSIDERATIONS

No blood donation will be allowed until 24 weeks after the end of the study.

Female participants of childbearing potential (excluding females with female partners) must agree to the use of an effective method of contraception from the screening visit until 40 days after the EOS visit at Day 28, as outlined in Section 4.1. For details on the existing data regarding the reproductive toxicity of the *Pf*SPZ-DVI Challenge, Riamet® and Malarone®, please see the current Investigator's Brochure [9] and SmPCs [22] and [12].

Information on prohibited therapies can be found in Section 6.

Participants will be confined from Day -1 in the morning until 2 h post inoculation on Day 1 and from Day 10 in the morning until the day of parasite clearance and at least 72 h after initiating antimalarial therapy (estimated to occur on or before Day 19 and on or before Day 22 for Cohort 1 and Cohort 2, respectively) or from Day 10 in the morning until Day 28 for participants that start antimalarial therapy on Day 24, unless they are withdrawn from the study.

Authorisation to leave the clinical unit will be given by the Investigator.

4.3.1 *Meals and Dietary Restrictions*

1. Safety clinical laboratory blood tests will be performed in a fasted state, i.e., after overnight fast for at least 8 h, except for the ones taken during antimalarial therapy for troponin T measurements, and except for the ones on Day of first PCR ≥5000 parasites/mL in Cohort 1 or ≥10000 parasites/mL in Cohort 2 and prior to initiating antimalarial therapy, where a non-fasted state is allowed.
2. The first dose of antimalarial drug should be taken as soon as possible according to the pre-defined cohort-specific PCR parasitemia/malaria clinical score thresholds (i.e., 5000 parasites/mL blood for Cohort 1 and 10000 parasites/mL blood for Cohort 2 and/or a malaria clinical score ≥6 for Cohorts 1 and 2) or on reaching Day 24. Antimalarial drug administration should be immediately followed by a meal or drinks rich in fat (e.g., milk).
3. Participants will abstain from eating or drinking grapefruit or grapefruit-related citrus fruits (e.g., Seville oranges, pomelos) from 2 weeks prior to the first Riamet® administration and for the whole duration of the study.
4. Participants should not consume poppy-seeds within 24 h before screening and before each urine drug screening because this can falsify the results of the opiate urine drug test.

4.3.2 *Caffeine, Alcohol, and Tobacco*

5. Participants will abstain from smoking a tobacco product or marijuana in the unit. Participants are non-smokers or ex-smokers for more than 90 days prior to screening, or smoke no more than 5 cigarettes per day (see Section 4.2).
6. Participants will not be allowed to take any alcohol-containing food or beverages and energy drinks containing taurine or glucuronolactone (such as Red Bull) from

24 h prior to screening and from 24 h prior to admission to the clinical unit on Day 10 until discharge.

4.3.3 *Activity*

7. Participants will abstain from any strenuous activity (e.g., weight lifting, aerobics, football, endurance training sessions) or unaccustomed physical exercise during the whole study (from 48 h prior to screening and from 48 h prior to admission to the unit on Day -1 until EOS).

4.3.4 *Other*

8. Transplantation is not permitted until successful antimalarial treatment will be given.

4.4 SCREEN FAILURES

Unscheduled visits may be planned to assess, confirm, and follow-up on out-of-range clinical laboratory test, vital sign, or ECG values that determine a participant's eligibility, or in case of a positive urine drug screen. The result of the retest must be considered for participant eligibility and must be available prior to inoculation (Day 1). Findings made during unscheduled visits should be reported in the eSource system.

If a participant does not meet all selection criteria (is a screen failure) but at some point in the future is expected to meet the eligibility criteria, the participant may be rescreened on 1 occasion only. Participants who are rescreened will undergo the informed consent process, be assigned a new participant number, and then restart a new screening phase.

5. STUDY INTERVENTIONS

5.1 STUDY INTERVENTIONS ADMINISTERED

Manufacturing, packaging, and labelling of *Pf*SPZ-DVI Challenge inoculum will be done under the responsibility of the biotechnology company Sanaria (USA) [9].

The clinical unit will be responsible for acquiring the registered antimalarial drugs artemether-lumefantrine (Riamet®) and atovaquone-proguanil (Malarone®):

- Participants will be prescribed with Riamet® according to the pre-defined cohort-specific PCR parasitaemia/malaria clinical score thresholds (i.e., 5000 parasites/mL blood for Cohort 1 and 10000 parasites/mL blood for Cohort 2 and/or a malaria clinical score ≥6 for Cohorts 1 and 2) to ensure parasite clearance prior to the end-of-study evaluation. In addition, participants will also be administered with Riamet®:
    - if they experience ≥1 inoculum-related SAE, irrespective of severity; or
    - if they experience ≥1 Common Terminology Criteria for Adverse Events (CTCAE) grade ≥3 (severe) AE deemed related to malaria and not self-resolved or relieved with concomitant medications; or
    - if the PI or delegate considers it necessary for participant safety. In this situation, the PI or delegate will consult with the independent infectious disease/malaria expert (Prof. Jean-Pierre Van geertruyden). However, antimalarial medication may be administered prior to consultation if immediate treatment is deemed necessary for participant safety.
- If an intolerance or contraindication to Riamet® develops, Malarone® will be administered.

All participants inoculated with PfSPZ-DVI Challenge will commence antimalarial therapy no later than Day 24 for both cohorts, regardless if they reach pre-defined cohort-specific PCR parasitaemia/malaria clinical score thresholds (i.e., 5000 parasites/mL blood for Cohort 1 and 10000 parasites/mL blood for Cohort 2 and/or a malaria clinical score ≥6 for Cohorts 1 and 2).

All participants must consent to receiving antimalarial therapy. Even in the case of withdrawal from the study, all participants administered the *Pf*SPZ-DVI Challenge are to receive antimalarial therapy as soon as possible, and to have all appropriate visits and assessments as required.

The *Pf*SPZ-DVI Challenge agent consists of a strain of *P. falciparum* NF54 sporozoites used for CHMI/VIS studies that is known to be sensitive to the registered antimalarial therapy treatments described above.

Table 2: Description of Study Interventions

Intervention Name	*Pf*SPZ-DVI Challenge	artemether-lumefantrine (Riamet®)	atovaquone-proguanil (Malarone®); only to be used if an intolerance or contraindication to Riamet® develops
Intervention Type	inoculum	drug	
Dose Formulation	cryovial	tablet	
Unit Dose Strength(s)	15000 or 50000 aseptic, cryopreserved *P. falciparum* sporozoites	20 mg artemether and 120 mg lumefantrine	250 mg atovaquone and 100 mg proguanil hydrochloride
Dosage Level	3200 *P. falciparum* sporozoites once	6 doses of 4 tablets over a period of 3 days at approximately 0, 8, 24, 36, 48 and 60 h	4 tablets as a single daily dose for 3 consecutive days
Route of Administration	intravenously by DVI	oral	
Meals in Relation to Dosing	Not applicable	see Section 4.3.1	
Use	challenge agent	antimalarial medication	
Sourcing	provided by Sanaria (USA)	provided locally by the clinical unit	
Packaging and Labeling	dispensed into screw-cap vials containing 15000 or 50000 sporozoites in a 20 µL-aliquot	light yellow, round tablet with 'NC' debossed on one side and 'CG' on the other	round, biconvex, pink film-coated tablets engraved 'GX CM3' on one side

Any deviation from the treatment regimen defined in the protocol must be documented in the eSource system.

5.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

The Investigator (or his/her designee) is responsible for the safe storage of *Pf*SPZ-DVI Challenge and all antimalarial drugs assigned to/acquired by the clinical site, in a locked, secure storage facility with access limited to those individuals authorized to dispense the *Pf*SPZ-DVI Challenge and antimalarial drugs, and maintained within the appropriate ranges of temperature. *Pf*SPZ-DVI Challenge and all antimalarial drugs must be stored as specified at delivery and in the original packaging.

Regular temperature logging of the *Pf*SPZ-DVI Challenge and antimalarial drug storage room at the clinical site should be performed. In case a deviation in storage conditions should occur, the clinical site must not further dispense the affected *Pf*SPZ-DVI Challenge and antimalarial drugs and notify the Sponsor.

The Investigator (or his/her designee) is responsible for ensuring that *Pf*SPZ-DVI Challenge and all antimalarial drugs received at the clinical site are inventoried and accounted for throughout the study.

*Pf*SPZ-DVI Challenge and antimalarial drugs should be dispensed under the supervision of the Investigator, a qualified member of the clinical staff, or by a hospital/clinic pharmacist. The Investigator must maintain accurate records demonstrating date and amount of *Pf*SPZ-DVI Challenge and drugs supplied to whom and by who. *Pf*SPZ-DVI Challenge and antimalarial drugs will be supplied only to participants participating in the study.

The Sponsor's designated site monitor will periodically check the supplies of *Pf*SPZ-DVI Challenge and antimalarial drugs held by the Investigator or pharmacist to ensure accountability and appropriate storage conditions of all products used.

Unused products must be available for verification by the site monitor during on-site monitoring visits.

After the last visit of the last participant in the study (LPLV), any unused *Pf*SPZ-DVI Challenge will be returned to Sanaria. Any unused antimalarial medication will be destroyed at the clinical site (via Emergency waste and chemical services [EWACS]) after Sponsor's approval (in this case a certificate of destruction will be provided and filed in the Trial Master File [TMF])

Hazardous materials such as used ampules, needles, syringes and vials containing hazardous liquids, should be disposed of immediately in a safe manner and therefore will not be retained for drug accountability purposes.

5.2.1 *PfSPZ-DVI Challenge*

In brief, the manufacturing process includes the production, under traditional environmental condition, of eggs from a colony of *A. stephensi* mosquitos housed in a controlled environmental chamber. Surface disinfection of the eggs is performed by exposure to chemical agents in a Class II biosafety cabinet (BSC). Thereafter, all materials and product are handled using aseptic methods to ensure that contaminating microorganisms are not introduced to, and carried through, the process.
Surface-disinfected eggs are inoculated into sterile, vented flasks containing aseptic growth medium. The eggs hatch and develop into pupae, which are transferred to an adult mosquito container from which the adult mosquitoes emerge.

These adult mosquitos, which have been raised under aseptic conditions, are fed *P. falciparum* gametocyte-infected blood in a BSC in a high-security insectary in Rockville, Maryland, USA. The *P. falciparum* gametocyte-infected blood is produced from cultures of the *P. falciparum* strain NF54, derived from a master cell bank of the well characterised *P. falciparum* strain NF 54. Infected adult mosquitos are maintained under aseptic conditions until *P. falciparum* sporozoites migrate to the salivary glands. The salivary glands from the *P. falciparum* sporozoite infected mosquitos are removed by hand dissection and then triturated to release the *P. falciparum* sporozoites.

The sporozoites are purified, counted, and at a specified concentration, cryopreserved. Cryopreservation commences with the addition of cryoprotective additives to the purified sporozoites to produce the *Pf*SPZ-DVI Challenge agent.

The diluent for *Pf*SPZ-DVI Challenge is composed of phosphate buffered saline (PBS) and human serum albumin (HSA). Vials of PBS and HSA will be shipped to the clinical unit, where diluent composed of PBS and HSA is prepared according to a local Standard Operational Procedure, as described in the Laboratory Manual of Sanaria (Storage, Preparation and Administration of *Pf*SPZ-DVI Challenge).

PBS that is manufactured in compliance with Good Manufacturing Practice (GMP) and according to upstream processing specifications is purchased by Sanaria. Every lot of PBS is supplied with a batch certification that is reviewed and approved upon receipt of Sanaria. The PBS is stored at ambient temperature in a controlled room.

HSA (25%), approved for parenteral, intravenous administration to humans, is purchased by Sanaria. Every lot of HSA is supplied with a Certificate of Analysis that is reviewed and approved upon receipt of Sanaria. HSA vials are stored at ambient temperature in a controlled room.

The *Pf*SPZ-DVI Challenge agent is dispensed into screw-cap vials containing 15000 or 50000 sporozoites in a 20 µL aliquot. The *Pf*SPZ-DVI Challenge is stored in liquid nitrogen vapour phase at -140°C to -196°C until it is shipped to the clinical unit. Shipment is in compliance with U.S. Food and Drug Administration (FDA), U.S. Department of Transportation and United Nations transport guidelines for shipping bio-hazardous materials on dry ice and liquid or vapour phase nitrogen.

Transfer of *Pf*SPZ-DVI Challenge from its storage site to the clinical unit will follow local Standard Operational Procedure, as described in the Laboratory Manual of Sanaria.

At the clinical unit, the liquid nitrogen vapour phase container will be monitored. Receipt of the *Pf*SPZ-DVI Challenge will be documented on a tracking log by study staff.

Immediately prior to use, the *Pf*SPZ-DVI Challenge in cryovials will be thawed individually by partial submersion of the vials for 30 s in a 37°C ± 1°C water bath. Designated, trained study staff will then prepare, dilute (if necessary) and dispense the *Pf*SPZ-DVI Challenge to clinical staff at the clinical unit according to local standard Operational Procedures and as described in the Laboratory Manual of Sanaria.

The *Pf*SPZ-DVI Challenge (3200 *P. falciparum* sporozoites per participant) will be administered intravenously by DVI. The study staff administering the *Pf*SPZ-DVI Challenge will wear gloves and eye protection. Advanced life support drugs and resuscitation equipment will be immediately available in the event of any participants experiencing an anaphylactic reaction to the challenge.

5.2.2 *Registered Antimalarial Therapy*

Riamet® and Malarone® must be stored at ambient conditions (15-30°C or 59-86°F), should not be exposed to freezing temperatures, and should be protected from light during storage at the clinical site.

Procedures for handling and storage are detailed on the study-specific labels [22,12].

5.3 RANDOMISATION AND BLINDING

Participants are not randomised. As participants are confirmed to be eligible for the study, they will be assigned a single unique identifier across the study and will be enrolled sequentially in 1 of the 2 cohorts.

All participants will be inoculated with *Pf*SPZ-DVI Challenge and receive antimalarial therapy. *Pf*SPZ-DVI Challenge will be packaged and labelled by Sanaria in line with local labelling regulations. The antimalarial drugs (Riamet® and Malarone®) will be labelled by the clinical unit pharmacy with an appropriate study label in line with local labelling regulations. The pharmacist or appropriate qualified member of the study staff, assigned by the PI, will prepare the *Pf*SPZ-DVI Challenge and antimalarial drugs identifying the products with the correct participant unique identifier according to the applicable procedures.

As this is an open-label study, blinding is not applicable.

5.4 DOSE MODIFICATION

No dose modification is allowed.

5.5 TREATMENT COMPLIANCE

To ensure treatment compliance, *Pf*SPZ-DVI Challenge and Riamet®/Malarone® will be administered/intakes will be supervised by the Investigator or his/her designee.

Any deviation from the treatment regimen defined in the protocol must be documented in the eSource system.

6. PRIOR AND CONCOMITANT THERAPY

All therapies (prescriptions and over-the-counter medications, including herbal preparations/treatments) other than the antimalarial medication administered from the time of informed consent until the last study visit must be recorded in the eSource system (name of the drug, dosage, route and dates of administration).

Medications taken during the 28 days prior to *Pf*SPZ-DVI Challenge will be recorded in the eSource system as previous medications. Medications taken after this time will be recorded as concomitant medications.

Female participants of childbearing potential (excluding females with female partners) must agree to the use of an effective method of contraception throughout the study, as outlined in Section 4.1. The use of oral, injectable and implantable hormonal contraceptives is to be recorded in the eSource system.

6.1 PERMITTED CONCOMITANT THERAPIES

Participants will abstain from using any medications (prescription or over-the-counter) or herbal remedies from 7 days or 5 half-lives (whichever is longer) prior to *Pf*SPZ-DVI Challenge, as described in the exclusion criteria of this protocol (see Section 4.2). If necessary, the incidental use of NSAIDs and paracetamol (2 g/day, 10 g/week), and the use of nutritional supplements may be acceptable at the Investigator's discretion.

Except for the antimalarial medication and medication considered essential to treat AEs, all medications (prescription and non-prescription), herbal remedies and nutritional supplements should be avoided within 7 days or 5 half-lives (whichever is longer) prior to the *Pf*SPZ-DVI Challenge until after the EOS visit on Day 28. Incidental and limited use of medications not believed to affect participant safety, nor the overall results of the study may be permitted on a case-by-case basis, following approval by the Sponsor in consultation with the Investigator, as described in the exclusion criteria of this protocol (see Section 4.2).

Due to possible transient asymptomatic ALT and AST elevations after malaria inoculation, ibuprofen (at doses of up to 1.8 g/day) is the preferred treatment over paracetamol for possible emergent malaria symptoms. In the case paracetamol is used, the recommended dose should not exceed 2 g/day.

6.2 PROHIBITED CONCOMITANT THERAPIES

The following medications are not allowed during the study and should be washed-out within 5 half-lives of *Pf*SPZ-DVI Challenge:

- Drugs with known antimalarial activity (trimethoprim-sulfamethoxazole, tetracycline, doxycycline, erythromycin, clarithromycin, azithromycin, clindamycin, rifampicin, or newer quinolones, benzodiazepines, flunarizine, fluoxetine, methotrexate, chloroquine and hydroxychloroquine);
- Any drug susceptible to interaction with breast cancer resistance proteins (BCRP) (cyclosporin, elacridar, eltrombopag, gefitinib).

7. ASSESSMENTS

7.1 TIMING OF ASSESSMENTS

An overview of the timing of *Pf*SPZ-DVI Challenge, treatment and assessments is given in the Time and Events Schedule.

If assessments are planned at the same time point in the study, the order of assessments should be as follows:

1. 12-lead ECG recordings;
2. vital signs measurements;
3. blood sampling;
4. other assessments (except for urinalysis assessments, morning void allowed).

The following time-related windows for parasitaemia assessments will be acceptable based on logistical and operational considerations:

- ± 60 min on Days 7, 8 and 9;
- ± 10 min during confinement;
- ± 1 day at the EOS visit.

The following time-related windows for blood pressure, pulse rate, ECG, haematology, blood chemistry and urinalysis assessments will be acceptable based on logistical and operational considerations:

- pre-inoculation observations within 3 h prior to inoculation;
- a 5% deviation from theoretical post inoculation times will be allowed for all other post inoculation assessments.

All assessments scheduled for a particular time point should be performed within these windows.

Any deviations from the above-mentioned window periods will be documented as protocol non-compliances and will be evaluated case-by-case for significance at the data review meeting prior to database lock.

Adverse events and the intake of concomitant medication(s) will be monitored continuously from informed consent until the last study-related activity.

7.1.1 *Screening Period*

Screening Visit

Screening for eligible and consenting participants will be performed within approximately 4 weeks prior to first confinement.

Participants will be given a full explanation of the nature of the study and written informed consent (approved by the local ethics committee) will be obtained according to local requirements before any study-related assessment will be carried out.

At screening, participants will be asked to attend the clinical unit to have assessments performed as indicated in the Time and Events Schedule.

All results from the screening procedure needed to evaluate eligibility, including the clinical laboratory results, must be available before *Pf*SPZ-DVI Challenge on Day 1. Any abnormal assessment at the screening visit will be assessed according to its clinical relevance, and if found relevant, the participant will not be included in the study (see also Section 4.4).

Up until Day -2, testing for SARS-CoV-2 using real-time reverse transcription PCR (rRT-PCR) on nasopharyngeal swabs will also be performed for participants who completed the rest of the screening procedure and are still eligible to be enrolled in the study. The rRT-PCR test will be performed by ZNA Middelheim or by SGS Clinical Pharmacology Unit, depending on the availability of appropriate equipment.

Unscheduled visits may be planned to assess, confirm and follow-up on out-of-range clinical laboratory test, vital sign or ECG values that determine a participant's eligibility, or in case of a positive urine drug screen. The result of the retest will be considered for participant eligibility. Findings made during unscheduled visits should be reported in the eSource system.

Pre-treatment Day -1

Participants will be admitted to the clinical unit in the morning of Day -1 (the day before *Pf*SPZ-DVI Challenge) and participants will remain at the clinical unit until 2 h post inoculation on Day 1.

Eligibility of the participants will be confirmed and assessments will be performed as indicated in the Time and Events Schedule. All assessments must be completed and safety assessment outcomes must be available before *Pf*SPZ-DVI Challenge on Day 1.

7.1.2 *Treatment Period*

Day 1

On Day 1, assessments begin as of 3 h before *Pf*SPZ-DVI Challenge (except for urinalysis assessments, morning void allowed) and will be performed as indicated in the Time and Events Schedule. All assessments must be completed and safety assessment outcomes must be available before inoculation with approximately 3200 *P. falciparum* sporozoites (NF54 strain) by DVI. After assessments are performed as indicated in the Time and Events Schedule, participants will be discharged 2 h post inoculation.

Day 2 Until Day 6

Participants will be monitored daily via phone call from Day 2 until Day 6 to solicit any AEs.

Day 7 Until Day 9

Participants will come to the clinical unit daily from Day 7 until Day 9 and the presence of parasites will be assessed once daily by qPCR.

Before confinement to the study site (on Day 10), participants will be tested again for SARS-CoV-2 using rRT-PCR on nasopharyngeal swabs. If a participant tests positive for SARS-CoV-2, he or she will be withdrawn from the study and will be administered

registered antimalarial therapy (i.e., Riamet®) immediately. The SARS-CoV-2 infection will be managed further according to national laws.

Day 10 Until Day of Pre-Defined Cohort-Specific Thresholds (Including Day of First PCR ≥250 Parasites/mL)

Participants will be confined to the clinical unit from Day 10 in the morning and the presence of parasites will be assessed twice daily by qPCR until Day of first PCR ≥5000 parasites/mL blood for Cohort 1 or until Day of first PCR ≥10000 parasites/mL blood for Cohort 2.

Assessments will be performed as indicated in the Time and Events Schedule.

Day of Pre-Defined Cohort-Specific Thresholds Until ≥72 h After Initiating Antimalarial Therapy

Participants will be administered registered antimalarial therapy, i.e., Riamet®, as soon as possible when the pre-defined cohort-specific PCR parasitaemia/malaria clinical score thresholds (i.e., 5000 parasites/mL blood for Cohort 1 and 10000 parasites/mL blood for Cohort 2 and/or a malaria clinical score ≥6 for Cohorts 1 and 2) are reached or at the Investigator's discretion (see Section 5.1).

All participants inoculated with PfSPZ-DVI Challenge will commence antimalarial therapy no later than Day 24 for both cohorts, regardless if they reach pre-defined cohort-specific PCR parasitaemia/malaria clinical score thresholds (i.e., 5000 parasites/mL blood for Cohort 1 and 10000 parasites/mL blood for Cohort 2 and/or a malaria clinical score ≥6 for Cohorts 1 and 2).

Antimalarial therapy will be further administered and monitored. qPCR assessments of parasitaemia will be carried out at multiple time points (2, 6, 8, 12, 16, 24, 36, 48 and 72 h) following initiation of Riamet®.

Assessments will be performed as indicated in the Time and Events Schedule.

Day of Parasite Clearance And ≥72 h After Initiating Antimalarial Therapy

Upon parasite clearance and at least 72 h after initiating antimalarial therapy (estimated to occur on or before Day 19 and on or before Day 22 for Cohort 1 and Cohort 2, respectively), and if clinically well, participants will be discharged from the clinical unit.

Participants who start antimalarial therapy on Day 24 will only be discharged from confinement at the end of the EOS visit on Day 28.

Assessments will be performed as indicated in the Time and Events Schedule.

7.1.3 *Follow-up Period*

After discharge from the clinical unit, or after confinement until the EOS on Day 28 in case antimalarial therapy was initiated on Day 24, all participants will be followed up for safety assessments, clinical evaluation and malaria qPCR in the clinical unit at the EOS visit on Day 28.

All participants will be assessed for parasitaemia and will be asked non-leading questions to determine the occurrence of any AEs at the EOS visit on Day 28. In order to provide some flexibility for the participants regarding the site visit and to maintain the integrity

of the study design, a time window of ±1 day is permitted, in case of time conflict or unforeseen circumstances.

Assessments will be performed as indicated in the Time and Events Schedule.

7.1.4 *Unscheduled Visits*

Unscheduled visits can be planned for instance:

- to obtain additional information to ensure safety to the participant. Additional blood and urine samples may be taken at the discretion of the Investigator;
- to assess, confirm and follow-up on out-of-range clinical laboratory test, vital sign or ECG values that will determine a participant's eligibility, or in case of a positive drug screen. The result of the retest will be considered for participant eligibility.

Findings made during unscheduled visits should be reported in the eSource system.

7.2 PHARMACODYNAMIC ASSESSMENTS

For an overview of pharmacodynamic endpoints, see Section 2.

7.2.1 *Parasitaemia*

A blood sample will be collected via direct venepuncture from each participant at time points where parasitological assessments are scheduled (Time and Events Schedule). The assessment of malaria parasitology by qPCR will be as follows: *var*ATS multigenic family targeted qPCR assay of parasite load will be performed in accordance with the centre of excellence for tropical medicine (ITM) standard operating procedure and the Laboratory Manual. Given the high sensitivity of qPCR, this method will be used to confirm parasite clearance after definitive antimalarial therapy for all participants. The results of the PCR at the ITM will be available in approximately 6 h from arrival of samples at the ITM. A participant will be considered cured following completion of the course of antimalarial therapy and after qPCR indicates 0 parasites per mL blood.

7.3 SAFETY ASSESSMENTS

The safety assessment in this study will be based on AEs, the malaria clinical score, clinical laboratory tests, vital signs, ECG, physical examination and Beck Depression Inventory (screening only) as described in the following sections.

7.3.1 *Adverse Events*

Adverse events will be monitored continuously from informed consent until the last study-related activity. At regular intervals during the study, participants will be asked non-leading questions to determine the occurrence of any AEs. All AEs reported spontaneously during the course of the study will be recorded as well.

For detailed definitions and reporting procedures of AEs, see Section 10.

Close monitoring of expected signs and symptoms associated with malaria infection (listed in Attachment 1) form part of the safety evaluation of the study and will be used as part of the decision criteria to administer registered antimalarial therapy (see

Section 5.1 and Section 7.3.2). These events will be classified as AEs, and may also be further sub-classified as inoculum-related AEs:

An inoculum-related AE is a sign or symptom associated with malaria infection, i.e., confirmed by a protocol-defined PCR positivity (defined for the purpose of this study as a qPCR outcome ≥250 parasites per mL blood) at

- the onset of the event; or
- if the event started within 1 day of parasite clearance (defined as a qPCR value of 0 parasites per mL blood after initiating antimalarial therapy) or laboratory-defined PCR positivity (a qPCR outcome of 50-249 parasites per mL blood).

If the presence of *P. falciparum* malaria is confirmed (see above), then these AEs will be further sub-classified as inoculum-related AEs, following the guidelines on AE reporting, i.e., followed-up until resolution with the same causality assessment (see Section 10), and reported as such in the final clinical study report.

If the PCR results for *P. falciparum* are between 0 and <250 parasites per mL blood at the time of the onset of the event and the day before, usual AE/SAE reporting procedures and criteria will apply (see Section 10), but the event will not be classified as an inoculum-related AE.

Final classification of signs and symptoms as AE and inoculum-related AE, or as AE only, will occur after PCR results are available.

7.3.2 *Malaria Clinical Score*

The malaria clinical score, which assesses malaria signs and symptoms (see Attachment 1) at the time points indicated in the Time and Events Schedule, will be part of the close monitoring for known/identified risks related to *Pf*SPZ-DVI Challenge/malaria. The malaria clinical score has been widely used in the conduct of IBSM CHMI/VIS studies in Australia and also implemented in the recently conducted *Pf*SPZ-DVI Chemoprotection Challenge study with P218 (MMV_P218_17_01 study [EudraCT number: 2018-003004-39]) at the SGS Clinical Pharmacology Unit in Antwerp, Belgium [2]. This quantitative tool will be used as part of the decision criteria to administer registered antimalarial therapy along with the level of parasitaemia (see Section 5.1).

The following 14 signs/symptoms frequently associated with malaria will be graded using a 4-point scale (absent: 0; mild: 1; moderate: 2; severe: 3) and summed to generate a total malaria clinical score (maximum score possible is 42): headache, myalgia (muscle ache), arthralgia (joint ache), fatigue/lethargy, malaise (general discomfort/uneasiness), chills/shivering/rigors, sweating/hot spells, anorexia, nausea, vomiting, abdominal discomfort, fever, tachycardia and hypotension. To determine severity of the 14 signs/symptoms we use the CTCAE grading scale grade 1 - 5. Mild (1) equates to CTCAE grade 1, Moderate (2) equates to CTCAE grade 2 and Severe (3) equates to CTCAE grade 3 or above. Individual scores for each symptom as well as the total score will be recorded.

For the grading of fever, the vital sign parameter body temperature (sublingual) will be assessed (see Section 7.3.4).

7.3.3 *Clinical Laboratory Tests*

Blood samples will be collected by venepuncture or via indwelling cannula at the time points indicated in the Time and Events Schedule. Biochemistry and haematology testing will be performed on these samples, as well as viral serology testing (hepatitis A IgM antibody, HbsAg, anti-HCV antibody, hepatitis D antibody [only in subjects positive for HbsAg], hepatitis E IgM antibody, CMV IgM antibody, EBV IgM antibody and HIV) on the sample from screening. In all female participants, also serum β-HCG assessments at screening and at the EOS visit and a urine β-HCG assessment on Day-1 will be performed. FSH will be measured at screening in all women.

All blood samples for safety assessments should be taken in a fasted state (after overnight fast for at least 8 h for unbiased glucose determination), except for the ones taken during antimalarial therapy for troponin T measurements, and except for the one on Day of first PCR ≥5000 parasites/mL in Cohort 1 or ≥10000 parasites/mL in Cohort 2 and prior to initiating antimalarial therapy, where a non-fasted state is allowed.

Standard laboratory tests will be performed by ZNA Middelheim.

The following biochemistry and haematology tests will be performed on the safety blood samples:

- Liver biochemistry: albumin, AST, ALT, alkaline phosphatase (ALP), gamma glutamylaminotransferase (GGT), total and direct bilirubin and total serum proteins;
- Biochemistry other than liver: sodium, potassium, chloride, bicarbonate, urate, inorganic phosphate, creatinine, estimated glomerular filtration rate (eGFR), glucose, lactate dehydrogenase (LDH), blood urea nitrogen (BUN) and creatine phosphokinase (CPK);
- High sensitive troponin T and;
- C-reactive protein (CRP);
- Haematology: haemoglobin, haematocrit, RBC count, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), white blood cell (WBC) count, platelet count, reticulocytes, neutrophils, eosinophils, basophils, lymphocytes and monocytes;
- Coagulation: prothrombin time (PT) and activated partial thromboplastin time (aPTT) (at screening and on Day -1 only), and international normalized ratio (INR).

A midstream urine sample will be collected for urinalysis by dipstick for glucose, protein, nitrite, pH and occult blood at the time points for biochemistry other than liver indicated in the Time and Events Schedule. Microscopic examination for WBC, RBC and casts will be performed.

A urine drug screen (amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine and opiates) and an alcohol breath test will be performed at the time points indicated in the Time and Events Schedule.

The Investigator must review the laboratory report, document this review and record any change occurring during the study he/she considers to be clinically relevant in the

eSource system. Laboratory values outside the normal range will be flagged and their clinical relevance will be assessed by the Investigator.

7.3.4 *Vital Signs*

Vital sign parameters will be assessed after at least 10 min in supine position at the time points indicated in the Time and Events Schedule. The vital sign parameters that will be assessed are supine systolic and diastolic blood pressure (SBP and DBP, respectively) and pulse rate. Body temperature (sublingual) will also be assessed, and this whenever the malaria clinical score will be evaluated (see Section 7.3.2). Orthostatic changes to BP and pulse rate will also be assessed at screening: participants will be requested to stand after completion of the supine measurements and blood pressure and pulse rate will be recorded after at least 2 min in the standing position.

These parameters will be measured using a completely automated device consisting of an inflatable cuff and an oscillatory detection system. All values will be registered on a built-in recorder so that measurements are observer-independent.

Any change from baseline in vital sign values occurring during the study that is considered to be clinically relevant by the Investigator should be recorded in the eSource system.

7.3.5 *Electrocardiogram*

Twelve-lead ECG recordings will be recorded in triplicate after at least 10 min in supine position at the time points indicated in the Time and Events Schedule.

Paper speed will be 25 mm/s, so that the different ECG intervals can be measured manually.

The interpretations of the ECGs will be performed by the Investigator or his/her designee at the clinical unit. Any change from baseline ECG occurring during the study that is considered to be clinically relevant by the Investigator should be recorded in the eSource system.

7.3.6 *Physical Examination*

Physical examination will be performed at the time points indicated in the Time and Events Schedule.

Height is to be measured barefoot and at screening only. Body weight to be measured as indicated in the Time and Events Schedule. To obtain the actual body weight, participants must be weighed lightly clothed.

Any change in physical examination occurring during the study that is considered to be clinically relevant by the Investigator should be recorded in the eSource system.

Full physical examination will be conducted at screening, on Day -1, within ±24 h after first PCR ≥250 parasites/mL blood and at the EOS visit on Day 28. Targeted, symptom-driven physical examination will be performed in case malaria clinical score >6 on Day 10 (upon confinement to the clinical unit), within 24 h after first PCR ≥5000 (Cohort 1) or ≥10000 (Cohort 2) parasites/mL blood and at pre-discharge from clinical unit; and will be focused on changes since the previous examination, but will

always include at least: chest/respiratory, general appearance, heart/cardiovascular, abdomen, neurological and skin/mucous membranes examination.

7.3.7 *Beck Depression Inventory*

The Beck Depression Inventory is performed at screening only (see Attachment 2).

The questionnaire is scored by the participant. The inventory completed by the participant will be reviewed/checked for completeness and the total score calculated by the study personnel.

7.4 PARASITE TRANSCRIPTOMICS

Different environmental conditions that occur during a human infection (such as the presence of drugs) can select for parasites with different expression patterns [16,17]. We aim to evaluate the effect of in-host factors on parasite transcriptional/epigenetic responses during experimental infections.

To this aim, blood samples will be collected via direct venepuncture at the time points indicated in the Time and Events Schedule. Sample aliquots will be stored in Trizol for RNA preservation and cryopreserved for parasite culture in case higher parasite densities are needed to increase starting material for transcriptomic analysis of parasite isolates.

Protocols for transcriptomic analysis of parasite isolates have been validated and are currently in use at the Malariology Unit at the ITM.

7.5 TREATMENT OF OVERDOSE

In this study, all dosing will be supervised by study personnel and consequently the risk of overdose will be minimal.

7.6 TOTAL VOLUME OF BLOOD SAMPLING

The total volume of blood drawn per the Time and Events Schedule from each participant will not exceed the maximum allowable volume of approximately 460 mL.

7.7 APPROPRIATENESS OF MEASUREMENTS

The assessments which will be made in this study are standard, and are generally recognized as reliable, accurate and relevant.

8. PARTICIPANT WITHDRAWAL FROM THE STUDY

8.1 WITHDRAWAL OF PARTICIPANTS FROM STUDY

Participants **may** be withdrawn from the study following inoculation with *Pf*SPZ-DVI Challenge in the event of:

- a severe AE or a SAE considered related to the *Pf*SPZ-DVI Challenge.

Participants **may** be withdrawn from the study following inoculation with *Pf*SPZ-DVI Challenge and administration of Riamet® in the event of:

- a severe AE or a SAE considered related to either Riamet® or the *Pf*SPZ-DVI Challenge.

Participants **must** be withdrawn from the study in the event of:

- withdrawal of consent;
- safety reasons, it being in the best interest of the participant that he/she be withdrawn, in the Investigator's opinion or in the opinion of the SRT (see Section 3.4);
- a positive pregnancy test (the participant or, in case of a male participant, his female partner), or if the participant/partner is non-compliant with the contraception requirements (see Section 4.1);
- a positive SARS-CoV-2 test (see Section 7.1.2);
- development of a medical condition that requires concomitant treatment with a prohibited therapy (see Section 6.2);
- failure of the participant to comply with the protocol requirements or to cooperate with the Investigator resulting in a significant risk to the participant's safety.

In the event a participant has to discontinue Riamet®, the monitor and Sponsor should be informed: in case of withdrawal due to an SAE (for details on AE reporting see Section 10), the Sponsor should be notified within 24 h; in case of withdrawal for other reasons, the Sponsor should be notified within 2 days from the event.

If there is a medical reason for withdrawal, the participant will remain under the supervision of the Investigator until satisfactory health has returned.

All participants must consent to receiving antimalarial therapy if inoculated with the *Pf*SPZ-DVI Challenge agent, i.e., the registered 3-day Riamet® regimen approved for treatment of uncomplicated malaria. Even in the case of withdrawal from the study, all participants administered the *Pf*SPZ-DVI Challenge are to receive antimalarial therapy as soon as possible (see Section 5.1), and to have all appropriate visits and assessments as required (see Time and Events Schedule). Participants will be followed up for safety assessments, clinical evaluation and malaria qPCR in the clinical unit at the EOS visit on Day 28. Participants who fail to return for visits will be traced as described in Section 8.2.

Participants who have to discontinue Riamet® prior to completion of the scheduled study procedures for safety reasons/due to intolerance must complete antimalarial therapy with Malarone® and should be invited to complete the assessments as much as possible: as

long as the participant consents, all relevant assessments of the day on which the participant discontinued should be completed, at least those related to safety, and the participant should come for a safety follow-up visit 4 weeks after the last administration of antimalarial drug. In the event of discontinuation due to an AE, the appropriate follow-up will be done.

Participants have the right to withdraw from the study at any time for any reason, including personal reasons. A participant can withdraw without giving a reason. This will not affect his/her future care. The Investigator should however try to find out why a participant withdraws from the study and document the reason for withdrawal in the source documents and in the eSource system.

If the participant withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.

Participants who are withdrawn from the study for reasons other than safety may be replaced. This decision will be made based upon discussion and mutual agreement

*Pf*SPZ-DVI Challenge and antimalarial drugs assigned to a participant who withdraws must not be assigned to another participant.

8.2 LOST TO FOLLOW-UP

Before a participant is considered lost to follow-up, every reasonable effort must be made by the study site personnel to contact the participant, if only to determine the reason for discontinuation. The measures taken to follow up must be documented.

The following measures will be taken to assure traceability of the participants:

- Following daily contact from Day 2 to 9 and scheduled, in-house confinement within the clinical unit, participants will be followed up for safety assessments, clinical evaluation and malaria qPCR in the clinical unit at the EOS visit on Day 28.
- All participants will commit to remaining contactable 24/7 throughout the study and this will be recorded on the Informed Consent Form (ICF).
- Participants will be required to provide current contact details (two telephone numbers including a mobile number and a responsible adult as an emergency contact) and a relevant email address.
- Should a participant not be contactable by the unit, all alternative methods possible (e.g., sending someone to home address of participant) will be used to locate and communicate with the participant.
- Should a participant still be uncontactable the situation will be discussed with the Sponsor and escalated as appropriate including alerting the regulatory authorities to a deviation from protocol.

Should the participant remain unreachable, he/she will be considered to have discontinued the study.

9. STATISTICAL METHODS

9.1 STATISTICAL ANALYSIS

All statistical analysis will be performed by SGS Life Sciences, under the supervision and responsibility of the Sponsor, using SAS® (SAS Institute Inc., Cary, NC, USA; version 9.4 or higher) software for statistical computations.

All statistical methods shall be detailed in a Statistical Analysis Plan (SAP) that will be finalised before database lock.

The final analysis will be performed once all participants have completed the follow-up visit or have discontinued earlier.

Data from all participants enrolled and inoculated with *Pf*SPZ-DVI Challenge will be included in the data analysis. The following analysis populations will be defined:

- Inoculation Set: all participants who were inoculated with *Pf*SPZ-DVI Challenge;
- PD Analysis Set: all inoculated participants with at least one available PD data who received all Riamet® doses and who experienced no protocol deviations with relevant impact on PD data;
- Safety Analysis Set: all inoculated participants who received any treatment.

Unless specified otherwise, the Inoculation Set will be used for analysis of demographics, the PD analysis set will be used for pharmacodynamic statistical analysis and the Safety Analysis Set will be used for safety/tolerability analysis.

9.1.1 *Initial Characteristics Data of the Participant Sample*

For all participants who receive *Pf*SPZ-DVI Challenge, descriptive statistics will be provided for demographic (e.g., age, height, weight, BMI, race, gender) and other initial participant characteristics (alcohol and drug screening tests, pregnancy test, orthostatic changes to blood pressure and pulse rate, serology, medical and social history, concomitant diseases, Beck Depression Inventory). Standard descriptive statistics for continuous variables are the number of participants (N), mean, standard deviation (SD), median, minimum and maximum values. The standard descriptive statistics for categorical variables are the number of participants in the category and the proportion expressed as a percentage.

Prior and concomitant medications will be coded using the WHO_DRUG Dictionary.

9.1.2 *Pharmacodynamic Data*

Parasitaemia

Primary endpoints:

To characterise key stages in the parasite growth:

- first PCR positivity;
- threshold of 5000 parasites/mL for Cohort 1 and threshold of 10000 parasites/mL for Cohort 2; and
- parasitaemia levels at drug administration.

This will be done by calculating:

- Descriptive statistics (geometric mean, SD, 95% CI, range) of time to first PCR positivity. In the absence of positive PCR, the duration will be set to a maximum of 28 days;
- Descriptive statistics (N, geometric mean, SD, 95% CI, range) of parasitaemia at first PCR positivity;
- Descriptive statistics (geometric mean, SD, 95% CI, range) of time to parasitaemia of ≥5000 parasites per mL blood (Cohorts 1 and 2);
- Descriptive statistics (geometric mean, SD, 95% CI, range) of parasitaemia at the time parasitaemia ≥5000 parasites per mL blood (Cohorts 1 and 2);
- Descriptive statistics (geometric mean, SD, 95% CI, range) of time to parasitaemia of ≥10000 parasites per mL blood (Cohort 2);
- Descriptive statistics (geometric mean, SD, 95% CI, range) of parasitaemia at the time parasitaemia ≥10000 parasites per mL blood (Cohort 2);
- Descriptive statistics per cohort (geometric mean, SD, 95% CI, range) of time to first dose of treatment with Riamet® (Cohorts 1 and 2); and
- Descriptive statistics per cohort (geometric mean, SD, 95% CI, range) of parasitaemia at first dose of treatment with Riamet® (Cohorts 1 and 2).

The number and proportion of participants with presence of positive PCR and parasitaemia of ≥5000 or ≥10000 parasites per mL blood between inoculation with *Pf*SPZ-DVI Challenge and Day 28 will be summarised per cohort. Corresponding two-sided 90% Exact Clopper-Pearson confidence limits will be presented as well.

Secondary endpoints:

To characterise the blood-stage parasite profile, a model will be fitted to the measured parasitaemia data prior to Riamet® administration to be able to predict the growth of parasites as a function of time. This model may be a log-linear model or, if the parasitaemia profile shows a cyclic behaviour due to synchronicity of the parasites and their sequestration during the late stages of their lifecycle, it may include a sinus function. The latter will allow the estimation of the period of the observed waves in

addition to the parasite growth. The following parameters will be calculated from the individual fits of the data:

- Descriptive statistics per cohort and overall (geometric mean, SD, 95% CI, range) of parasite growth rate expressed as the PMR_{48};
- If cycles are observed and if their estimated period is not 48 h, descriptive statistics per cohort and overall (geometric mean, SD, 95% CI, range) of parasite growth rate expressed as the PMR_{LC}; and
- Descriptive statistics (geometric mean, SD, 95% CI, range) of predicted time to reach parasitaemia threshold of first positive PCR, ≥5000 parasites per mL blood, ≥10000 parasites per mL blood for Cohorts 1 and 2.

To characterise the blood-stage parasite clearance profile of Riamet® in malaria infection in naïve healthy participants after *Pf*SPZ-DVI Challenge; by calculating:

- Descriptive statistics per cohort and overall (geometric mean, SD, 95% CI, range) of time to parasite clearance, i.e., time between drug administration and first PCR below the limit of quantification;
- Descriptive statistics per cohort and overall (geometric mean, SD, 95% CI, range) of $\log_{10}PRR_{48}$;
- Descriptive statistics per cohort and overall (geometric mean, SD, 95% CI, range) of $\log_{10}PRR_{48,max}$; and
- Descriptive statistics per cohort and overall (geometric mean, SD, 95% CI range) of $PC_{½}$.

The parasite reduction ratios (PRRs) and the clearance half-life will be estimated from a log-linear model that will be fitted to the parasitaemia data observed from the administration of the treatment onwards. The PRR_{48}, $PRR_{48,max}$ and $PC_{½}$ will be calculated from the individual parameters and summarised by cohort.

Exploratory endpoints:

To characterise the individual variability of blood-stage parasite profile:

- Obtain the inter-individual variability of the parasite growth rate; and
- 5^{th}, 50^{th} and 95^{th} percentiles of predicted time to reach parasitaemia threshold of first positive PCR, ≥5000 parasites per mL blood, ≥10000 parasites per mL blood including their 95% CI.

Both these estimates will be based on the mixed model on parasite growth data described above.

9.1.3 *Safety Data*

Safety parameters will be tabulated and analysed descriptively.

Adverse Events

The original terms entered in the eSource system by Investigators to identify AEs will be fully described and coded according to the Medical Dictionary for Regulatory Activities (MedDRA).

The reported AEs will be allocated to phases based on their start date. All AEs will be listed. All AEs with onset after *Pf*SPZ-DVI Challenge will be summarised by cohort. Summaries will be made per MedDRA primary system organ class, MedDRA preferred term, severity, with the number and percentage of participants and the number of events. Similar summaries will be prepared for AEs considered to be related to Riamet® and AEs considered to be related to the *Pf*SPZ-DVI Challenge, for serious AEs and AEs of special interest.

Special attention will be paid to those participants who died, discontinued the investigational products due to an AE or experienced a severe or serious AE. Summaries, listings and narratives (also see Section 12.11) may be provided, as appropriate.

Malaria Clinical Score

For the malaria clinical score that will be administered by the PI or his/her trained delegate, actual values and changes from baseline will be evaluated by means of descriptive statistics. Additionally, expected signs and symptoms (see Attachment 1) will be summarised by score.

Clinical Laboratory Tests

Each continuous biochemistry and haematology laboratory test will be evaluated by means of descriptive statistics on the actual values, at each assessment time point and by cohort. Changes from baseline will also be summarised using descriptive statistics by assessment time point and by cohort.

Relative changes in clinical laboratory test values compared to values at baseline will be evaluated in accordance with the normal ranges of the clinical laboratory (below, within or above normal range). The percentage of participants with clinical laboratory test abnormalities will be summarised by cohort.

The number and percentage of participants with liver enzyme elevations after inoculation with the *Pf*SPZ-DVI Challenge as defined below will be summarised:

- ALT or AST >3 x Upper Limit of Normal (ULN);
- ALT or AST >5 x ULN;
- ALT or AST >8 x ULN;
- ALT or AST >3 x ULN and bilirubin >2 x ULN at the same time point, together with a conjugated bilirubin fraction> 35% (Potential Hy's law cases).

A listing of participants with any clinical laboratory test result outside the reference ranges will be provided.

Vital Signs

Vital signs parameters will be assessed after at least 10 min in supine position at the time points indicated in the Time and Events Schedule. Pulse rate, SBP and DBP will be evaluated by means of descriptive statistics (actual values and changes from baseline).

The percentage of participants with vital signs abnormalities will be summarised by cohort in a cross-tabulation of post-baseline versus baseline abnormalities to the normal ranges (as defined in Attachment 3).

Electrocardiogram

Twelve-lead ECG recordings will be performed in triplicate after participants remained in a supine position for at least 10 min.

All ECG data automatically measured by ECG devices (PR, QRS, QT, QTcB, QTcF and HR) and overall ECG evaluation will be listed. The ECG data, along with changes from baseline will be summarised by means of descriptive statistics at each assessment time point and by cohort.

The percentage of participants with ECG abnormalities will be summarised by cohort in a cross-tabulation of post-baseline versus baseline abnormalities to the normal ranges (as defined in Attachment 3). This cross-tabulation will include categorical assessment on actual values and changes from baseline of QTcB and QTcF prolongation.

Physical Examination

Abnormal findings in physical examination will be listed.

9.2 DETERMINATION OF SAMPLE SIZE

Up to 16 participants will be enrolled in 2 cohorts of 8 participants per cohort. In agreement with the Sponsor, additional participants may be recruited in each cohort, to replace discontinuations for non-safety reasons and achieve the required cohort size.

This is an exploratory study focusing on the methodology of malaria inoculation in healthy participants, thus no formal sample size calculation is performed.

10. ADVERSE EVENT REPORTING

10.1 DEFINITIONS

Adverse Event

An AE is any untoward medical occurrence in a clinical study participant administered a medicinal (investigational or non-investigational) product. An AE does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal finding), symptom or disease temporally associated with the use of a medicinal (investigational or non-investigational) product, whether or not related to that medicinal (investigational or non-investigational) product.

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal result of diagnostic procedures, including clinical laboratory test abnormalities.

Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose meets any of the following conditions:

- results in death;
- is life-threatening, i.e., the participant was at risk of death at the time of the event (e.g., ventricular fibrillation and anaphylaxis). The term does not refer to an event which hypothetically might have caused death if it were more severe.
- requires inpatient hospitalization or prolongation of existing inpatient hospitalisation:
  Hospitalisation refers to an overnight admission into hospital for the purpose of investigating and/or treating the AE. Hospitalisation for an elective procedure, or routinely scheduled treatment for a pre-existing condition that is unrelated to the study and has not worsened, is not an SAE. Treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of an SAE given above and not resulting in hospital admission, is not an SAE. Cosmetic surgery or for social reasons or respite care in the absence of any deterioration in the participant's general condition, is not an SAE;
- results in persistent or significant disability/incapacity, i.e., causing substantial disruption of the participant's ability to conduct normal life;
- is a congenital anomaly/birth defect;
- is medically significant, i.e., may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the participant's health or may require intervention to prevent one of the above outcomes. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalisation or development of drug dependency or drug abuse.
- Constitutes a possible Hy's Law case (defined as a participant with any value of ALT or AST greater than or equal to 3x the Upper Limit of Normal (ULN)

together with an increase in bilirubin to a value greater than 2xULN [>35% direct] and NOT associated with an ALP value greater than 2xULN).

Adverse Events of Special Interest (AESIs)

An AESI (serious or non-serious) is of scientific and medical concern specific to the Sponsor's product or programme, for which ongoing monitoring and rapid communication by the Investigator to the Sponsor could be appropriate. Such an event might require further investigation in order to characterise and understand it. Depending on the nature of the event, rapid communication by the study Sponsor to other parties (e.g., regulators) might also be warranted (CIOMS VI, ICH E2F, 2010). For the purpose of this study, any abnormalities listed below should be reported as AESI:

Hepatic

- Any ALT or AST above 3x ULN;
- Any elevation in bilirubin 2x ULN;
- Any AST or ALT above 2x ULN AND
    - Total bilirubin level (TBL) >1.5x ULN OR
    - INR >1.4
- Any AST or ALT above 2x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (eosinophil count above the ULN).

Cardiac

- Chest pain
- High sensitive troponin T ≥12 ng/L;
- QTcB or QTcF at any time >480 ms;
- Bundle branch block (except right bundle branch block that was present prior to Riamet® administration);
- Any arrhythmia, except:
    - sinus bradycardia that is clinically asymptomatic, and not associated with any other relevant ECG abnormalities;
    - sinus tachycardia that is clinically asymptomatic, and associated with a body temperature >38.0 °C, and not associated with any other relevant ECG abnormalities;
    - respiratory sinus arrhythmia;
    - wandering atrial pacemaker;
    - isolated, single premature atrial/ventricular complex (i.e., no bigeminy, trigeminy, couplets, triplets or salvos) that does not occur more than once in a particular ECG tracing.

Haematological

- Haemoglobin drop >2 g/dL and under lower limit of normal from baseline prior to inoculation;
- Absolute neutrophil count <1000/µL;
- Platelet count <100000/mm^3.

Dermatological:*

- Any suspected cutaneous AE, e.g., rash

* if one of these cutaneous reactions is observed and when feasible, pictures of the lesions should be obtained.

Unlisted (Unexpected) Adverse Event

An AE is considered unlisted if the nature or severity is not consistent with the applicable product reference safety information (Investigator's Brochure for an unapproved IMP or SmPC or approved Package Insert for an authorized product).

For this protocol, the reference documents for the assessment of expectedness for *Pf*SPZ-DVI Challenge is the Investigator's Brochure [9]. Expected reactions to the antimalarial therapies are described in the product's prescribing information [22,12]:

- *Pf*SPZ-DVI Challenge can be associated with itching, pain and swelling at the site of inoculation. This usually resolves within 24-48 hours.
- Riamet® (artemether and lumefantrine) may be associated with the following AEs; these may occur with a varying periodicity:

Frequency of Undesirable Effects of Riamet®

	Adults and adolescents above 12 years of age
Immune system disorders	
Hypersensitivity	Not known
Metabolism and nutrition disorders	
Decreased appetite	Very common
Psychiatric disorders	
Sleep disorders	Very common
Insomnia	Common
Nervous system disorders	
Headache	Very common
Dizziness	Very common
Paraesthesia	Common
Ataxia, hypoaesthesia	Uncommon
Somnolence	Uncommon
Clonus	Common
Cardiac disorders	
Palpitations	Very common
Electrocardiogram QT prolonged	Common
Respiratory, thoracic and mediastinal disorders	
Cough	Common
Gastrointestinal disorders	
Vomiting	Very common
Abdominal pain	Very common
Nausea	Very common
Diarrhoea	Common
Hepatobiliary disorders	
Liver function tests increased	Uncommon
Skin and subcutaneous tissue disorders	
Rash	Common
Pruritus	Common
Urticaria	Uncommon
Angioedema*	Not known
Musculoskeletal and connective tissue disorders	
Arthralgia	Very common
Myalgia	Very common
General disorders and administration site conditions	
Asthenia	Very common
Fatigue	Very common
Gait disturbance	Common

- Malarone® (antimalarial therapy if an intolerance or contraindication to Riamet® develops: atovaquone and proguanil hydrochloride) may be associated with the following AEs; these may occur with a varying periodicity:

System Organ Class	Very Common	Common	Uncommon	Rare	Not known[2]
Blood and lymphatic disorders		Anaemia Neutropenia [1]			Pancytopenia
Immune system disorders		Allergic reactions			Angioedema[3] Anaphylaxis (see section 4.4) Vasculitis[3]
Metabolism and nutrition disorders		Hyponatraemia[1] Anorexia	Elevated amylase levels[1]		
Psychiatric disorders		Abnormal dreams Depression	Anxiety	Hallucinations	Panic attack Crying Nightmares Psychotic disorder
Nervous system disorders	Headache	Insomnia Dizziness			Seizure
Cardiac disorders			Palpitations		Tachycardia
Gastrointestinal disorders	Nausea[1] Vomiting Diarrhoea Abdominal pain		Stomatitis		Gastric intolerance[3] Oral ulceration[3]
Hepatobiliary disorders		Elevated liver enzymes[1]			Hepatitis Cholestasis[3]
Skin and subcutaneous tissue disorders		Pruritus Rash	Hair loss Urticaria		Stevens-Johnson Syndrome Erythema multiforme Blister Skin exfoliation Photosensitivity reactions
General disorders and administration site conditions		Fever			
Respiratory, thoracic and mediastinal disorders		Cough			

An **Inoculum-Related AE** is a sign or symptom associated with malaria infection, i.e., confirmed by a protocol-defined PCR positivity (defined for the purpose of this study as a qPCR outcome ≥250 parasites per mL blood) at

- the onset of the event; or
- if the event started within 1 day of parasite clearance (defined as a qPCR value of 0 parasites per mL blood after initiating antimalarial therapy) or laboratory-defined PCR positivity (a qPCR outcome of 50-249 parasites per mL blood).

Close monitoring of expected signs and symptoms associated with malaria infection (listed in Attachment 1) form part of the safety evaluation of the study and will be used as part of the decision criteria to administer registered antimalarial therapy. These events will be classified as AEs, and may also be further sub-classified as inoculum-related AEs (see Section 5.1, Section 7.3.1, Section 7.3.2 and Section 9.1.3).

An **Antimalarial Therapy-Related AE** is a sign or symptom associated with the antimalarial drugs. Expected reactions to the antimalarial drugs are described in the product's prescribing information [22,12].

10.2 INTENSITY OF ADVERSE EVENTS

Each AE must be rated on a 5-point scale of increasing intensity according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0:

Note: the semi-colon within the description of the grade indicates 'or'.

Grade 1:

Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2:

Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily life (preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.).

Grade 3:

Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated, disabling; limiting self-care activities of daily life (bathing, dressing and undressing, feeding self, using the toilet, taking medications and not bedridden).

Grade 4:

Life-threatening consequences; urgent intervention indicated.

Grade 5:

Death related to AE.

10.3 CAUSALITY ASSESSMENT

The Investigator must assess the relationship of each event to the *Pf*SPZ-DVI Challenge agent and the antimalarial treatments (separately) and decide whether, in his or her

medical judgement, there is a reasonable possibility that the event may have been caused by any of the study agents. Where possible, a distinction should be made between events considered related to the *Pf*SPZ-DVI Challenge agent and the antimalarial treatments. If there is no valid reason for suggesting a relationship, then the AE should be classified as "not related". Alternatively, if there is any valid reason for suspecting a possible cause-and-effect relationship between the investigational product(s) and the occurrence of the AE (even if undetermined or untested), then the AE should be considered as "related" to whichever product as relevant. This should be documented in the participant's source document and eSource system.

The following may guide this assessment:

- **Related** – the temporal relationship between the event and the administration of the *Pf*SPZ-DVI Challenge agent and/or antimalarial treatment is compelling and/or follows a known or suspected response pattern to that product, and the event cannot be explained by the participant's medical condition, other therapies or accident;
- **Not related** – the event can be readily explained by other factors such as the participant's underlying medical condition, concomitant therapy or accident and no plausible temporal or biologic relationship exists between the *Pf*SPZ-DVI Challenge agent and/or antimalarial treatment and the event.

In addition to the assessments of relationship to the investigational products, the Investigator should comment on the adverse event record in the eSource system whether an AE is related to the study participation of the participant (study procedures etc.).

10.4 ACTION TAKEN REGARDING THE INVESTIGATIONAL PRODUCTS

The action taken towards the investigational products must be described as follows:

- Permanently discontinued;
- No action taken;
- Unknown/Not applicable.

10.5 OUTCOME

The outcome of each AE must be rated as follows:

- Recovered/resolved;
- Recovering/resolving;
- Not recovered/not resolved;
- Recovered with sequelae/resolved with sequelae;
- Fatal;
- Unknown.

10.6 RECORDING OF ADVERSE EVENTS

All (S)AEs occurring during the clinical investigation (i.e., monitored continuously from informed consent until the last study-related activity at the EOS on Day 28) must be documented in the eSource System.

Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (e.g., cough, runny nose, sneezing, sore throat, and head congestion should be reported as "upper respiratory infection"). Investigators must record their opinion concerning the relationship of the (S)AE to the investigational products in the eSource System. All measures required for (S)AE management must be recorded in the source documents and reported according to Sponsor's instructions.

All AEs occurring at any time during the study (including the follow-up period) will be followed by the Investigator until satisfactory resolution (e.g., value back to baseline value) or stabilisation or until final database lock. If necessary, in order to obtain additional information to ensure safety to the participant, additional blood and urine samples may be taken at the discretion of the Investigator. Certain long-term AEs related to therapy cannot be followed until resolution within the setting of this study. In these cases, follow-up will be the responsibility of the treating physician.

10.7 REPORTING OF SERIOUS ADVERSE EVENTS TO PRIMEVIGILANCE LTD.

All SAEs and AESIs, independent of the circumstances or suspected cause, must be reported on a Serious Adverse Event Form by the investigator to PrimeVigilance Ltd. within 24 h of their knowledge of the event, preferably by fax (+44 800 471 5694) or by e-mail (MMV@primevigilance.com).

The SAE form should include a clearly written narrative describing signs, symptoms, and treatment of the event, diagnostic procedures, as well as any relevant laboratory data and any sequelae, in order to allow a complete medical assessment of the case and independent determination of the possible causality.

Follow-up and outcomes should be reported for all participants who experience an SAE.

It is critical that the information provided on the Serious Adverse Event Form matches the information recorded in the source documents and in the eSource system for the same event.

Copies of additional laboratory tests, consultation reports, postmortem reports, hospital case reports, autopsy reports, and other documents should be sent when requested and applicable. Follow-up reports relative to the participant's subsequent course must be submitted to PrimeVigilance Ltd. until the event has subsided or, in case of permanent impairment, until the condition stabilises.

10.8 PREGNANCY

All initial reports of pregnancy in participants or in partners of male participants must be reported by the Investigator to PrimeVigilance Ltd. within 24 h of his/her knowledge of the event using a Pregnancy Form. Any participant who becomes pregnant during the study must be promptly withdrawn from the study (cfr. Section 8).

The Investigator will contact the participant at the expected time of delivery for follow-up. Abnormal pregnancy outcomes (e.g., spontaneous abortion, stillbirth, neonatal death, congenital abnormality, birth defect) are considered SAEs and must be reported using the Serious Adverse Event Form.

10.9 REPORTING OF SERIOUS ADVERSE EVENTS TO COMPETENT AUTHORITIES/ETHICS COMMITTEES

PrimeVigilance Ltd. assumes responsibility for appropriate reporting of AEs to the regulatory authorities. PrimeVigilance Ltd. will also report to the Investigator all SAEs that are unlisted (unexpected) and associated with the use of the drug. The Investigator (or PrimeVigilance Ltd. where required) must report these events to the appropriate Independent Ethics Committee/Institutional Review Board (IEC/IRB) that approved the protocol, unless otherwise required and documented by the IEC/IRB.

Adverse events reporting, including suspected unexpected serious adverse reactions (SUSARs), will be carried out in accordance with applicable local regulations.

After termination of the clinical study (determined as LPLV), any unexpected safety issue that changes the risk-benefit analysis and is likely to have an impact on the participants who have participated in the study, together with proposed actions, will be reported by the Sponsor/PrimeVigilance Ltd. to the competent authority(ies) concerned as soon as possible.

11. ETHICAL ASPECTS

11.1 STUDY-SPECIFIC DESIGN CONSIDERATIONS

Potential participants will be fully informed of the nature of the study and of the risks and requirements of the study before any study-related assessment will be carried out. During the study, participants will be given any new information that may affect their decision to continue participation. They will be informed that their participation in the study is voluntary and that they may withdraw from the study at any time with no reason given and without penalty or loss of benefits to which they would otherwise be entitled. Only participants who are fully able to understand the risks, benefits, and potential AEs of the study, and who provide their consent voluntarily will be enrolled in the study.

11.2 REGULATORY ETHICS COMPLIANCE

11.2.1 *Investigator Responsibilities*

The Investigator(s) should be qualified by education, training, and experience to assume responsibility for the proper conduct of the study, should meet all the qualifications specified by the applicable regulatory requirement(s), and should provide evidence of such qualifications through up-to-date curriculum vitae or other relevant documentation requested by the Sponsor, the IRB/IEC or the regulatory authority(ies).

The Investigator is responsible for ensuring that the clinical study is performed in accordance with the protocol, current International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines on Good Clinical Practice (GCP), and applicable regulatory and country-specific requirements.

Good Clinical Practice is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human participants. Compliance with this standard provides public assurance that the rights, safety, and well-being of study participants are protected, consistent with the principles originating from the Declaration of Helsinki (1964 and revisions), and that the clinical study data are credible.

11.2.2 *Independent Ethics Committee or Institutional Review Board (IEC/IRB)*

An IRB/IEC should safeguard the rights, safety, and well-being of all study participants. Special attention should be paid to studies that may include vulnerable participants.

Before the start of the study, the Investigator (or Sponsor where required) will provide the IEC/IRB with current and complete copies of the following documents:

- final protocol and, if applicable, amendments;
- Sponsor-approved ICF (and any updates or any other written materials to be provided to the participants);
- Investigator's Brochure (or equivalent information) and addenda;
- available safety information;

- information on compensation for study-related injuries or payment to participants for participation in the study, if applicable;
- Investigator's current curriculum vitae or other documentation evidencing qualifications (unless not required, as documented by the IEC/IRB);
- information regarding funding, name of the Sponsor, institutional affiliations, other potential conflicts of interest and incentives for participants;
- any other documents that the IEC/IRB may require to fulfill its obligation.

This study will be undertaken only after the IEC/IRB has given full written approval of the final protocol and amendments (if any), the ICF(s) and updates (if any), applicable recruiting materials and any other written information to be provided to the participants, and the Sponsor has received a copy of this approval. This approval letter must be dated and must clearly identify the IEC/IRB and the documents being approved.

During the study, the Investigator (or Sponsor where required) will send the following documents and updates to the IEC/IRB for its review and approval, where appropriate:

- protocol amendments;
- revision(s) to the ICF and any other written materials to be provided to the participants;
- revisions to compensation for study-related injuries or payment to participants for participation in the study;
- Investigator's Brochure addenda or new edition(s);
- summaries of the status of the study at intervals stipulated in guidelines of the IEC/IRB (at least annually);
- reports of AEs that are serious, unlisted, and associated with the IMP;
- new information that may adversely affect the safety of the participants or the conduct of the study;
- deviations from or changes to the protocol to eliminate immediate hazards to the participants;
- report of death of any participants under the Investigator's care;
- notification if a new Investigator is responsible for the study at the clinical site;
- Development Safety Update Report, Short-Term Study Specific Safety Summary and Line Listings, where applicable;
- any other requirements of the IEC/IRB.

For all protocol amendments (excluding the ones that are purely administrative, with no consequences for participants, data or study conduct), the amendment and applicable ICF revisions must be submitted promptly to the IEC/IRB for review and approval before implementation of the change(s), except when necessary to eliminate immediate hazard to the study participants. If a deviation from or a change to the protocol was implemented to eliminate an immediate hazard to study participants, then the implemented deviation or change, the reasons for it, and, if appropriate, the protocol amendment should be submitted to the IEC/IRB as soon as possible.

The Investigator (or Sponsor where required) will notify the IEC/IRB about the study completion within 90 days after the end of the study (defined as LPLV).

11.2.3 *Informed Consent*

Each participant must give written consent according to local requirements after the nature of the study has been fully explained. The consent form must be signed before performance of any study-related activity. The consent form that is used must be approved by both the Sponsor and the reviewing IEC/IRB. The informed consent should be in accordance with the principles that originated in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and Sponsor policy.

Before enrolment in the study, the Investigator or an authorised member of the clinical staff must explain to potential participants the aims, methods, reasonably anticipated benefits and potential hazards of the study, and any discomfort participation in the study may entail. Participants will be informed that their participation is voluntary and that they may refuse to participate or withdraw consent to participate at any time, without penalty or loss of benefits to which the participant was entitled. Finally, they will be told that the Investigator will maintain a participant identification register for the purposes of long-term follow-up if needed and that their records may be accessed by health authorities and authorised Sponsor staff without violating the confidentiality of the participant, to the extent permitted by the applicable law(s) or regulations. By signing the ICF the participant is authorising such access, and agrees to allow his or her study physician to recontact the participant for the purpose of obtaining consent for additional safety evaluations, if needed.

The language about the study used in the oral and written information, including the ICF, should be non-technical and practical and should be understandable to the participant (or the participant's legally acceptable representative). The participant will be given sufficient time to read the ICF and the opportunity to ask questions. After this explanation and before entry into the study, consent should be appropriately recorded by means of the participant's personally dated signature. After having obtained consent, a copy of the ICF must be given to the participant.

If a participant (or legally acceptable representative) is unable to read or write, an impartial witness should be present during the entire informed consent discussion. After the written ICF and any other written information to be provided to the participants, is read and explained to the participant (or legally acceptable representative), and after the participant (or legally acceptable representative) has orally consented to the participant's participation in the study and, if capable of doing so, has personally dated and signed the ICF, the witness should personally date and sign the consent form. By signing the ICF, the witness attests that the information in the ICF and any other written information was accurately explained to, and apparently understood by, the participant (or legally acceptable representative), and that informed consent was freely given by the participant (or legally acceptable representative).

11.2.4 *Privacy of Personal Data*

The collection and processing of personal data from participants enrolled in the study will be limited to those data that are necessary to investigate the safety, quality and utility of the IMP used in the study.

These data must be collected and processed with adequate precautions to ensure confidentiality and compliance with applicable data privacy protection laws and regulations. Appropriate technical and organizational measures to protect the personal data against unauthorized disclosures or access, accidental or unlawful destruction, or accidental loss or alteration must be put in place. Sponsor personnel whose responsibilities require access to personal data need to agree to keep the identity of the study participants confidential.

During the informed consent process the participants will be informed that, if they participate in this study, the Investigator may be required to allow direct access to participants' original medical records for study-related monitoring, audit, IEC/IRB review, and regulatory inspection. The participants will also be informed of possible transfer of the collected personal data to other entities and to other countries and that should such transfer take place, the Sponsor will ensure that technical and organisational measures are put in place to protect the data.

12. ADMINISTRATIVE REQUIREMENTS

12.1 PROTOCOL AMENDMENTS

Neither the Investigator nor the Sponsor will modify this protocol without a formal amendment. All protocol amendments must be issued by the Sponsor and signed and dated by the Investigator. Protocol amendments must not be implemented without prior IEC/IRB approval nor when the relevant competent authority has raised any grounds for non-acceptance, except when necessary to eliminate immediate hazard to the participants, in which case an amendment must be promptly submitted to the IEC/IRB and relevant competent authority. Documentation of amendment approval by the Investigator and IEC/IRB must be provided to the Sponsor or his designee. When the change(s) involves only logistic or administrative aspects of the study, the IRB (and IEC where required) only needs to be notified.

12.2 PARTICIPANT IDENTIFICATION, ENROLMENT AND SCREENING LOGS

The Investigator agrees to complete a participant identification and enrolment log to permit easy identification of each participant during and after the study. This document will be reviewed by the Sponsor site contact for completeness.

The participant identification and enrolment log will be treated as confidential and will be filed by the Investigator in the study file. To ensure participant confidentiality, no copies will be made. All reports and communications related to the study will identify participants by initials and/or assigned number only.

The Investigator must also complete a participant screening log which reports on all participants who were seen to determine eligibility for inclusion in the study.

12.3 SOURCE DOCUMENTATION

The Clinspark system is an electronic data capturing and information management system that will also serve as an eSource system for this study. The system combines all aspects of source data capturing with process control and clinical study management. All clinical and laboratory data, except those that are paper-based, will be collected directly in Clinspark. The Source Document Identification Overview will specify which information will be eSource and which will be paper-based. The monitor will check data at the monitoring visits to the clinical site. The Investigator will ensure that the data collected are accurate, complete and legible. Data will be monitored within Clinspark by the study monitor who has only reading rights. Any changes required following monitoring will be made by site personnel or the Investigator and will be documented with a full audit trail within Clinspark.

At a minimum, source documentation must be available for the following: participant identification, eligibility and study identification; date of informed consent, dates of visits, results of safety and efficacy parameters as required by the protocol, record of all AEs, follow-up of AEs, concomitant medication, drug receipt/dispensing/return records, investigational product administration information, laboratory and ECG printouts (if not available digitally), date of study completion and reason for early discontinuation of investigational products or withdrawal from the study, if applicable.

It is recommended that the author of an entry in the (e)Source documents be identifiable.

Source data may be directly captured from devices, transferred from third parties (e.g., laboratory data), or entered manually into the eSource system in use at the clinical site. In such case, the majority of the source data will only be available electronically. The remainder of the data, captured initially on paper, may be entered retrospectively into the eSource system.

Following the ICH-GCP guidelines, direct access to (e)Source documentation (medical records) must be allowed.

12.4 CASE REPORT FORM COMPLETION

All source data, except those that are paper-based, will be collected directly into the eSource system. Data must be entered in English. Paper-based source data will be manually transcribed to the eSource system. Only the data required for the clinical database will be transferred electronically from the eSource system to the clinical database. The Investigator must verify that all data entries in the eSource system are accurate and correct.

12.5 MONITORING

Medical and clinical monitoring of the study will be done under the responsibility of the Sponsor by ICON Clinical Research and IQVIA RDS & Integrated Services Belgium NV/SA, respectively.

The monitor will perform on-site monitoring visits as frequently as necessary. The monitor will record the dates of the visits in a study site visit log that will be kept at the clinical site. The first post initiation visit will be made as soon as possible after enrolment has begun. At these visits, the monitor will compare the data captured in the eSource system for completeness and accuracy and perform data source verification to any data that has been captured as paper source or entered in the system later on. The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the eSource system are known to the Sponsor and clinical staff and are accessible for verification by the Sponsor site contact. If electronic records are maintained at the investigational site, the method of verification must be discussed with the clinical staff.

Direct access to eSource documentation (medical records) must be allowed at all times for the purpose of verifying that the data recorded in the eSource system are consistent with the source documentation data. Findings from this review of captured data will be discussed with the clinical staff. During on-site monitoring visits (notified and agreed in advance with the clinical staff), the relevant clinical staff will be available, the (e)Source documentation will be accessible, and a suitable environment for review of study-related documents will be provided. The monitor will meet with the Investigator on a regular basis during the study to provide feedback on the study conduct.

12.6 DATA MANAGEMENT

Data management of the study will be performed under Sponsor delegation by SGS Life Sciences.

After the data entered in the eSource system are released by the Investigator, the data will be uploaded into the clinical database to perform cleaning activities. Computerised data cleaning rules will be used in addition to manual review to check for discrepancies and to ensure consistency and completeness of the data. Queries emerging during data cleaning will be generated by the clinical data manager in the eSource system. The Investigator or his designee will answer the queries and update the source data, if needed.

The clinical database will be locked as soon as it is considered clean. Before the clinical database will be locked, the study eSource system will be locked by the clinical staff. Only authorised and well-documented updates to the study data are possible after database lock. The locked database is used in the final statistical analysis for study reporting. Measures will be undertaken to protect participant data handed over by the Investigator to the data management department and during inspections against disclosure to unauthorised third parties. Participant confidentiality will be maintained at all times.

12.7 DATA QUALITY ASSURANCE

The accuracy and reliability of the study data will be assured by the selection of qualified Investigators and appropriate study sites, review of protocol procedures with the Investigator and associated personnel prior to the study, and by periodic monitoring visits by the Sponsor or designate.

The Sponsor or his designee will review the eSource system for accuracy and completeness during (on-site) monitoring visits and after transmission to the Sponsor; any discrepancies will be resolved with the Investigator or designee, as appropriate. After upload of the data into the clinical study database, their accuracy is verified using appropriate validation programs.

In accordance with Good Clinical Research Practice Guidelines and Recommendations, the Sponsor will be entitled to audit the facilities used in the clinical and laboratory parts of the study, as well as to access all the data files pertaining to the study. Similar procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The Investigator should immediately notify the Sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

12.8 ON-SITE AUDITS

Representatives of the Sponsor’s quality assurance department may visit the clinical site at any time during or after completion of the study to conduct an audit of the study in compliance with regulatory guidelines and company policy. These audits will require access to all study records, including source documents, for inspection and comparison with the eSource system. Participant privacy must, however, be respected. The Investigator and clinical staff are to be present and available for consultation during routinely scheduled site audit visits conducted by the Sponsor or his designee.

Similar procedures may also be conducted by agents of any regulatory body, either as part of a national GCP compliance program or to review the results of this study in support of a regulatory submission. The Investigator should immediately notify the

Sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

12.9 STUDY TERMINATION

The Sponsor has the right to terminate the study at any time. In case of an early termination of the study for safety reasons, or temporary halt by the Sponsor, the IEC/IRB and competent authorities should be notified within 15 calendar days and should be provided with a detailed written explanation for the termination/halt.

An end-of-study declaration will be submitted to the regulatory authorities and IEC/IRB after the complete study has ended. This notification will be submitted within 90 days after the end of the study.

12.10 RECORD RETENTION

In compliance with the ICH/GCP guidelines, the Investigator/Institution will maintain all eSource and all paper source documents that support the data collected from each participant, as well as all study documents as specified in ICH/GCP Section 8, Essential Documents for the Conduct of a Clinical Trial, and all study documents as specified by the applicable regulatory requirement(s). The Investigator/Institution will take measures to prevent accidental or premature destruction of these documents.

Essential documents must be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the IMP. These documents will be retained for a longer period if required according to the applicable regulatory requirements or per agreement with the Sponsor. It is the responsibility of the Sponsor to inform the Investigator/Institution as to when these documents no longer need to be retained.

If the responsible Investigator retires, relocates, or for any other reasons withdraws from his responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The Sponsor must be notified in writing of the name and address of the new custodian. Under no circumstance shall the Investigator relocate or dispose of any study documents without having obtained written approval from the Sponsor.

If it becomes necessary for the Sponsor or the appropriate regulatory authority to review any documentation related to the study, the Investigator must permit access to such reports.

12.11 USE OF INFORMATION AND PUBLICATION

All information, including but not limited to, information regarding investigational product or the Sponsor's operations (e.g., patent application, formulas, manufacturing processes, basic scientific data, prior clinical data, formulation information) supplied by the Sponsor to the Investigator and not previously published, and any data generated as a result of this study are considered confidential and remain the sole property of the Sponsor. The Investigator agrees to maintain this information in confidence, to use this

information only to accomplish this study, and not to use it for other purposes without the Sponsor's prior written consent.

The Investigator understands that the information generated in this clinical study will be used by the Sponsor in connection with the continued development of the investigational product, and thus may be disclosed as required to other clinical Investigators or regulatory agencies. To permit information derived from the clinical studies to be used, the Investigator is obliged to provide the Sponsor with all data obtained in the study.

The results of the study will be reported in a Clinical Study Report generated under the responsibility of the Sponsor and will contain eSource system data from all clinical sites that participated in the study.

Clinical narratives may be written for the following events (for example):

- All deaths (irrespective of drug relationship);
- All other SAEs and AESIs during treatment with the investigational products;
- All discontinuations of the investigational products due to AEs (irrespective of drug relationship);
- At the discretion of the team and after statistical analysis of the data, certain discontinuations not related to AEs or treatment failure, i.e., related to lost to follow-up or withdrawal of consent (irrespective of treatment group);
- Any events of special interest explicitly requested by the regulatory agencies.

The PI will sign off the final version of the Clinical Study Report. A summary of this final version will be provided to the Investigators, the applicable regulatory authorities, and the IECs/IRBs, if required by the applicable regulatory requirements, within 1 year after the end of the study (LPLV).

The Sponsor shall have the right to publish study data and information without approval from the Investigator. If an Investigator wishes to publish information from the study, a copy of the manuscript must be provided to the Sponsor for review at least 60 days before submission for publication or presentation. Expedited reviews will be arranged for abstracts, poster presentations or other materials. If requested by the Sponsor in writing, the Investigator will withhold such publication for up to an additional 60 days to allow for filing of a patent application. In the event that issues arise regarding scientific integrity or regulatory compliance, the Sponsor will review these issues with the Investigator. The Sponsor will not mandate modifications to scientific content and does not have the right to suppress information. For multicentre study designs and substudy approaches, results may need to be published in a given sequence (e.g., substudies should generally not be published before the primary endpoints of a study have been published). Similarly, Investigators will recognise the integrity of a multicentre study by not publishing data derived from an individual clinical site until the combined results from the completed study have been published in full, within 12 months after conclusion, abandonment or termination of the study at all clinical sites, or the Sponsor confirms there will be no multicentre study publication. Authorship of publications resulting from this study will be based on the guidelines on authorship, such as those described in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, which state that the named authors must have made a significant contribution to the design of the

study or analysis and interpretation of the data, provided critical review of the paper, and given final approval of the final version.

12.12 REGISTRATION OF CLINICAL STUDIES AND DISCLOSURE OF RESULTS

Public disclosure of the study is under the responsibility of the Sponsor. The study will be registered on the ClinicalTrials gov site.

12.13 CONFIDENTIALITY

All study documents are provided by the Sponsor to the Investigator and appointed clinical staff in confidence. None of this material may be disclosed to any party not directly involved in the study without the Sponsor's written permission.

The Investigator must assure that participants' anonymity will be maintained. The Investigator will keep a separate list with at least the initials, the participants' study numbers, names, addresses, and telephone numbers. The Investigator will maintain this for the longest period of time allowed by his/her own institution and, in any case, until further communication from the Sponsor.

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ATTACHMENT 1: CLINICAL SCORE FOR MALARIA

Grading of the signs and symptoms specified in the table beneath according to the CTCAE grading scale will be performed at all relevant protocol-specified time points, during the previous 24 h (continuously) if once daily assessments are required or during the previous 12 h (continuously) if assessments are performed twice daily, in accordance with the following:

- Absent = 0
- Mild = 1; equates to CTCAE grade 1
- Moderate = 2; equates to CTCAE grade 2
- Severe = 3; equates to CTCAE grade 3 or above

Participant ID:	**Date:**	**Time of Onset:** **Stop:**
Symptom/Sign	**Score (0 to 3)**	**qPCR**
Headache		☐ positive ☐ negative
Myalgia (muscle ache)		
Arthralgia (joint ache)		
Fatigue/lethargy		
Malaise (general discomfort/uneasiness)		
Chills/Shivering/Rigors		
Sweating/hot spells		
Anorexia		
Nausea		
Vomiting		
Abdominal discomfort		
Fever		
Tachycardia		
Hypotension		
TOTAL SCORE	**…/42**	

ATTACHMENT 2: BECK DEPRESSION INVENTORY

Beck's Depression Inventory

This depression inventory can be self-scored. The scoring scale is at the end of the questionnaire.

1.
- 0 I do not feel sad.
- 1 I feel sad
- 2 I am sad all the time and I can't snap out of it.
- 3 I am so sad and unhappy that I can't stand it.

2.
- 0 I am not particularly discouraged about the future.
- 1 I feel discouraged about the future.
- 2 I feel I have nothing to look forward to.
- 3 I feel the future is hopeless and that things cannot improve.

3.
- 0 I do not feel like a failure.
- 1 I feel I have failed more than the average person.
- 2 As I look back on my life, all I can see is a lot of failures.
- 3 I feel I am a complete failure as a person.

4.
- 0 I get as much satisfaction out of things as I used to.
- 1 I don't enjoy things the way I used to.
- 2 I don't get real satisfaction out of anything anymore.
- 3 I am dissatisfied or bored with everything.

5.
- 0 I don't feel particularly guilty
- 1 I feel guilty a good part of the time.
- 2 I feel quite guilty most of the time.
- 3 I feel guilty all of the time.

6.
- 0 I don't feel I am being punished.
- 1 I feel I may be punished.
- 2 I expect to be punished.
- 3 I feel I am being punished.

7.
- 0 I don't feel disappointed in myself.
- 1 I am disappointed in myself.
- 2 I am disgusted with myself.
- 3 I hate myself.

8.
- 0 I don't feel I am any worse than anybody else.
- 1 I am critical of myself for my weaknesses or mistakes.
- 2 I blame myself all the time for my faults.
- 3 I blame myself for everything bad that happens.

9.
- 0 I don't have any thoughts of killing myself.
- 1 I have thoughts of killing myself, but I would not carry them out.
- 2 I would like to kill myself.
- 3 I would kill myself if I had the chance.

10.
- 0 I don't cry any more than usual.
- 1 I cry more now than I used to.
- 2 I cry all the time now.
- 3 I used to be able to cry, but now I can't cry even though I want to.

11.

0	I am no more irritated by things than I ever was.
1	I am slightly more irritated now than usual.
2	I am quite annoyed or irritated a good deal of the time.
3	I feel irritated all the time.

12.

0	I have not lost interest in other people.
1	I am less interested in other people than I used to be.
2	I have lost most of my interest in other people.
3	I have lost all of my interest in other people.

13.

0	I make decisions about as well as I ever could.
1	I put off making decisions more than I used to.
2	I have greater difficulty in making decisions more than I used to.
3	I can't make decisions at all anymore.

14.

0	I don't feel that I look any worse than I used to.
1	I am worried that I am looking old or unattractive.
2	I feel there are permanent changes in my appearance that make me look unattractive
3	I believe that I look ugly.

15.

0	I can work about as well as before.
1	It takes an extra effort to get started at doing something.
2	I have to push myself very hard to do anything.
3	I can't do any work at all.

16.

0	I can sleep as well as usual.
1	I don't sleep as well as I used to.
2	I wake up 1-2 hours earlier than usual and find it hard to get back to sleep.
3	I wake up several hours earlier than I used to and cannot get back to sleep.

17.

0	I don't get more tired than usual.
1	I get tired more easily than I used to.
2	I get tired from doing almost anything.
3	I am too tired to do anything.

18.

0	My appetite is no worse than usual.
1	My appetite is not as good as it used to be.
2	My appetite is much worse now.
3	I have no appetite at all anymore.

19.

0	I haven't lost much weight, if any, lately.
1	I have lost more than five pounds.
2	I have lost more than ten pounds.
3	I have lost more than fifteen pounds.

20.

0	I am no more worried about my health than usual.
1	I am worried about physical problems like aches, pains, upset stomach, or constipation.
2	I am very worried about physical problems and it's hard to think of much else.
3	I am so worried about my physical problems that I cannot think of anything else.

21.

0	I have not noticed any recent change in my interest in sex.
1	I am less interested in sex than I used to be.
2	I have almost no interest in sex.
3	I have lost interest in sex completely.

INTERPRETING THE BECK DEPRESSION INVENTORY

Now that you have completed the questionnaire, add up the score for each of the twenty-one questions by counting the number to the right of each question you marked. The highest possible total for the whole test would be sixty-three. This would mean you circled number three on all twenty-one questions. Since the lowest possible score for each question is zero, the lowest possible score for the test would be zero. This would mean you circles zero on each question. You can evaluate your depression according to the Table below.

Total Score	Levels of Depression
1-10	These ups and downs are considered normal
11-16	Mild mood disturbance
17-20	Borderline clinical depression
21-30	Moderate depression
31-40	Severe depression
over 40	Extreme depression

ATTACHMENT 3: NORMAL RANGES FOR VITAL SIGNS AND ECG

NORMAL RANGES FOR VITAL SIGNS

Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Pulse rate (bpm)	Oral temperature (°C)
90≤ SBP ≤150	45≤ DBP ≤90	40≤ pulse ≤100	35.0≤ t° ≤37.5

These normal ranges are applicable in supine and sitting position (after at least 10 min) and in the standing position (after at least 2 min in the standing position).

NORMAL RANGES FOR ECG PARAMETERS

PR (ms)	QRS (ms)	QTc F (ms)	Heart rate (bpm)
120≤ PR ≤220	QRS ≤120	QTc ≤450 for males, ≤470 for females.	40≤ HR ≤100

These normal ranges are applicable in supine and sitting position (after at least 10 min).

ATTACHMENT 4: CLINICALLY ACCEPTABLE RANGES FOR CLINICALLY IMPORTANT STUDY INCLUSION LABORATORY TESTS

Test	Unit	Acceptable Inclusion Range	
		Low	High
Sodium	mmol/L	130	150
Potassium	mmol/L	3.0	5.5
Chloride	mmol/L	85	120
Calcium (Corrected)	mg/dL	0.95 x LLN	1.05 x ULN
Phosphate	mmol/l	0.95 x LLN	1.05 x ULN
Bicarbonate	mmol/l	0.95 x LLN	1.05 x ULN
Glucose Fasted	mg/dL	N/A	1.0 x ULN
Urea	mg/dL	N/A	1.75 x ULN
Uric acid	mg/dL	N/A	1.75 x ULN
Creatinine	mg/dL	N/A	1.0 x ULN
Creatine kinase	U/L	N/A	< 2.5 x ULN
*eGFR	mL/min/1.73m2	60	N/A
Total Protein	g/L	≥ 0.85 x LLN	≤ 1.25 x ULN
Albumin	g/L	≥ 0.85 x LLN	≤ 1.25 x ULN
Total Bilirubin	mg/dL	N/A	1.25 x ULN
Direct Bilirubin	mg/dL	N/A	1.25x ULN
ALP	U/L	N/A	1.5 x ULN
AST	U/L	N/A	1 x ULN
ALT	U/L	N/A	1 x ULN
GGT	U/L	N/A	1.5 x ULN
Lactate Dehydrogenase	U/L	0.9x LLN	1.1 x ULN
Prothrombin time INR	INR	1.0 x LLN	1.0 x ULN
Cholesterol	mg/dL	N/A	1.2 x ULN

Test	Unit	Acceptable Inclusion Range	
		Low	High
HDL Cholesterol	mg/dL	0.9x LLN	N/A
LDL Cholesterol	mg/dL	N/A	1.25 x ULN
Haemoglobin	g/dL	0.9x LLN	1.1.x ULN
Platelets	10E9/L	0.9x LLN	1.1 x ULN
White Blood Cells	10E9/L	0.9x LLN	1.1 x ULN
Neutrophils	10E9/L	1.0 x LLN	1.0x ULN
Lymphocytes	10E9/L	1.1 x LLN	1.1 x ULN
Monocytes	10E9/L	N/A	1.2 x ULN
Eosinophils	10E9/L	N/A	1.0 x ULN
Basophils	10E9/L	N/A	2.0 x ULN
C-reactive protein (CRP)	mg/L	N/A	1.0 x ULN
Troponin-T (high sensitivity)	ng/L	N/A	< 12
Protein (dipstick)		N/A	Trace
Ketones (dipstick)		N/A	Positive
Red Blood Cells (MCS)	µL	N/A	<19
White Blood Cells (MCS)	µL	N/A	<15
Hyaline Casts (MCS)		N/A	1+