MIST 1

STATISTICAL ANALYSIS PLAN

A clinical study to assess the feasibility of a controlled human Plasmodium vivax malaria infection model through experimental sporozoite infection in Thai adults.

Short title:

Version 1.0
Date: 10 05 2021

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1. INTRODUCTION

To date, most vivax malaria challenge models have been successfully performed in non-endemic settings such as in the US, UK and Australia. To date the challenge studies in UK in healthy vivax-naïve volunteers have not had any serious adverse event, malaria relapse, or lost to follow-up (unpublished data obtained directly from lead investigator). However, the findings in high income settings may not be extrapolated to the target population for future vaccine deployment, which is more heterogeneous both in terms of vivax immunity and genetic background. The best volunteer population to test new vaccines is the eventual target population for vaccine deployment.

This study aims to assess the safety and feasibility of controlled human *P. vivax* malaria infection in Thailand. It also aims to build on the limited knowledge that exists on parasite growth dynamics, transmission and the human immune response to infection following infection by the natural route of delivery – mosquito bite. **A major objective of the study is to provide a source of *P. vivax* infected blood to use in future vaccine efficacy trials of a sexual and asexual blood- and transmission-stage vaccines – making these studies more feasible in the future**.

For MIST 1 study, healthy vivax-naïve volunteers will be recruited at FTMCTU, Bangkok.

This documents sets out the statistical analysis plan for a MIST 1 to assess the feasibility and safety study and providing banked infected blood inocula for future blood stage human challenge studies.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 PRIMARY OBJECTIVE

a. To assess the feasibility and safety of controlled human *P. vivax* malaria infection in six healthy human volunteers, through experimental sporozoite infection (mosquito bite).

b. To obtain up to 250 mL of blood from each infected volunteer and freeze down the blood to create inocula for use in future *P. vivax* challenge studies.

2.2 PRIMARY ENDPOINT

a. Feasibility and safety of *P. vivax* sporozoite human challenge, as measured by successful infection (development of detectable persistent parasitaemia +/- clinical symptoms) and AE(S) occurrences.

b. Collection and freezing down of up to 250 mL *P. vivax*-infected blood from each of the 6 volunteers.

2.3 SECONDARY OBJECTIVES AND ENDPOINTS

a. To assess the immune response to primary *P. vivax* infection delivered by mosquito bite. The endpoint for this objective is cellular and humoral Immune response to primary *P. vivax* infection.

b. To assess gametocytaemia following primary *P. vivax* infection delivered by the mosquito. The endpoint for this objective is gametocytaemia pre-treatment, as measured by qPCR

3. STUDY DESIGN

3.1 GENERAL

This is a sporozoite-challenge clinical study with the **primary objectives** of assessing the **feasibility and safety** of controlled human sporozoite *P. vivax* malaria infection in six healthy volunteers, and **developing a bank of *P. vivax*-infected blood for use as inocula in future** controlled human blood stage *P. vivax* malaria infection studies. Secondary objectives are to assess the growth of and the immune response to *P. vivax* infection, and assess the induction of sexual gametocytaemia post-CHMI via the natural route of malaria infection (mosquito bite).

Up to six healthy, malaria-naïve adults aged between 20 and 55 years with weight more than 50 kg will be recruited at the FTMCTU in the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok. Volunteers will be recruited and receive mosquito-bite sporozoite challenge in two batches. The first batch will consist of **2** volunteers, and the second batch of **4** volunteers. A *P. vivax* mosquito-bite controlled human challenge infection (CHMI) by sporozoites will be delivered to the first batch of volunteers at the insectarium unit, Department of Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok. The procedures will be performed strictly following the approved Standard Operating Procedures (SOPs) and Work Instructions (WIs) and clinical monitoring will be performed during the post-challenge period at the FTMCTU as an inpatient until volunteers have two consecutive negative of malaria blood films and antimalarial medications (chloroquine) are completed. Chloroquine and primaquine will be prescribed according to current Thai national guideline. The second batch of volunteers will receive mosquito bite CHMI after the careful evaluation of the safety profiles / outcomes of the first 2 volunteers (at 28 days after treatment initiation as the minimum time interval).

During admission, the volunteers will have blood taken at regular intervals post-challenge to assess parasitemia, immune response following *P. vivax* infection, and they will be monitored closely until they meet the criteria for blood donation and treatment. Up to 250 mL of blood will be taken prior to treatment from the **successfully** infected volunteers. The volunteers will then be treated with a standard course of oral chloroquine and a 2-week course of directed observed therapy (DOT) of oral primaquine for radical cure of *P. vivax* hypnozoites. Volunteers will be followed up for 1 year after antimalarial treatment initiation. They will be asked to come back to the outpatient clinic on D 28, D 90, and 1 year after treatment initiation. During this phase, the volunteers will be contacted fortnightly by email/ phone call / other social communications e.g message (SMS), WhatsApp, Line, to ensure they remain well and asymptomatic.

3.1 DETERMINATION OF SAMPLE SIZE

The number of volunteers undergoing malaria challenge in this CHMI study will be up to 6, deemed sufficient to meet the primary objective of assessing the safety and feasibility of controlled human *P. vivax* malaria infection in Thailand.

4. ANALYSIS

4.1 GENERAL CONSIDERATIONS

The feasibility and safety of *P. vivax* sporozoite human challenge, will be declared if there will be a successful infection in at least one of the 6 volunteers i.e. if there will be development of detectable persistent parasitaemia +/- clinical symptoms.

The success of this study will also be declared if we will be able to obtain up to 250 mL of blood from each successfully infected volunteer and freeze down the blood to create inocula for use in future *P. vivax* challenge studies.

The safety of this challenge study will be assessed by summarising and evaluating the SAEs and the AEs regarding severity and relatedness to the challenge.

4.1.1 Data integrity

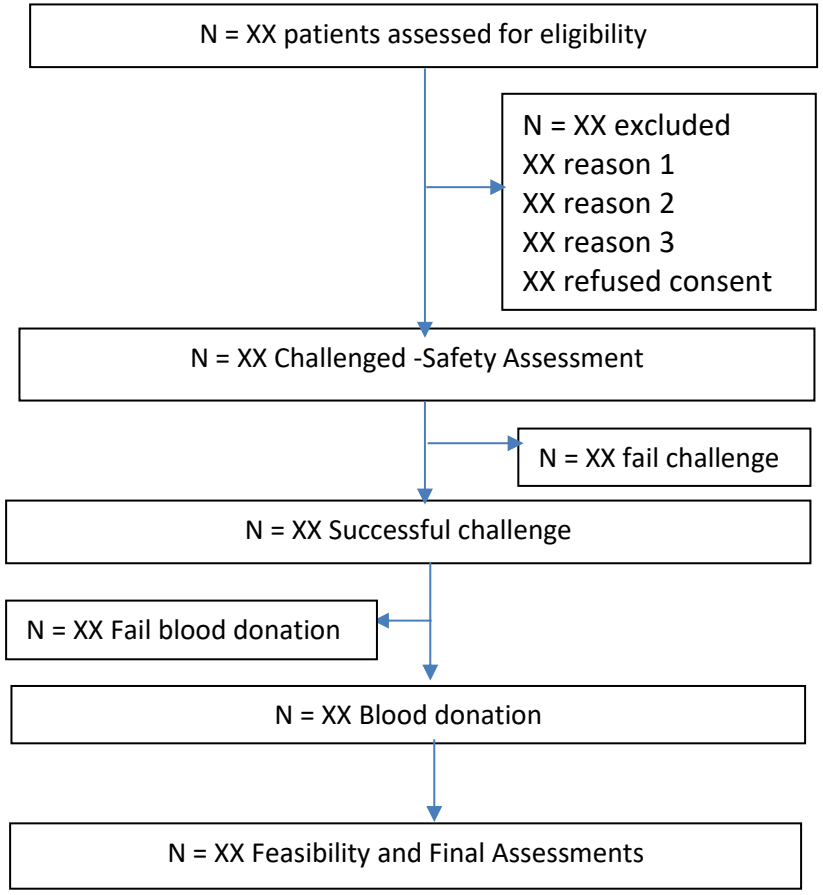
This study will be conducted in compliance with the protocol, relevant Standard Operating Procedures (SOPs), Work Instructions (WIs), Good Clinical Practice (GCP) and the applicable regulatory requirement(s). All the analyses will be performed on clean data only.

4.1.2 Data cleaning and verification

All data will be cleaned and verified prior to statistical analysis. The study site will be visited by the Monitor periodically at times agreed on with the Investigator. At the time of each monitoring visit, the Monitor will review the completed CRFs to ascertain that all items have been completed and that the data provided are accurate and obtained in the manner specified in the protocol. The Monitor will also check that the data in the CRF are consistent with the clinical records (Source Data Verification [SDV]) and that study results are recorded completely and

correctly. Study Specific Audit will also done to assess the clinical and laboratory study activities compliance with study protocol, SOPs and Plans, local QMS procdures, applicable standards and guidelines. The data manager will ensure that clean data is submitted to the statistician for analysis. The statistician will cross-check that the available data for analysis is clean. Any data cleaning queries will need to be resolved before statistical analyses.

4.2 DUMMY FIGURE 1 – STUDY PROFILE



4.3 STUDY ASSESSMENTS

4.3.1 Demographics and other baseline characteristics

The following baseline characteristics for each participant will be described and summarized for all partcipants in table 1 below. Variables such as age, heart rate, respiratory rate, and laboratory data will be summarized using median and range. Continuous variables such as weight, height and haemoglobin will be summarized using mean and standard deviation. Categorical variables such as sex and symptoms will be summarized using frequencies.

Table 1 Baseline Characteristics of the Subjects

Characteristics	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Total, Median (Range) or Mean (SD) or n/N (%)
Age (years)	xx	xx	xx	xx	xx	xx	xx (xx – xx)
Male (Y/N)	x	x	x	x	x	x	x/x
Body temperature (°C)	xx.x	xx.x	xx.x	xx.x	xx.x	xx.x	xx (xx – xx)
Blood pressure (mmHg), (Systolic/Diastolic)	xxx/xxx	xxx/xxx	xxx/xxx	xxx/xxx	xxx/xxx	xxx/xxx	xx (xx – xx)
Heart rate (beats/min)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
Respiratory rate (breaths/min)	xx	xx	xx	xx	xx	xx	xx (xx – xx)
Weight (kg)	xx	xx	xx	xx	xx	xx	xx (xx)
Height (cm)	xx	xx	xx	xx	xx	xx	xx (xx)
Haematology							
WBC (103/µL)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
RBC (10^6/µL)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
Heamoglobin (g/dL)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx)
Pletelets (103/µL)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
Neutrophil (%)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
Lymphocyte (%)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
Monocyte (%)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)

Eosinophil (%)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
Basophil (%)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
Other abnormal WBC	xx	xx	xx	xx	xx	xx	xx (xx)
Biochemistry							
BUN (mg/dL)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
Creatinine (mg/dL)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
Total bilirubin (mg/dL)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
Cholesterol (mg/dL)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
Triglyceride (mg/dL)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
HDL (mg/dL)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
LDL (mg/dL)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
Alkaline Phosphatase (U/L)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
AST (U/L)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
ALT (U/L)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
Albumin (g/dL)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
Globulin (U/L)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
Sodium (mmol/L)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
Potassium (mmol/L)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
Calcium (mg/dL)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
Magnesium (mg/dL)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
Chloride (mmol/L)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
Bicarbonate (mmol/L)	xxx	xxx	xxx	xxx	xxx	xxx	xx (xx – xx)
FBS (mg/dL)							
etc ...							

4.3.2 Clinical efficacy assessments (an example of a possible heading)

No clinical efficacy planned for MIST 1

4.3.3 Pharmacokinetic assessments (an example of a possible heading)

No PK analyses planed for MIST 1

4.3.4 Adverse events

Adverse events will be summarized as shown in table 3 below. Firstly, all events will be captured.ie. including multiple events in an individual. Thereafter the number of individuals with at least an event will be recorded and the highest grade will be presented.

Table 3.1 Adverse eventsduring the admission phase (Day 0 to day of blood donation)

Adverse event	Number of event (grade 1-2), n	Number of participants (grade 1-2), n	Number of event (grade 3-4), n	Number of participants (grade 3-4), n
n				
Symptom or disease				
Fever	x	x	x	x
Headache	x	x	x	x
Myalgia	x	x	x	x
Palpitation	x	x	x	x
Dizziness	x	x	x	x

Chills	x	x	x	x
Abdominal pain	x	x	x	x
Profuse sweating	x	x	x	x
Anorexia	x	x	x	x
Nausea	x	x	x	x
Vomitting	x	x	x	x
Diarrhea	x	x	x	x
Muscle pain	x	x	x	x
Anemia	x	x	x	x
etc ...				
Abnormal laboratory finding, n	x	x	x	x
Creatinine	x	x	x	x
Total bilirubine	x	x	x	x
Alkaline phosphatase	x	x	x	x
Alanyl transfarase (ALT)	x	x	x	x
Aspartate transfarase (AST)	x	x	x	x
WBC	x	x	x	x
Platelet	x	x	x	x
etc...				

Table 3.2 Adverse events during the treament phase

Adverse event	Number of event (grade 1-2), n	Number of participants (grade 1-2), n	Number of event (grade 3-4), n	Number of participants (grade 3-4), n
n				
Symptom or disease				
Fever	x	x	x	x

Headache	x	x	x	x
Myalgia	x	x	x	x
Palpitation	x	x	x	x
Dizziness	x	x	x	x
Chills	x	x	x	x
Abdominal pain	x	x	x	x
Profuse sweating	x	x	x	x
Anorexia	x	x	x	x
Nausea	x	x	x	x
Vomitting	x	x	x	x
Diarrhea	x	x	x	x
Muscle pain	x	x	x	x
Anemia	x	x	x	x
etc ...				
Abnormal laboratory finding, n	x	x	x	x
Creatinine	x	x	x	x
Total bilirubine	x	x	x	x
Alkaline phosphatase	x	x	x	x
Alanyl transfarase (ALT)	x	x	x	x
Aspartate transfarase (AST)	x	x	x	x
WBC	x	x	x	x
Platelet	x	x	x	x
etc ...				

Relationship of adverse events to either treatment or infection will be summarized in table 4 below.

Table 4 Distribution of adverse events by **relationship t**o study procedures.

	During admission (infection) phase					During treatment phase				
	Unrelated	Unlikely	Probably	Possibly	Definitely	Unrelated	Unlikely	Probably	Possibly	Definitely
AE term	n	n	n	n	n	n	n	n	n	n
Abdominal discomfort	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Abdominal pain.	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Anorexia	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Back pain	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Bruising	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Chills	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Diarrhea	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Dizziness	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Dry mouth	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Dyspepsia	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Fatigue	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Fever	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Flushing	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Gingivitis	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Headache	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Insomnia	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Malaise	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Maculopapular	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Mucositis oral	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Myalgia	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Nausea	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Neck pain	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx

Palpitation	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Profuse sweating on palms & soles	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Pruritus.	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Sore throat	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Tachycardia	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Urinary tract infection	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Urticaria	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx
Vomiting etc ...	xx	xx	xx	xx	xx	xx	xx	xx	xx	xx

Serious adverse events will be summarized in table 5 below.

Table 5 Serious adverse events

Adverse event	Number of event	Number of participants
Death		
Life-threatening event	x	x
Persistent or significant disability or incapacity	x	x
Transfer of inpatient care to the intensive care unit	x	x
An important medical event	x	x
Congenital anomaly or birth defect	x	x

A detailed listing will also be shown.

Immune response and gametocytaemia assessment

The assessment of the immune response and gametocytaemia to primary *P. vivax* infection delivered by mosquito bite will be analysed as detailed below:

4.3.5 Cellular Response

Cellular response will be investigated using whole blood and/or peripheral blood mononuclear cells (PBMCs). The data will be summarized as frequencies, percentages (%), and expression level (median fluorescence intensity (MFI)) as shown in the table below. Furthermore a spaghetti plot will be constructed and presented to show the profile of each of the important parameters over time across the participants. The profiles will also be presented as a plot of box and whisker plot indicating the profile of median and quartiles of the absolute parameters over-time. Where tests of hypothesis will be necessary, paired tests such as Wilcoxon's signed rank test will be used to compare parameters at a specified day with the baseline will be performed. Caution will be taken into account when interpreting significant tests considering that sample sizes are very small. Tests of significance will be performed at 5% significance level. Stata software of at least version 15.0 will be used to analyse the data and make plots. Some plots will be done in Graphpad Prism where necessary. MFI value will be analysed and plotted by FlowJo software.

Table 6 Immunological profile (frequency, %, MFI)

Immune cell subset	Baseline (Pre)	Challenge phase			Post challenge/ Treatment, Follow-up			
	Day-1	Day 2	Day 5	Day_{BD}	Day_{Rx7}	Day_{Rx28}	Day_{Rx90}	1 year
Granulocyte subsets								
Neutrophil								
Basophil								
Eosinophil								
etc ...								
Monocyte subsets								
Myeloid Dendritic cell (MDC)								
Plasmacytoid Dendritic cell (PDC)								
etc ...								
Lymphocyte subsets								
Natural Killer (NK)								
Natural Killer T (NKT)								
Mucosal Associated Invariant T cell (MAIT)								
Helper T-cell								
Cytotoxic T-cell								
Memory T-cell								
Regulatory T-cell								
γδ T-cell								
Naïve B-cell								
Memory B-cell								
Plasmablast etc ...								

4.3.6 Humoral Response

Cytokine response (median fluoresence intensity, relative antibody unit)

The level of cytokine responses related to sporozoite challenge will be assessed by using Multiplex Bead Based Immunoassay (Luminex) and/or ELISA. Luminex and/or ELISA will be used to explore the level of antibodies and cytokines responses during different phases of infection. P-values for the test of significance will be indicated where applicable. However, Caution will be taken into account when interpreting such significant tests considering that sample sizes are very small.

Table 7 Cytokine response profile (MFI, RAU)

Cytokine	Baseline (Pre)	Challenge phase			Post challenge/ Treatment, Follow-up			
	Day-1	Day 2	Day 5	Day_{BD}	Day_{Rx7}	Day_{Rx28}	Day_{Rx90}	1 year
FGF basic								
Eotaxin								
G-CSF								
GM-CSF								
IFN-γ								
IL-1β								
IL-1ra								
IL-2								
IL-4								
IL-5								
IL-6								
IL-7								
IL-8								
IL-9								
IL-10								
IL-12 (p70)								
IL-13								
IL-15								
IL-17A								
IP-10								
MCP-1 (MCAF)								
MIP-1α								
MIP-1β								
PDGF-BB								
RANTES								
TNF-α								
VEGF								

TGF-β1								
TGF-β2								
TGF-β3 etc ...								

4.3.7 Gametocyte Analysis

Another secondary objective of MIST1 study relating to gametocyte is to assess gametocytaemia following primary *P. vivax* infection delivered by the mosquito bite and the endpoint is gametocytaemia pre-treatment, as measured by qRT-PCR.

The endpoint for gametocyte detection will be the Pvs25 gene transcript copy number/ul blood collected at each time point as follows: challenge day; day 1 to 5 or up to day of treatment and during subsequent days of follow-up. The data will be summarized using the geometric mean and standard deviation/error at each time point. The trend in geometric means will be assessed and where necessary will be presented graphically using trend lines.