Sars

Abbreviated Title: Blood-stage *Plasmodium vivax* cell bank

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Title: Induced blood-stage Plasmodium vivax infection with HMPBS02-Pv Challenge Agent in healthy malaria-naïve adults to produce a Plasmodium vivax parasite cell bank for future studies

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Investigational Agents:

Drug Name:	HMPBS02-*Pv* Challenge Agent
IND Number:	IND 27245
Sponsor:	Office of Clinical Research Policy and Regulatory Operations (OCRPRO), Division of Clinical Research (DCR), NIAID, NIH
Manufacturer:	Queensland Institute of Medical Research, Berghofer (QIMRB) and NIH Department of Transfusion Medicine

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice(GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies(45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFRPart 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the institutional review board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: Induced blood-stage *Plasmodium vivax* infection with HMPBS02-*Pv* Challenge Agent in healthy malaria-naïve adults to produce a *Plasmodiumvivax* parasite cell bank for future studies

Study Description: This is a single-center, open-label study to infect healthy volunteers using induced blood-stage malaria (IBSM) so that blood can be collected to enable production of a human malaria parasite (HMP) bank for use in future studies. This study will be conducted in up to two participants, with an accrual ceiling of 50. Participants will be inoculated intravenously (IV) with human malaria parasite blood stage *P. vivax* parasite–infected erythrocytes (HMPBS02-*Pv*) challenge agent (Day 0) and then monitored closely via outpatient clinic visits, phone visits, and while inpatient at the NIH Clinical Center (CC) for symptoms and signs of malaria to characterize the safety, tolerability, and infectivity in healthy malaria-naïve participants inoculated with *P. vivax*. Blood sampling will be done periodically to measure parasitemia via quantitative polymerase chain reaction (qPCR) targeting the *P. vivax* 18S rRNA gene.

The threshold for the commencement of collection of blood for production of the HMP bank and subsequent antimalarial rescue treatment with artemether/lumefantrine will occur when the Malaria Clinical Score is >6 (admission within 24 hours of notification), or parasitemia is >20,000 parasites/mL, or at the investigator's discretion. When this threshold is reached, the participant will be admitted to the NIH CC for further safety assessments before undergoing the blood collection procedure.

After blood collection, the first dose of artemether/lumefantrine will be administered and the participant will remain inpatient for 48-72 hours to monitor for safety and tolerability of rescue therapy, and to ensure adequateclinical and parasitological response to treatment. In the unlikely and unprecedented event that artemether/lumefantrine fails to clear parasitemia,participants will be treated with chloroquine. If oral administration of eitherartemether/lumefantrine or chloroquine is not possible (e.g., the participant is vomiting), the participant will receive IV treatment with artesunate. Afterdischarge, participants will be followed on an outpatient basis for monitoring of safety and parasite clearance. Follow-up for safety assessments will be performed on Day 28±3, Day 56±7 (phone call only), and Day 90±7 (End of Study).

Participants may also be evaluated for the presence of sexual parasite stages(gametocytes) and other parasite lifecycle stages in the blood during the study using quantitative reverse transcriptase PCR (qRT-PCR).

Objectives:

Primary Objective: The collection of blood from healthy participants experimentally infected with *P. vivax* isolate HMPBS02-Pv for the production of a *P. vivax* blood-stage parasite bank for use in future studies.

Secondary Objective: To assess the safety and tolerability of the *P. vivax* IBSM model following inoculation of healthy malaria-naïve participants with *P. vivax*.

Exploratory Objective: To further characterize blood- and sexual-stage parasite growth profiles following inoculation with *P. vivax* isolate and treatment with artemether/lumefantrine.

Endpoints:

Primary Endpoint: Collection of blood for the production of a *P. vivax* blood-stage parasite bank from study participants following experimental infection with *P. vivax* isolate HMPBS02-Pv.

Secondary Endpoint: Occurrence of solicited and unsolicited adverse events(AEs) following *P. vivax* inoculation prior to the initiation of antimalarial treatment.

Exploratory Endpoint: The rate of growth of blood- and sexual-stage *P. vivax* isolate following inoculation and treatment with artemether/lumefantrine as determined by blood smear and/or qPCR.

Study Population: Healthy malaria-naïve US adults between 18 and 50 years of age

Phase: 1

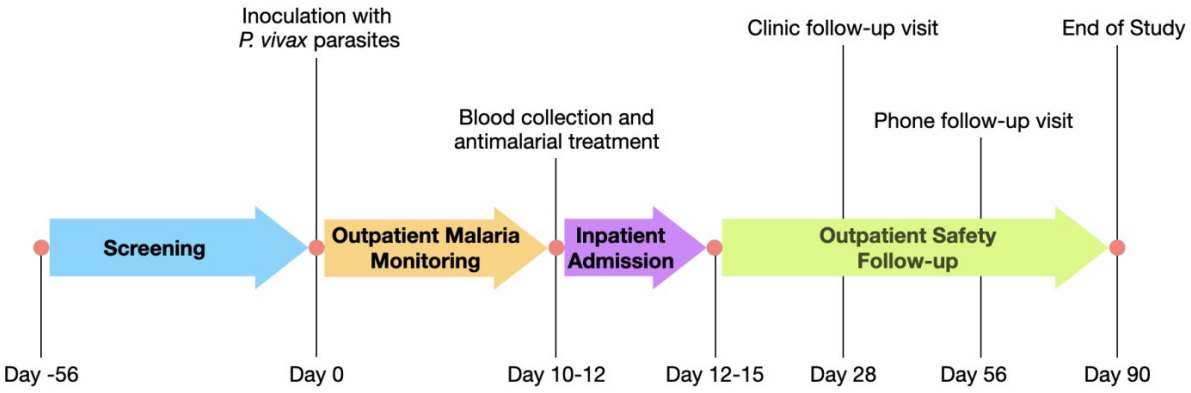
Description of Sites/Facilities Enrolling Participants: NIH CC, Bethesda, Maryland, USA

Description of Study Intervention: A single dose of HMPBS02-*Pv* Challenge Agent will be administered IV.

Study Duration: Approximately five months

Participant Duration: Three months

1.2 SCHEMA


Inoculation with
P. vivax parasites
Clinic follow-up visit
End of Study
Blood collection and
antimalarial treatment
Phone follow-up visit
Screening
Outpatient Malaria
Monitoring
Inpatient
Admission
Outpatient Safety
Follow-up
Day -56
Day 0
Day 10-12
Day 12-15
Day 28
Day 56
Day 90

1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	Screening	Eligibility Confirmation[a]	Malaria Inoculation	Phone Contact[b]	Malaria Monitoring[c]	Blood Collection for Banking and Rescue Treatment[d]	Outpatient Monitoring[e]	Safety Visit	Follow-up Phone Call	EOS Visit
Study Day	D-56 to D-1	D-3 to D-1	D0	D1 to D3	D4 to blood collection for banking	Day of collection	Post-admission to D28 Safety visit[e]	D28±3[b]	D56±7	D90±7
Eligibility and Safety Assessments										
Assessment for HMP bank	X									
Informed consent	X									
PHQ-2 questionnaire	X									
Malaria Comprehension Exam	X									
Assessment of CV disease risk	X									
Medical history & prior medications	X		X							
Drug & alcohol screen[g]	X		X			X[h,]				
Full physical examination	X							X		
Symptom-directed physical examination[i]		X	X		X	X	X[e]			X
Vital signs assessment	X	X	X		X	X	X[e]	X		X
ECG	X		X			X[j]				
Urinalysis	X	X				X[h]		X		
Hematology & biochemistry[k]	X	X			X	X	X[e]	X[l]		X[m]
Pregnancy test[n]	X		X			X[h]				
Blood type and alloantibodies[v]	X							X		X[m]
HIV pre-test counseling	X									
Bloodborne infection screening[w]	X					X				
DTM NIH CC donor assessment		X[f]								
SARS-Cov-2[Y]		X								
Safety serum storage			X							X
Safety EDTA storage						X				
AEs & concomitant medications			X	X	X	X	X[e]	X	X	X
Malaria clinical score					X	X	X[e,o]			
Solicited (local/systemic) reactogenicity			X	X						
Verify ongoing eligibility			X							
Confirm pregnancy prevention compliance			X	X	X	X	X			
Blood collection for banking						X[p]				
Research sample collection[x]			X		X	X		X		
Malaria Monitoring										
Malaria 18S qPCR blood sampling			X		X[q]	X	X[e]	X[l]		X[m]
Parasite lifecycle stage qRT-PCR blood sampling			X		X	X	X[e]	X[l]		
Malaria blood smear[r]			X		X	X[s]	X	X		X
Rescue Drug Treatment										
Artemether/lumefantrine treatment						X[k]				
Chloroquine treatment						X[t]				
Artesunate treatment						X[u]				

AEs: Adverse events; CV: cardiovascular; DTM NIH CC: Department of Transfusion Medicine NIH Clinical Center; ECG: Electrocardiogram; EOS: End of Study; HIV: human immunodeficiency virus; HMP: human malaria parasite; PHQ-2: Patient Health Questionnaire-2; qPCR: quantitative polymerase chain reaction; qRT-PCR: quantitative reverse transcriptase polymerase chain reaction; RBC: red blood cell

[a] This visit is not required in the event that the screening visit is conducted within this period.
[b] A phone call will also be made on Day 56±7 to monitor participant well-being and to solicit any AEs.
[c] Daily visits until Day 9. Up to 3 visits per day may be required, at the discretion of the investigator, between Day 9 and treatment day.
[d] Admission is expected to start on Day 9, 10, 11 or 12 and last 48 to 72 hours.
[e] If participant is released from confinement before 72 hours post–artemether/lumefantrine treatment.
[f] Routine donor screening visit by DTM NIH CC service providers may occur any time between screening visit and day of eligibility confirmation visit.
[g] Urine drug screen, serum ethanol test. Blood ethanol at screening only. Urine drug screen at screening, Day 0, Admission. Day 0/admission results assessed retrospectively.
[h] At time of admission to clinical unit only.
[i] Physical examinations should be performed at screening and at Day 28±3. At all other times, symptom-directed physical examinations will only be performed when signs or symptoms of malaria are identified, and it isclinically indicated at the investigator's discretion. Before discharge, nursing staff must confirm with the study doctor or investigator if any participant requires symptom-driven examination.
[j] ECG may be performed at any time during the rescue treatment and observation period
[k] Required laboratory testing for hematology and biochemistry as per Section 8.2.2.1, Section 8.3.
[l] At the discretion of the investigator and if required for safety reasons.
[m] If not performed on Day 28±3.
[n] Serum pregnancy test at screening. Urine pregnancy test may be performed at malaria inoculation and time of admission to clinical unit.
[o] Only if vital signs are abnormal, or at the investigator's discretion.
[p] Collection of a target minimum 200 mL blood sample for cell bank processing. At the discretion of the investigator, extra volume may be collected, within maximum whole blood volume collection limit of 550 mL in any 8-week period.
[Q] Blood sampling for malaria may be done up to 3 times a day from Day 9 to treatment day (at the discretion of the investigator).
[R] Blood smear collection may occur in parallel with other malaria monitoring, at the discretion of the investigator.
[S] Blood smear collection prior to blood collection for banking.
[T] Chloroquine will only be administered to participants in the case of failure of artemether/lumefantrine therapy.
[U] Intravenous artesunate treatment will only occur in the event that a participant is unable to complete oral treatment with either artemether/lumefantrine or chloroquine (e.g., the participant is vomiting).
[V] Blood type including ABO, Rh status, Duffy antigen at screening only. Alloantibodies at screening and at Day 28 or 90/EOS visit.
[W] Eligibility screening for bloodborne infections at screening visit. Further screening for acute transfusion-transmissible agents at admission.
[X] A PaxGene (or equivalent) tube for transcriptional studies may be collected during the course of the study at Day 0, Day 4 (approximate), Admission, and Day 28.
[Y] A SARS-CoV-2 screening test will be performed at the eligibility confirmation visit (or at the screening visit if within -3 to-1 day before inoculation) and at future visits based on investigator discretion/ NIH CC policies.

2 INTRODUCTION

2.1 STUDY RATIONALE

Although *P. falciparum* is the most virulent *Plasmodium* species, *P. vivax* is the second-most prevalent, occurs over a wider geographical area, is the dominant species in Oceania and Asia, and altogether is estimated to cause 80 to 300 million clinical cases peryear. Despite this high burden, the tools available to study *P. vivax* malaria are sparse andthe prospects for elimination are more daunting than for *P. falciparum*. In addition, following inoculation of sporozoites by mosquitoes, *P. vivax* forms a hypnozoite that can lay dormant in hepatocytes for months or years and then cause a relapse of infection. Most of the studies aimed at investigating mosquito infection from human volunteers with *P. vivax* have been undertaken using *P. vivax* sporozoites. However, sporozoite inoculation in healthy volunteers carries the additional risk of relapse of *P. vivax* infection due to the formation of hypnozoites. This risk can be eliminated using the IBSM model that uses a blood-stage parasite challenge. To date, a total of 45 healthy volunteers have been challenged with two *P. vivax* blood-stage malaria isolates. The results of these studies have demonstrated that this approach is safe with no signs of relapse of *P. vivax* infection detected in any of the challenged volunteers. The availability of a *P. vivax* blood-stage challenge model and optimal use of such a model provides a unique opportunity to rapidly and cost-effectively test the efficacy of *P. vivax* vaccines and drugs in healthy volunteers,accelerating clinical development of new interventions for *P. vivax* malaria.

2.2 BACKGROUND

Despite the decrease in malaria incidence achieved in the last 15 years, this parasitic disease still threatens almost half of the world's population. According to a recent World Health Organization (WHO) report, in 2017 there were 219 million cases of malaria and 435,000 deaths. [1] Most malaria cases occurred in sub-Saharan Africa. However, Asia, Latin America, the Middle East and parts of Europe are also at risk. Whilst *P. falciparum* is the most prevalent malaria parasite in Africa, *P. vivax* has a wider geographical distribution. In 2017, 44% of the malaria cases that occurred outside the African continent were caused by *P. vivax*.[1] Moreover, 70 to 80 million cases per year of relapsing malariaoccur due to infection with *P. vivax*.

The WHO has declared that the response to malaria is a global development priority and has changed their recommendation from control to eradication programs. A robust development pipeline of potential drug and vaccine candidates is required in order to meet this target. The development process for determining safety and clinical efficacy of potential antimalarial interventions requires fast, efficient test systems.

Controlled human malaria infection (CHMI) is increasingly being used to evaluate antimalarial drug and vaccine candidates.[2-9] Most CHMI studies use sporozoite parasite forms to induce infection. However CHMI studies with *P. falciparum* and *P. vivax* have also been conducted with the IBSM model, whereby participants are infected with blood-stage malaria parasites.[5, 7, 10] The IBSM models offer a pathway to test efficacyof *P. falciparum* and/or *P. vivax* vaccines and drugs in healthy volunteers in a rapid and cost effective manner. One of the advantages of the IBSM model is its ability to facilitate analysis of antimalarial and vaccine efficacy against blood-stage parasite growth by

providing a standardized inoculum of blood-stage parasites. Validation studies have shown a high correlation between natural and experimental infections, which further justifies theuse of IBSM

for testing new vaccines and drugs.[4, 8] The safety and utility of this approach for assessment of antimalarial and vaccine efficacy has been augmented by the implementation of rapid, sensitive, and robust real-time PCR assays for quantification of parasitemia.[4, 11]

Another significant benefit of the IBSM model for *P. vivax* studies is the exclusion of the liver stage of the parasite that is the source of relapses. Until recently, primaquine was the only Food and Drug Administration (FDA)-approved medication available for preventing relapse of malaria, but poor regimen adherence and other factors can lead to relapse rates of up to 32% in patients treated with the standard 15 mg daily dose of primaquine for 14 days.[12] In addition, primaquine is not fully metabolized to its active form in persons with low activity of cytochrome P450 isozyme (CYP) 2D, which can also lead to treatment failure. Persons with glucose-6-phosphate-dehydrogenase deficiency are at elevated risk of hemolysis when taking primaquine, limiting its usefulness. Since July 2018, tafenoquine has also been approved by the FDA for the prevention and treatment of hypnozoites.[13] The major advantage of tafenoquine over primaquine is single-dose treatment of hypnozoites, but otherwise many of the drawbacks of primaquine remain. Therefore, excluding the liver stage of the parasite by using an IBSM model removes the risk of recurring *P. vivax* infection from occult hypnozoites. As such, the approach of experimental infection using the IBSM model provides a system for assessing the efficacy and safety of novel antimalarial drugs and vaccines against *P. vivax* infection with significantly reduced safety issues associated with recurrent *P. vivax* infections in the healthy participants.

In the absence of a method of in vitro culture of *P. vivax*, the only way to source parasites is ex vivo. This hampers all aspects of development of tools to eliminate this parasite. For example, to test and develop candidate *P. vivax* vaccines or hypnozoiticidal drugs, a reliable source of *P. vivax* sporozoites is required. Currently this entails an expensive, logistically complex and unreliable process of sourcing *P. vivax*-infected mosquitoes from endemic areas of Asia. In addition to these logistical issues, the parasites are not genetically homogenous. As a result, the dose and composition of *P. vivax* sporozoite challenge material is extremely difficult to standardize. The establishment of a ready supply of homogenous *P. vivax* parasites in a blood-stage challenge model would facilitate rapid and cost-effective studies of *P. vivax* vaccines and drugs in healthy volunteers, accelerating clinical development of new interventions for *P. vivax* malaria.

2.2.1 Previous Human *P. vivax* CHMI Experience

Challenge models have previously been developed for assessment of the efficacy of vaccine or drug candidates against *P. vivax*. Initial studies entailed challenge of healthy volunteers with live *P. vivax* sporozoites. Herrera et al. reported challenge of 18 malaria-I volunteers via mosquito bites.[14] The mosquitoes were first infected by feeding with blood donated by *P. vivax* infected patients: 17 out of 18 volunteers developed malaria as determined by thick blood smear and PCR. All l7 infected volunteers developed symptoms consistent with malaria on day 9 post-challenge and all volunteers were treated with chloroquine and primaquine. All volunteers cleared parasitemia between 24 and

48 hours after initiating antimalarial treatment and recovered within 2 to 3 days without any severe or serious adverse reactions. Furthermore, the authors reported that none of the volunteers developed parasite relapses within an 18 month follow up.

Similarly, Herrera et al. reported 17 malaria-naive volunteers receiving sporozoite challenge with all volunteers developing symptoms and signs consistent with malaria infection between day 8 and 15 post-challenge.[6] Malaria infection was also confirmed by thin blood smears. The prepatent

periods recorded in this study varied between 9 and 16 days with a median of 9 days. Volunteers were treated with chloroquine and primaquineone month after the day of challenge. Although no serious adverse events (SAEs) were reported in this study and all of the volunteers recovered after the antimalaria treatment, one of the volunteers developed symptoms consistent with a relapse of malaria infection 2 months after treatment. This infection was also confirmed with thin blood smear. The re-treated volunteer recovered completely.

These studies have reported that the sporozoite challenge model is safe and can be used forassessing the efficacy of antimalarial vaccines.[6, 14] However, the use of a mosquito challenge system in *P. vivax* has the additional risk of relapse of malaria infection despite primaquine therapy. In addition to the experience of Herrera et al. above, the relapse of *P. vivax* malaria infection during *P. vivax* sporozoite challenge studies was also recorded in a similar trial conducted at the Walter Reed Army Medical Research in the US, in which33 participants underwent sporozoite challenge. All participants developed parasitemia byday 13 and parasitemia was cleared following treatment with chloroquine and primaquine.However, two participants had multiple relapses of malaria, and both were subsequently found to have low CYP2D6 activity compared with the participants who did not suffer relapses.[3]

2.2.2 Previous Human *P. vivax* IBSM Experience

A cryopreserved *P. vivax* isolate manufactured by QIMRB will be used to manufacture the *P. vivax* challenge agent at the NIH for this study. *P. vivax* parasite banks (HMPBS-*Pv* and HMPBS02-*Pv*) from QIMRB have been previously used to inoculate 45 malaria study participants.[15-21]

2.2.2.1 QIMRB Isolate HMPBS-*Pv*

The naturally occurring or wild type *P. vivax* master cell bank (MCB) containing HMPBS-*Pv,* was produced using blood collected from an individual with malaria infection presenting to hospital after travel to the Solomon Islands. The donor was an adult with laboratory-confirmed *P. vivax* malaria who consented to the proposed study. Once the patient consented, blood was collected, and the patient was successfully treated with artemether/lumefantrine.

A total of 8 healthy participants have been infected with *P. vivax* isolate HMPBS-*Pv*.[10, 22] The most frequent symptoms reported in *P. vivax* challenge studies have been those consistent with early malaria infection, including headache, malaise, chills, myalgia,fatigue, sweats and rigors. Less commonly reported symptoms include muscular pain, sensitive skin, paresthesias, nausea, and vomiting. In one volunteer who received HMPBS-*Pv* challenge agent, mild non-tender splenomegaly was also recorded 24 hours following initiation of the antimalarial treatment. This completely resolved by day 28 post-inoculation.[22] Four of the eight volunteers also experienced transient significant derangements of liver function tests (LFTs).[10] These derangements appeared as elevations greater than five times the upper limit of normal (ULN) of both alanine transaminase (ALT) and aspartate transaminase (AST). No volunteer reported any symptoms arising from or accounting for the LFT derangement. The derangements all resolved completely with no specific intervention by day 50 post-inoculation. Thorough investigation that included viral serologies for human immunodeficiency virus (HIV), hepatitis B and C, cytomegalovirus (CMV), Epstein-Barr virus (EBV), alphaviruses, and flaviviruses, as well as acetaminophen levels, creatine kinase (CK), and liver ultrasound screen indicated that the observed derangement was likely caused by the inoculum itself. External hepatologists were also consulted and no other possible cause could be identified.The recorded derangements were reviewed at the completion of each cohort and given the complete resolution and lack of sinister features

(particularly a significant elevation of bilirubin, see Figure 1), these abnormalities were deemed to not preclude proceeding withsubsequent cohorts nor with further studies utilizing this inoculum. In addition to the LFT derangements, the only other laboratory abnormalities observed were expected derangements of full blood count parameters, which were consistent with malaria infection.[10]

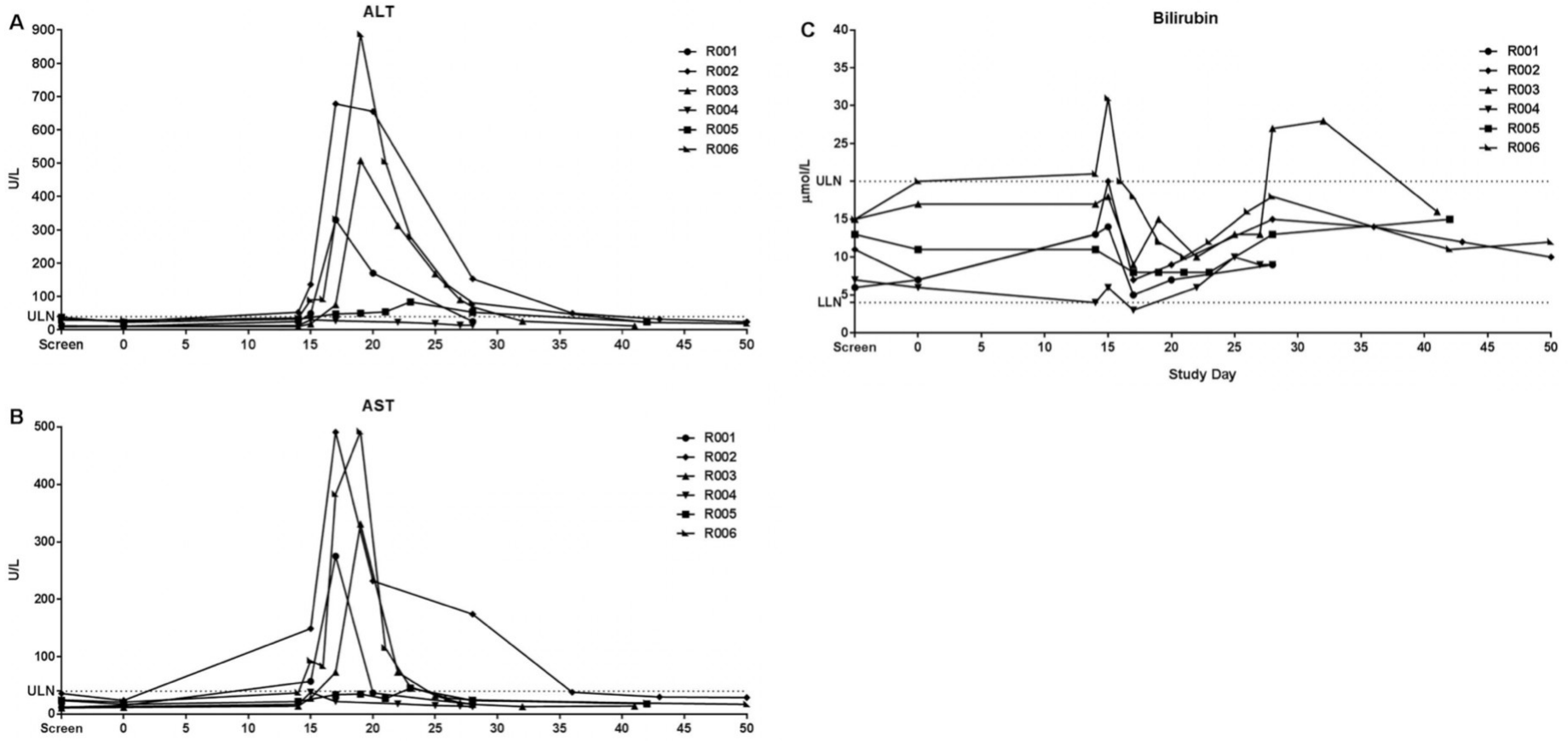


Figure 1: Liver Function Tests in Six Volunteers who Received HMPBS-Pv Challenge Agent (ANZCTR Trial ID: 12613001008718)

Adapted from Griffin et al. (2016). **Liver function tests.** Levels of (**A**) ALT (alanine transaminase), (**B**) AST (aspartate transaminase), and (**C**) total bilirubin versus study day for each of the six subjects. The horizontal dotted line indicates normal range. Challenge agent was administered on Day 0. Antimalarial treatment was administered on Day 14. Abbreviations: ULN: upper limit of normal; LLN: lower limit of normal).[10]

2.2.2.2 QIMRB Isolate HMPBS02-*Pv*

The blood-stage *P. vivax* MCB containing HMPBS02-*Pv* was also produced using blood collected from a consenting patient with naturally acquired malaria infection. The malaria infection was confirmed to be *P. vivax* by species-specific PCR at Pathology Queensland (accredited by the Australian National Association of Testing Authorities) and further confirmed by deep sequencing at the Sanger Institute. The clinical response to antimalarial chemotherapy was also demonstrated with this donor, who was successfully cured with artemether/lumefantrine.

This second *P. vivax* isolate, HMPBS02-*Pv*, has been used to inoculate 37 healthy volunteers to date in four studies.[15, 18-21] In the first study where two volunteers were inoculated with the HMPBS02-*Pv* isolate, the recorded AEs were all mild in severity with the exception of one moderate headache that was reported by one volunteer.[18] No significant liver enzyme derangements were recorded in either of these two volunteers.

Comparison of the parasitemias that developed in the participants following inoculation revealed no significant differences between the cohorts. The results showed consistency of the dosage strength and no evidence for reduction in the potency of the bank during storage. The results also demonstrated rapid and complete parasitological response following treatment with either artemether/lumefantrine or chloroquine. Parasitemia cleared within 2 to 3 days following the initiation of the treatment. The AEs recorded in these studies were consistent with malaria infection and included headache, fever, myalgia, arthralgia, presyncope, and rigors. The majority of the recorded AEs were transient in nature, mild in severity, and resolved either with antimalarial treatment alone or with doses of acetaminophen or ibuprofen. There were no clinically significant changes in hematology, biochemistry, or urinalysis over time in any participants on these studies.

Asymptomatic derangement in LFTs, however, have been recorded in some study participants inoculated with HMPBS02-*Pv* challenge agent. In one of the studies, one out of the eight challenged participants showed an increase in AST/ALT/lactate dehydrogenase >1.25 times the ULN, with peak ALT of 2.6 times the ULN on day 15 and day 18, resolving by day 22.[16] This participant used ibuprofen on days 9 and 10 of the study. Moderate transient neutropenia, mild transient lymphopenia, and mild transient thrombocytopenia on day 13 were also recorded in this participant.

In another recent study, seven out of eight participants within a single cohort who were treated with an investigational drug following inoculation with HMPBS02-*Pv* challenge agent developed elevated LFTs >1.25 times the ULN (ALT>AST with no elevation in bilirubin). In this cohort, four participants had peak malaria clinical scores ≥10.[21] All participants with elevated LFTs received symptomatic treatment with ibuprofen and acetaminophen. All participants had normalization of LFTs, seven by day 28 and one by day 44, the latter owing to delayed follow up.

All of the volunteers who have received HMPBS-*Pv* or HMPBS02-*Pv* challenge agent fully recovered and remained well when reviewed at their end of study visits. The recorded AEs for both HMPBS-Pv and HMPBS02-Pv challenge agent are summarized in Table 1.

Table 1. Challenge Agent-Related Adverse Events for HMPBS02-Pv and HMPBS-Pv Isolate

Malaria-Related Symptoms and Signs	Study QP12C14 [22] HMPBS-Pv(n = 2)	Study QP13C09 [10] HMPBS-Pv(n = 6)	Study QP14C10 [18] HMPBS02-Pv (n = 2)	Study QP15C11 [21] HMPBS02-Pv (n = 8)	Study QP15C19 [19] HMPBS02-Pv (n = 24)	Study QP17C14 [20] HMPBS02-Pv (n=3)
Abdominal discomfort	1(1)			7(2)	1(1) -–co1 6(4) -–co2 4(4) - co3	
Abdominal tenderness–					1(1) - co3	
Anorexia/ Decreased appetite		2(2)		3(3)	2(2) – co1 7(3) – co2 5(4) – co3	2(2)
ALT increased		8(4)		10(5)	2(2) – co2 3(2) – co3	1(1)
AST increased		7(4)		5(5)	1(1) – co3	
Arthralgia		4(2)	1(1)	9(6)	1(1) – co1 11(4) – co2 7(4) – co3	1(1)
Chills / Rigor	2(2)	5(5)	1(1)	7(5)	1(1) – co1 8(4) – co2 8(4) – co3	4(3)
Dehydration					2(2) – co2	
Diarrhea		1(1)		1(1)	1(1) – co2	

Dizziness	1(1)	1(1)		1(1)	2(2) – co2 1(1) – co3	1(1)
Feeling Hot						1(1)
Fatigue/Lethargy	3(1)	3(2)		19(8)	5(3) – co1 7(4) – co2 14(7) - co3	2(2)
Headache	7(2)	10(6)	5(2)	2(8)	8(5) - co1 30(7) - co2 15(7) – co3	5(2)
Hot Flush				1(1)		
Hyperhidrosis /sweat	1(1)	1(1)		5(4)		2(2)
Hypocalcemia					1(1) – co2	
Hypophosphatemia						2(2)
Influenza likeillness	1(1)	4(4)			2(1) – co1 7(4) – co2 4(2) –co3	
Insomnia		1(1)				
Lactate dehydrogenas eincreased		2(2)				
Lymphocyte countdecreased				8(8)	6(6) – co2 5(5) – co3	5(4)
Malaise	1(1)			8(6)	6(4) – co2 6(6) – co3	2(2)
Muscle rigidity				2(2)		

Myalgia	2(2)	2(2)	2(1)	16(7)	6(4) – co1 11(6) – co2 9(6) – co3	3(3)
Nausea	1(1)	6(6)		6(4)	6(3) – co1 7(3) – co2 11(6) – co3	1(1)
Neutrophil count decreased		2(2)			4(3) – co2 2(2) – co3	4(2)
Oral Herpes						1(1)
Oropharyngeal pain	1(1)				1(1) – co3	
Paresthesia	1(1)					
Platelet count decreased					1(1) – co2	
Presyncope			1(1)			
Pyrexia		22(6)	2(2)	30(8)	4(3) – co1 10(7) – co2 15(7) – co3	19(4)
Rhinorrhea					1(1) – co3	
Sensitive skin	1(1)					
Splenomegaly					2(2) – co2 1(1) – co3	
Tachycardia				2(2)	2(2) – co2 1(1) – co3	
Extreme Thirst	1(1)					

Thrombocytopenia		4(3)				
Vomiting	1(1)	1(1)		1(1)	1(1) – co1 4(3) – co2 2(2) – co3	
While blood cell count decreased		2(2)			3(3) – co2 2(2) – co3	5(4)
Total	26	88	12	170	37 – co1 140 – co2 119 – co3 296 - all	61

ALT: alanine transaminase; AST: aspartate transaminase; co: cohort; LDH: lactate dehydrogenase.

The number between brackets represent the number of subjects in which a given AE was observed. For study QP15C19, treatment was administered on Day 8 for cohort 1 and Day 10 for cohorts 2 and 3. For cohort 1, ibuprofen or paracetamol (acetaminophen) were administered for symptoms relief. For cohort 2, ibuprofen was administered preferably and for cohort 3, it was paracetamol.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 Known Potential Risks

2.3.1.1 Venipuncture and IV Placement

Risks occasionally associated with venipuncture and IV line placement include pain, bruising, bleeding, and infection at the site of venipuncture, lightheadedness, and rarely, syncope.

2.3.1.2 Challenge Agent Injection

In this study, HMPBS02-*Pv* challenge agent containing cryopreserved *P. vivax* isolate HMPBS02-*Pv* will be used. Information regarding the HMPBS02-*Pv* challenge agent is available in the Investigator's Brochure. The *P. vivax* MCB containing HMPBS02-*Pv* has been previously used to challenge 37 healthy study participants using the IBSM model. No SAEs related to the challenge agent have been reported in the participants exposed to date.

Possible local reactions include pain, swelling, erythema, induration, limitation of limb movement for several days, lymphadenopathy, or pruritus at the injection site. Systemic reactions such as fever, chills, headache, fatigue, malaise, myalgia, and joint pain may also possibly occur, with some reactions potentially being moderate or severe.

The challenge agent contains a small number of red blood cells (RBCs) from the original donor. The risk for development of RBC antibodies in this study is considered extremely low. The donor of the malaria cell bank used in this study was confirmed to be blood group O, Rh (D) positive. People with blood group O are generally considered "universal donors" as recipients of their blood have minimal risk of developing RBC alloantibodies when given much larger volumes of blood than is routinely used for IBSM. However, it is possible that participants could suffer a transfusion

reaction after they receive the inoculum or develop antibodies to the donor RBCs that may make blood transfusion more difficult in the future. Therefore, the participants will be monitored in the period immediately after the administration of the malaria parasite dose and screened at the end of the study for occurrence of RBC alloantibodies as part of the safety monitoring. In addition, eligible females of childbearing potential must not be Rh (D) negative.

The risk of bloodborne infection from the blood transfused in this study is expected to be very low for a number of reasons. First, the donor was screened and tested negative for the presence of acute bloodborne infections. Furthermore, the volume of blood used in IBSM challenge studies for transmitting malaria is significantly lower than in a transfused unit of blood. In addition, the white blood cells are removed from the MCB during the production process, which lowers the risk of any past/latent EBV and CMV infection due to transfusion. The bank has tested PCR negative for both CMV and EBV even though the donor of the bank was antibody positive for both CMV and EBV. As part of the safety monitoring, all study participants will have serum stored for testing of blood-borne virus infections before entry on and after the completion of the study.

As with any investigational product, immediate hypersensitivity reactions including urticaria, anaphylaxis, or other IgE-mediated responses are possible. There is a theoretical possibility of risks about which we have no present knowledge. Participants will be informed of any such risks should further data become available.

2.3.1.3 *P. vivax* IBSM

The number of blood-stage parasites in the challenge agent is much lower than what reaches the blood after the bite of a single malaria-infected mosquito, where approximately 30,000 parasites are released into the blood when they break out of a single infected liver cell. Following administration of the challenge agent, the growth of the parasites as well as any symptoms in the participants will be closely monitored. The threshold for commencement of treatment will be when the clinical symptom score is >6 (admission within 24 hours of notification that this threshold has been reached), or parasitemia is >20,000 parasites/mL, or at the investigator's discretion. This treatment threshold has been selected because it is below the point at which advanced and severe clinical symptoms of malaria infection are likely to occur.

The parasites used in the challenge agent for this study are known to be sensitive to the standard antimalarial drugs artemether/lumefantrine and chloroquine. As such, there is no serious risk of inadequately treated clinical malaria provided that the inoculated participants comply with the curative antimalarial regimen as directed.

Symptoms related to malaria infection are expected to occur. The AEs include headache, fever, myalgia, arthralgia, presyncope, fatigue, and rigors. The majority of the recorded AEs reported have been transient in nature, mild in severity, and resolved either with antimalarial treatment or with acetaminophen or ibuprofen and overall last approximately 2-3 days.

Transient, asymptomatic LFT derangements have been reported in several participants in IBSM studies.[23, 24] These LFT derangements, which consisted of ALT/AST elevation with no change in bilirubin, did not require treatment and resolved by the end of each study. Similar elevations in LFTs have been reported following naturally acquired malaria infection. [25, 26] Following an independent review involving experts in drug-induced liver injury, it was concluded that these LFT elevations were most likely a consequence of the malaria infection. As a precaution, all participants in this study will undergo regular safety monitoring to assess for asymptomatic LFT abnormalities.

Participants are required to limit their intake of possibly hepatotoxic substances during the course of the study, including alcohol and acetaminophen (acetaminophen can be used as second-line symptom control after ibuprofen and will not exceed 4 g/day in the study).

Other laboratory abnormalities observed in the studies in some volunteers were expected derangements of full blood count parameters consistent with malaria infection. Such abnormalities include but are not limited to decreased counts of total white blood cells, neutrophils, and/or lymphocytes. These aberrations may also be expected to occur to similar degrees described in studies of natural Pv infections even if not previously reported in Pv IBSM trials. For example, a study of Brazilian adults presenting with symptomatic Pv infections found that 85% of individuals had decreased lymphocytes, 16% had a decrease that would correspond to a Grade 3 adverse event based on the criteria in Appendix C, and 3% had a decrease that would correspond to a Grade 4 adverse event [32]. These derangements in white blood count parameters (ANC, ALC, etc) are transient, expected, and self-limited once antimalarial treatment is completed and levels of parasitemia drop.

As with any clinical investigation, even with extensive precautions there is always a risk of serious or even life-threatening allergic reactions to administration of the investigational product. These risks are minimized by eligibility criteria that exclude participants with a potential for adverse reaction (AR) to the malaria challenge, infection, or study drugs. Likewise, participants will be closely monitored during the administration of challenge agent and/or drugs and in the immediate period following administration by clinical staff trained and equipped to respond immediately to acute systemic reactions including anaphylaxis and clinical malaria.

2.3.1.4 Artemether/lumefantrine (Coartem®)

Participants will be treated with Coartem, which is a registered, oral, proven, and highly efficacious antimalarial treatment.

Coartem has an acceptable safety profile. Individuals who may have any contraindication for the use of this drug (e.g., known prolonged corrected QT interval (QTc) or taking other medications that can prolong QTc, history of myocardial infarction) will be excluded at screening. The most common side effects (i.e., >30%) in adults are headache, anorexia, dizziness, asthenia, arthralgia, and myalgia. Discontinuation of Coartem due to AE is rare (0.2%) in adults. Rare but serious hypersensitivity reactions (urticarial and angioedema) and skin reactions (bullous eruption) have been reported post marketing.

Published data from clinical studies and pharmacovigilance data have not established an association with artemether/lumefantrine use during pregnancy and major birth defects, miscarriage, or adverse maternal or fetal outcomes. The estimated background risk of major birth defects and miscarriage for the indicated population is unknown. Results of animal studies suggest the possibility of increased risk of fetal loss with Coartem dosing, but the relevance of these findings from animal reproductive studies to human risk is unclear.

Thus, all female participants will undergo pregnancy testing prior to receipt of Coartem. Also, per the package insert, Coartem may decrease the efficacy of hormonal birth control, so female volunteers who are on hormonal birth control will be counseled about back-up pregnancy prevention methods.

2.3.1.5 Chloroquine Phosphate

Participants may be treated with chloroquine phosphate, which is a registered, oral, proven, and

highly efficacious antimalarial treatment.

The most commonly reported side effects of chloroquine dosing include gastrointestinal disturbance, headache, dizziness, blurred vision, insomnia, tinnitus, and pruritus, but generally these effects do not require discontinuation of the drug. High doses of chloroquine, as used to treat rheumatic diseases (>3.5 mg/kg/day over 1 year or more), have been associated with retinopathy, although this generally requires a cumulative dose exceeding 100 g,[27] and thus is extremely unlikely at doses used for routine weekly malaria prophylaxis especially for the limited duration in this study. Chloroquine is reported to exacerbate psoriasis; therefore, subjects with a history of psoriasis are excluded.

2.3.1.6 IV Artesunate

Participants may be treated with IV artesunate, which is a registered, proven, and highly efficacious antimalarial treatment.

Artesunate is generally well tolerated. The most commonly reported side effects of artesunate use include rare reports of anemia, allergic reactions, anxiety, dizziness, headaches, metallic taste, erythema, pruritus, skin rash, anorexia, diarrhea, nausea, vomiting, hemolysis, neutropenia, increased serum ALT, increased blood urea nitrogen, and dyspnea.

2.3.1.7 Other Risks

Women of reproductive potential will be required to agree to use birth control as outlined in Section 5. Malaria can cause pregnancy complications and antimalarial medications have a low but possible risk of harming a fetus. Because this is a research study, women of reproductive potential will be asked to notify the site immediately upon learning of pregnancy during this study and will be tested for pregnancy prior to administration of the challenge agent and periodically throughout the course of the study as outlined in the Schedule of Activities (Section 1.3).

Medications including ibuprofen and acetaminophen may be used for symptom control during this study. These medications are considered safe, available over the counter, and will be used in accordance with the manufacturer's instructions. The most common side effect of ibuprofen is dyspepsia. Rarely, mucosal bleeding has been reported related to excessive use. Acetaminophen, which may be used as a second-line agent, is typically well tolerated but may be associated with hepatotoxicity in excess.

Risks of a nasopharyngeal swab may include possible discomfort, bleeding or rarely infection at site of insertion.

2.3.1.8 Risk to the Community

Malaria is not contagious and cannot be spread by person-to-person contact but only via the bite of a female *Anopheles* mosquito. The window of time when participants can transmit to mosquitoes has been well documented in previous studies.[28] Based on these studies, there is negligible risk of volunteers transmitting to local mosquito vectors in the DC, Maryland, and Virginia area if they adhere to study procedures. The antimalarial treatment that study participants receive (Coartem, artemether/lumefantrine) is known to kill all parasite lifecycle stages, including gametocytes, and in contrast to *P. falciparum*, administration of primaquine as a gametocytocidal agent is not necessary. Additionally, field studies of *P. vivax* transmission have demonstrated that artemether/lumefantrine is an effective gametocytocidal agent.[29, 30]

There is a theoretical risk of bloodborne infection in study staff in the case of needlestick exposure

to blood product containing parasitized erythrocytes. This risk is considered extremely low for two reasons: the use of personal protective equipment and the small volume nature of a needlestick exposure.

2.3.2 Known Potential Benefits

Participants will not receive any direct benefit from participation in this study. It is hoped that information gained in this study will contribute to the development of a safe and effective malaria vaccine or other antimalarial interventions.

2.3.3 Assessment of Potential Risks and Benefits

The anticipated benefit of this study is the development of a reliable tool to facilitate the development antimalarial interventions. The development of a reliable *P. vivax* blood stage parasite MCB will allow for further future IBSM studies for the efficient testing of investigational antimalarial interventions for safety and efficacy. These benefits justify the risks incurred by participants in this study. While IBSM does expose a healthy volunteer to risk that they would not otherwise be subject to, there have been many participants in previous trials utilizing IBSM without significant or lasting adverse effects observed. Participants in this study will be closely monitored for safety throughout their participation in the trial and will be fully treated for malaria prior to completing the study. In addition, very few participants (only 2 are envisioned) will undergo IBSM on this trial, limiting the risk to a small number of healthy volunteers. Part of the rationale for developing a *P. vivax* MCB is to reduce the risk of future *P. vivax* challenge studies by eliminating the possibility of hypnozoites and the risk of relapsing malaria infection in future participants.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
The collection of blood from healthy participants experimentally infected with *P. vivax* isolate HMPBS02-Pv for the production ofa *P. vivax* blood-stage parasite bankfor use in future studies.	Collection of blood for the productionof a *P. vivax* blood-stage parasite bank from study participants following experimental infection with *P. vivax* isolate HMPBS02-Pv.	The blood bank to be produced is intended for use in future studiesof *P. vivax* vaccines, pathogenesis, or other antimalarial interventions.
Secondary		
To assess the safety and tolerabilityof the *P. vivax* IBSM model following inoculation of healthy malaria-naïve participants with *P. vivax.*	Occurrence of solicited and unsolicited AEs following *P. vivax* inoculation prior to the initiation ofantimalarial treatment.	Standard clinical safetyendpoints.
Tertiary/Exploratory		
To further characterize blood- and sexual-stage parasite growth profiles following inoculation with *P. vivax* isolate and treatment withartemether/lumefantrine.	The rate of growth of blood- and sexual-stage *P. vivax* isolate following inoculation and treatment with artemether/lumefantrine as determined by blood smear and/or qPCR	Blood smear and qPCRare considered the gold standards for measuringmalaria parasitemia and gametocytemia.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is a single-center, open-label study to infect healthy volunteers using induced blood-stage malaria (IBSM) so that blood can be collected to enable production of a human malaria parasite (HMP) bank for use in future studies.

This study involves infecting healthy volunteers using IBSM so that blood can be collected to produce a HMP bank. This study will be conducted in up to 2 participants, who are healthy adults aged between 18 and 50 years old. Participants will be screened for eligibility up to 56 days before inoculation. Eligible participants will be inoculated intravenously on Day 0 with approximately 564 viable *P. vivax* parasite-infected erythrocytes (HMPBS02-*Pv* challenge agent). A sample size of two participants has been selected to maximize the chances of at least one successful banking procedure and to minimize the volume of the HMPBS02-*Pv* cell bank, which is in limited supply.

Participants will be monitored daily via phone on Days 1 to 3 post-inoculation. Beginning on Day 4 post-inoculation, the participants will be seen in the outpatient clinical unit daily for blood sampling to measure parasitemia via qPCR targeting the *P. vivax* 18S rRNA gene (referred to as malaria 18S qPCR), to monitor for symptoms and signs of malaria, and to record AEs.

The threshold for the commencement of blood collection for banking and subsequent antimalarial rescue treatment with artemether/lumefantrine will occur when the Malaria Clinical Score is >6, when parasitemia is >20,000 parasites/mL, or at the investigator's discretion. Within 24 hours of notification that this threshold is reached, participants will be admitted to the clinical unit for initial safety assessments. The participant will then undergo the blood collection procedure with the Department of Transfusion Medicine as per the SOP. The participant will then receive the first dose of artemether/lumefantrine. The participant will remain inpatient within the clinical unit for a minimum of 48 hours, which may be extended to 72 hours or longer at the discretion of the investigator to monitor for safety and tolerability of rescue therapy and to ensure adequate clinical and parasitological response to treatment. For example, if qPCR results indicate unsatisfactory clearance of the parasitemia, defined as 2 consecutive qPCR time points showing less than 20% change from baseline (i.e., the parasitemia before drug treatment) by 72 hours, participants may be administered chloroquine.

In the unlikely event that artemether/lumefantrine fails to clear parasitemia, participants will be treated with chloroquine. If oral administration of either artemether/lumefantrine or chloroquine is not possible (e.g., the participant is vomiting), the participant will receive intravenous treatment with artesunate. After discharge from the inpatient unit, participants will be followed up on an outpatient basis for monitoring of safety and parasite clearance. Follow-up for safety assessments will be performed on Day 28 ±3, Day 56 ±7 (phone call only), and Day 90 ±7 (End of Study).

Participants may also be evaluated for the presence of sexual parasite stages (gametocytes) and other parasite lifecycle stages in the blood during the study using reverse transcriptase qPCR (qRT-PCR). Study procedures and the planned timeline of events are outlined in the protocol below and summarized in the Schedule of Activities (Section 1.3).

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

This is a single-center, open-label study using IBSM infection to establish a HMP bank using malaria parasites from the blood of two healthy malaria-naïve participants experimentally infected

with blood-stage *P. vivax*. Participants will be allowed to develop patent infection, at which time an estimated target minimum 200-mL whole blood sample will be collected prior to receipt of effective antimalarial therapy. This will allow for the local production of up to two *P. vivax* HMP banks for future IBSM model studies. The whole blood sample will undergo leukodepletion and aliquoting as per the standard operating procedure (SOP) to generate a bank (estimated 200 aliquots) of *P. vivax*– parasitized erythrocytes. This study will also explore the safety, tolerability, and infectivity of this IBSM model at the NIH CC. Local generation of HMP banks will allow access to reliable blood-stage *P. vivax* human challenge studies with a consistent parasite dose and strain that permits efficient assessment of antimalarial drugs and vaccine candidates.

4.3 JUSTIFICATION FOR DOSE

The inoculum dose, containing an estimated 564 viable *P. vivax*-infected erythrocytes in a volume of 2 mL, has been established to be sufficient to provoke a reliable *P. vivax* infection in previous studies by QIMRB [28]. The inoculum will be administered intravenously to each participant on study Day 0. The actual number of parasites inoculated will take into account the loss of viability resulting from cryopreservation, storage, and thawing. The dose administered will be confirmed retrospectively based on in vivo parasite growth kinetics.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

All of the following criteria must be fulfilled for a participant to participate in this trial:

1. Age ≥18 and ≤50 years.
2. In good general health and without clinically significant medical history.
3. Does not live alone from study Day 0 until the end of the antimalarial drug treatment (investigator discretion).
4. Malaria comprehension exam completed, passed (a score of ≥80% or per investigator'sdiscretion) and reviewed prior to enrollment.
5. Reliable access to the NIH CC and availability to participate for duration of study.
6. Females of childbearing potential must be willing to use reliable contraception (as definedbelow) from 21 days prior to study Day 0 to 28 days following IBSM.
    - **Subject to the judgment and discretion of the Principal Investigator (PI)**, female participants who meet ANY ONE of the criteria listed immediately below, may not be required to take any additional measures to avoid pregnancy. Such participants will be counseled on risks at the time of consent and at appropriate points (e.g., when pregnancy testing occurs) during the study:
        - Females who have had their uterus and/or BOTH ovaries removed.
        - Females who have undergone ligation or removal of BOTH fallopian tubes.
        - Females who are above the age of 45 and have spontaneously had no menses at any point during the past 12 or more consecutive months (i.e., have reached menopause).
        - Females who, in the conservative and reasonable judgment of the PI(e.g., due to sexual orientation, gender identity, or serious life choice [such as being celibate clergy]), during the entire trial will NOT participate in any potentially reproductive sexual contact.
        - Females who, in the conservative and reasonable judgment of the PI, are ina monogamous stable relationship with a male who has

undergone vasectomy at least 4 months prior or another procedure/medical condition that deems the male sterile.

- **Subject to the judgment and discretion of the PI**, female participants who DO NOT meet ANY of the criteria listed above, will be appropriately counseled on reproductive risks and pregnancy avoidance, and will be required to adhere to the following measures and agree to 2 methods of pregnancy prevention as noted below:

CATEGORY 1:

- a highly effective hormonal method to prevent pregnancy [e.g., CONSISTENT, CONTINUOUS use of contraceptive pill, patch, ring, implant or injection], and/or
- Intrauterine device or equivalent.

IN ADDITION TO

CATEGORY 2:

- a barrier method to be used at the time of potentially reproductive sexual activity (e.g. [male/female condom, 'cap,' or diaphragm] + spermicide).

7. Sign written informed consent prior to undertaking any study-related procedure.

5.2 EXCLUSION CRITERIA

A participant will be excluded from participating in this trial if any one of the following criteria is fulfilled:

1. Currently breastfeeding (if female).
2. Pregnant as determined by a positive urine or serum human choriogonadotropin (β-hCG)test (if female).
3. Planned travel to a malaria-endemic area during the study period and up to 2 weeks following the EOS visit (see www.cdc.gov/malaria/travelers/country_table).
4. History of recent travel to or residence in a *P. vivax* malaria–endemic region or prior participation in a malaria challenge study (investigator discretion).
5. Any history of confirmed malaria diagnosis on peripheral blood smear or by clinical history
6. Screening laboratory parameters outside of local lab normal range, to include serum-corrected calcium, creatinine, hepatic transaminase enzymes (ALT, AST), total bilirubin (unless the participant has documented Gilbert syndrome), and hemoglobin. Participants may be included at the investigator's discretion for "not clinically significant" values outside of normal range.
7. Abnormal urinalysis as defined by positive urine glucose, protein, and hemoglobin. Participant can be included if investigator determine the abnormality is "not clinically significant."
8. Duffy blood group negative (male or female).
9. ABO blood type other than O (male or female).
10. Rh blood group negative (females of childbearing potential).
11. Anticipated use during the study period, or use within the following periods prior to enrollment of any of the following (investigator discretion):
    a. Investigational malaria vaccine within the last 2 years.
    b. Malaria chemoprophylaxis within the past 6 months.
    c. Chronic systemic immunosuppressive medications (>14 days) within 6 months (e.g., cytotoxic medications, adrenocorticotrophic hormone, or oral/parental

corticosteroids equivalent to >0.5 mg/kg/day of prednisone). Corticosteroid nasal spray for allergic rhinitis and topical corticosteroids for mild, uncomplicated dermatitis are allowed.

d. Blood products or immunoglobulins within the previous 6 months.
e. Systemic antibiotics or medications with potential antimalarial effects within the past 6 weeks (e.g., clindamycin, chloroquine, piperaquine, benzodiazepines, fluoxetine, tetracycline, azithromycin, or doxycycline [if not used as an antimalarial prophylaxis agent]).
f. Investigational or non-registered product or vaccine within 30 days.
g. Receipt of any vaccination within 28 days prior to *P. vivax* IBSM.
h. Medications known to interact with artemether/lumefantrine.
i. Anticoagulants within the past 28 days.

12. History of:
    a. Sickle cell disease, sickle cell trait, or other hemoglobinopathies.
    b. Splenectomy or functional asplenia.
    c. Systemic anaphylaxis.
    d. Any allergic reactions to artemether/lumefantrine, chloroquine (or any 4-aminoquinolines), artemether or other artemisinin derivatives, or their excipients.
    e. History of malignancy of any organ system (other than localized basal cell carcinoma of the skin or in situ cervical cancer), treated or untreated, within 5 years of screening, regardless of whether there is evidence of local recurrence or metastases.
    f. Blood transfusion.
13. Clinically significant medical condition, physical examination findings, other clinically significant abnormal laboratory results, or past medical history that may have clinically significant implications for current health status and participation in the study in the opinion of the investigator. A clinically significant condition or process includes but is not limited to:
    a. A process that would affect the immune response, or requires medication that affects the immune response.
    b. Any contraindication to repeated phlebotomy.
    c. A condition or process in which signs or symptoms could be confused with reactions to malaria challenge and/or infection.
    d. A chronic or subclinical condition which could be exacerbated by malaria infection.
    e. Frequent headaches and/or migraines, recurrent nausea, and/or vomiting (more thantwice a month).
    f. A history of retinal abnormalities, disease of the retina or macula of the eye, visualfield defects, or hearing disorders (e.g., reduced hearing, tinnitus).
    g. History of coagulopathy or bleeding diatheses.
    h. Any condition or disease that might affect drug absorption, distribution or excretion (e.g., gastrectomy, diarrhea).
    i. Presence of current or suspected serious chronic diseases such as cardiac or autoimmune disease, insulin-dependent and non-insulin–dependent diabetes, progressive neurological disease, severe malnutrition, acute or progressive hepatic disease, acute or progressive renal disease, porphyria, psoriasis, rheumatoid arthritis, asthma, epilepsy, or obsessive compulsive disorder.

14. Vital signs taken after 5 minutes of resting in seated or supine position that fall outside the following ranges:
    a. Systolic blood pressure ≤90 mm Hg or ≥140 mm Hg.
    b. Diastolic blood pressure ≤50 mm Hg or ≥90 mm Hg.
    c. Heart rate ≤40 bpm or ≥100 bpm.
15. Body mass index less than 17.0 or greater than 35.0 kg/m^2 at the time of screening.
16. History of, or known active cardiac disease including: (1) prior myocardial infarction (heart attack); (2) angina pectoris; (3) congestive heart failure; (4) valvular heart disease; (5) cardiomyopathy; (6) pericarditis; (7) stroke or transient ischemic attack; (8) exertional chest pain or shortness of breath; (9) symptomatic cardiac arrhythmias; (10) clinically relevant bradycardia; or (11) other heart conditions under the care of a doctor.
17. Clinically significant electrocardiogram (ECG) findings as determined by the expert study cardiologist, or QTcF ≥450 ms, or presence of second- or third-degree atrioventricular block or abnormal T wave morphology.
18. Moderate or high risk for coronary heart disease based on National Health and Nutrition Examination Survey (NHANES) I cardiovascular risk assessment (Appendix A).
19. Family history of sudden death or of congenital prolongation of the QTc interval or known congenital prolongation of the QTc interval or any clinical condition known to prolong the QTc interval.
20. History of electrolyte disturbances, particularly hypokalemia, hypocalcemia, or hypomagnesemia.
21. Acute infectious disease or fever (e.g., temperature ≥38.5°C) within the 5 days prior to inoculation with malaria parasites.
22. Evidence of acute illness within the 4 weeks prior to screening that the investigator deems may compromise participant safety.
23. Positive result on any of the following tests: hepatitis B surface antigen (HBsAg), anti-hepatitis C virus (anti-HCV) antibodies, and anti-human immunodeficiency virus 1 and 2 antibodies (anti-HIV1 and anti HIV2 Ab).
24. Positive urine drug test for any drug listed in Section 8.2.2.1 (Drug Screening) unless there is an explanation acceptable to the investigator (e.g., the participant has stated in advance that they consumed a prescription or over-the-counter product that contained the detected drug) and/or the participant has a negative urine drug screen on retest by the pathology laboratory. Any participant testing positive for acetaminophen at screening may still be eligible for study participation, at the investigator's discretion.
25. Elevated serum ethanol level.
26. Psychiatric condition that precludes compliance with the protocol including but not limited to:
    a. Psychosis within the past 3 years.
    b. Ongoing risk for suicide, or history of suicide attempt or admission for danger to self or others.
    c. History of schizophrenia, bipolar disease, or other severe (disabling) chronic psychiatric diagnosis including depression or receiving psychiatric drugs or who has been hospitalized within the past 5 years prior to enrollment for psychiatric illness.
    d. History of other serious psychiatric conditions 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, 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.

e. The Patient Health Questionnaire-2 (PHQ-2) (Appendix F) 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 3 or more on the PHQ-2 will not be eligible for participation. These participants will be referred to a general practitioner or medical specialist as appropriate. Participants with a score of 1 or 2 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.

27. Suspected or known current alcohol or drug abuse as defined by the American Psychiatric Association in the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition, at the discretion of the investigator.
28. Smoking more than 5 cigarettes or equivalent per day and unable to stop smoking for the duration of admission. Participants may smoke up to 5 cigarettes or equivalent per day for the rest of the study.
29. History or presence of alcohol abuse (alcohol consumption of more than 4 standard drinks per day), drug habituation, or any prior intravenous usage of an illicit substance.
30. Excessive consumption of beverages or food containing xanthine bases (such as Red Bull, chocolate, etc.), or more than 400 mg of caffeine per day (equivalent to more than 4 cups of coffee per day, investigator discretion).
31. Ingestion of any poppy seeds within the 24 hours prior to the screening blood test (participants will be advised by phone not to consume any poppy seeds in this time period, investigator discretion).
32. Use of prescription drugs or non-prescription drugs and herbal supplements (such as St John's Wort) within 14 days or 5 half-lives (whichever is longer) prior to the inoculation administration. (Note: diazepam interferes with the analysis of blood levels of chloroquine and thus should not have been used for at least 8 weeks prior to administration of the study drug). If needed (i.e., an incidental and limited need), ibuprofen up to 1.2 g/day or acetaminophen up to 4 g/day is acceptable. The participant must inform the investigator of any ibuprofen or acetaminophen use at the next convenient time. Limited use of other non-prescription medications or dietary supplements not believed to affect participant safety or 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. Participants are requested to refrain from taking non-approved concomitant medications from recruitment until the conclusion of the study.
33. Clinical trial staff with direct involvement in the conduct of the trial are excluded from participation.
34. Participating in other clinical trials involving investigational interventions or off-label medication use during the study period or within the 12 weeks preceding study day 0 (investigator discretion). Participation in other trials such as observational or imaging studies will be discussed withthe investigators.
35. Blood donation of any volume within 4 weeks prior to study day 0, or participation in any

research study involving blood sampling (more than 450 mL/unit of blood), or blood donation to Red Cross (or other) blood bank within 8 weeks prior to study day 0.

36. Unwillingness to defer blood donations for at least 3 years.
37. Unwillingness to abstain from consumption of quinine-containing foods/beverages, such as tonic water or lemon bitter, from inoculation (study day 0) to the end of antimalarial treatment (investigator discretion).
38. Unwillingness to abstain from consumption of grapefruit or Seville oranges from inoculation (study day 0) to the end of antimalarial treatment (investigator discretion).
39. Any participant without a good peripheral venous access.
40. Participant who, in the judgment of the investigator, is likely to be noncompliant during the study, or is unable to cooperate because of a language or mental deficit.
41. Any other finding that, in the judgment of the investigator, would interfere with, or serve as a contraindication to, protocol adherence, assessment of safety or reactogenicity, or a participant's ability to give informed consent, or increase the risk of having an adverse outcome from participating in the study.

5.3 INCLUSION OF VULNERABLE PARTICIPANTS

5.3.1 Justification for Exclusion of Pregnant Women

This study will not enroll pregnant and/or breastfeeding women since malaria infection can have adverse effects on both the pregnant mother and fetus. Additionally, though observational studies have shown initial safety data on the use of artemether/lumefantrine (Coartem) during the first trimester and has been approved for use in the second and third trimester in malaria-endemic regions, artemether/lumefantrine (Coartem) is currently not recommended in healthy pregnant women. Thus, women who are pregnant, nursing, or plan to become pregnant during the study are excluded from the study.

5.3.2 Justification for Exclusion of Children

Children are excluded from this study because there are insufficient data regarding dosing or AEs available in adults to judge the potential risk in children. In addition, given the nature of malaria challenge trials in US malaria-naïve participants, the risk being more than minimal, and no direct benefit to the child, evaluation of IBSM with *P. vivax* in infants and children under the age of 18 would not be warranted when this can be completed successfully in healthy adults.

5.3.3 Justification for Exclusion of Adults Who Cannot Consent

Participants must be able to provide initial and ongoing informed consent to participate in this study. As with children, the risk of participation in this study is more than minimal, and this study offers no prospect of direct benefit to decisionally impaired adults. Therefore, participation by adults who cannot provide informed consent is not warranted when this study can be completed successfully in cognitively healthy adults.

5.3.4 Justification for Exclusion of Participants Older than 50

This study will not enroll participants aged over 50 years due the possibility of reduced erythrocyte survival following bank collection in older donors and the general lack of data and experience performing IBSM challenge studies in this age group. Thus, to optimize the production of a viable *P. vivax* cell bank from study participants, subjects aged over 50 will be excluded from this study.

5.3.5 Participation of NIH Staff or Family Members of Study Team

NIH staff and family members of the study team may be enrolled in this study as this population meets the study entry criteria. Neither participation nor refusal to participate as a subject in the research will have an effect, either beneficial or adverse, on the participant's employment or position at NIH.

Every effort will be made to protect participant information, but such information may be available in medical records and may be available to authorized users outside of the study team in both an identifiable an unidentifiable manner.

The NIH investigator will provide and request that the NIH staff member review the *Frequently Asked Questions (FAQs) for Staff Who are Considering Participation in NIH Research* and the *Leave Policy for NIH Employees Participating in NIH Medical Research Studies (NIH Policy Manual 2300-630-3)*. Considerations for consent of staff is described in Section 10.1.3.

5.4 LIFESTYLE CONSIDERATIONS

During this study, participants are asked to abstain from alcohol for 24 hours prior to serum ethanol assay and from inoculation until the end of antimalarial treatment.

Participants should not consume more than 400 mg of caffeine per day, equivalent to more than 4 cups of coffee, from inoculation until the end of the antimalarial treatment.

Participants should not consume tonic water, lemon bitter, or other drinks/foods containing quinine or grapefruit or Seville oranges, from inoculation until the end of antimalarial treatment.

Participants should not eat any poppy seeds in the 24 hours before the following time points: screening, inoculation day, and day of admission for antimalarial treatment.

5.5 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAE.

5.6 STRATEGIES FOR RECRUITMENT AND RETENTION

Healthy adult male and non-pregnant, non-lactating female participants will be recruited from a variety of sources including those previously screened or enrolled in other vaccine trials at the NIH CC or by the use of an IRB-approved screening protocol and study-specific print or media advertising. After an initial phone screen (using an IRB-approved phone screen), a screening visit will be scheduled.

During the screening process, which may require more than one visit, the participant will read the consent form, be encouraged to ask questions, and then complete a written comprehension evaluation questionnaire (Malaria Comprehension Exam). The questionnaire is used to identify the areas of the study and consent that the participant may not fully understand. The person administering consent will review the answers with the participant. If the participant gets a question wrong, the person administering the consent will review the portion of the consent form that relates to that particular question with the participant. The participant may either sign the

consent form during the screening visit, or return after further consideration.

The accrual ceiling will be up to 50 individuals to recruit two subjects for the study.

5.6.1 Costs

Participants in this study will not be billed for any study procedures outlined in this protocol. Participants are required to have outside medical insurance in order to facilitate follow-up of any medical conditions or abnormalities uncovered as a result of this study that are not related to the study procedures.

5.6.2 Compensation

Participants will be compensated for their time while participating in this trial. Compensation is prorated based upon completion of specific visits and is not contingent upon completing the entire study. A compensation schedule is provided below (Table 2). Compensation for interim visits to follow up clinical or laboratory abnormalities will be provided at the investigator's discretion, up to $80. If a participant withdraws from the trial, payment will be made based on the last completed visit. Information about compensation, including the amount and schedule of payment(s) and applicable reporting to the IRS, will also be described in the informed consent form. Participants will not be compensated for travel or lodging.

Table 2. Compensation Table

Study Activity	Number of Visits	Compensation Amount (USD)	Total (USD)
Screening Visit	1	$180	$180
DTM donor screening visit	1	$50	$50
Eligibility Confirmation Visit	1	$110	$110
Study Visits			
Administration of Challenge Agent (IBSM inoculation)	1	$440	$440
Malaria Monitoring	3 (by phone)	$15	$45
	~9 (outpatient)	$70	$630
Inpatient Stay Day 1 (including banking)	1	$550	$550
Inpatient Stay, beyond Day 1	1	$170	$170
Antimalarial treatment (whole course)	1	$100	$100
Outpatient Monitoring	1	$80	$80
Safety Monitoring	1 (Day 28)	$80	$80
	1 (Day 56, by phone)	$15	$15
	1 (Day 90)	$80	$80
Approximate TOTAL for Study			$2530

The number of malaria monitoring visits and the duration of inpatient stay may vary depending on the progress of parasitemia. Estimates provided are based on previous experience at QIMR-B. If a participant is diagnosed with malaria early, compensation may be less, or if more visits are needed before admission, compensation may be more, (diagnosis on Day 12 is presented here). An inpatient stay beyond the proposed two days will receive pro-rata reimbursement at the daily rate

for 'Inpatient Stay, beyond day 1.'

6 STUDY INTERVENTION

6.1 STUDY INTERVENTIONS ADMINISTRATION

6.1.1 *P. vivax* Challenge Agent

The *P. vivax* isolate HMPBS02-*Pv* was collected from blood group O Rhesus (Rh)-positive blood donated from a returned traveler from India who presented with clinical manifestations of malaria. The HMPBS02-*Pv* containing MCB was cryopreserved, aliquoted into cryovials, and stored under liquid nitrogen under controlled conditions. Refer to the HMPBS02-*Pv* Challenge Agent Investigator's Brochure for more details. A MCB cryovial containing HMPBS02-*Pv* will be retrieved from storage, thawed, and used to aseptically prepare the challenge agent.

6.1.2 Dosing and Administration

A challenge agent dose, containing an estimated 564 viable *P. vivax*-infected erythrocytes in a volume of 2 mL, will be administered IV to each participant on the morning of study day 0. The actual number of parasites inoculated will take into account the loss of viability resulting from cryopreservation, storage, and thawing. On inoculation day, participants may have food until at least half an hour prior to inoculation. Participants will undergo intravenous cannulation with an appropriate gauge cannula. Placement and patency will be checked by flushing the vein with 5-10 mL of clinical grade saline. The inoculum will be injected, and the cannula again flushed with 5- 10 mL of clinical grade saline. The cannula will then be removed, and hemostasis ensured by useof an appropriate dressing.

6.1.3 Antimalarial Treatment

Artemether/lumefantrine

Artemether/lumefantrine (Coartem) is a commercially available antimalarial drug. The package insert is provided. Artemether/lumefantrine (Coartem) will be purchased directly from a commercial source by the NIH CC pharmacy and will be held in appropriate locked storage conditions and labelled for study purposes in accordance with all applicable regulatory requirements. The standard treatment will be dispensed and accounted for in accordance with NIH CC pharmacy standard procedures. All used medications will be fully documented.

Chloroquine phosphate

Chloroquine phosphate is a commercially available antimalarial drug. The package insert is provided. Chloroquine phosphate will be purchased directly from a commercial source by the NIH CC pharmacy and will be held in appropriate locked storage conditions and labelled for study purposes in accordance with all applicable regulatory requirements. The standard treatment will be dispensed and accounted for in accordance with NIH CC pharmacy standard procedures. All used medications will be fully documented.

Intravenous artesunate

As of April 1, 2019, artesunate is the sole recommended parenteral treatment for malaria in the United States. Currently, it is not commercially available but can be obtained through an expanded access Investigational New Drug (IND) protocol administered by the US Centers for Disease Control (CDC). Please see the CDC's website for further information on the process by which artesunate will be obtained, if needed: www.cdc.gov/malaria/diagnosis_treatment/artesunate.html.

6.1.3.1 Dosing and Administration

Artemether/lumefantrine

All participants will receive treatment with artemether/lumefantrine. The threshold for treatment will occur when the Malaria Clinical Score is >6, parasitemia is >20,000 parasites/mL, or at the investigator's discretion. Artemether/lumefantrine tablets containing 20 mg of artemether and 120 mg of lumefantrine will be administered as 6 doses of 4 tablets (total course of 24 tablets) given over a period of 60 hours (total dose of 480 mg of artemether and 2.88 g of lumefantrine). Each dose of tablets administered orally should be taken with food or drinks rich in fat (e.g., milk). Participants will be reminded of the potential side effects of artemether/lumefantrine.

Chloroquine (only if required)

Participants will only be administered chloroquine if artemether/lumefantrine fails to clear the malaria parasites. For example, if qPCR results indicate unsatisfactory clearance of the parasitemia, defined as two consecutive qPCR time points showing less than a 20% decrease from baseline (i.e., the parasitemia before drug treatment) by 72 hours, participants may be administered chloroquine. Chloroquine tablets containing 250 mg of chloroquine phosphate (equivalent to 155 mg of chloroquine base) will be administered as an initial oral dose of 4 tablets, followed by 2 tablets at 6, 24, and 48 hours (total dose of 2.5 g of chloroquine phosphate [1550 g base]).

Artesunate (only if required)

Treatment of participants with IV artesunate will only occur in the event that participants are unable to complete oral treatment with either artemether/lumefantrine or chloroquine (e.g., the participant is vomiting). This would be done at the recommended dose regime of 2.4 mg/kg at approximately 0, 12, 24 hours and then daily for up to 7 days or until able to take oral drugs.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 Acquisition and Accountability

Transfer, receipt and maintenance of the *P. vivax* MCB from its storage site to the clinical trial site will follow SOP. During transport and at the study site, the liquid nitrogen vapor phase shipper will be monitored. Receipt of the malaria inocula will be documented on a tracking log by study staff. Final accountability will be performed at the conclusion of the Day 0 and at the end of the study. Final disposition of any remaining *P. vivax* vials will be determined and documented following SOP.

The investigator, clinical site pharmacist, or designee, as nominated by the investigator, is responsible for maintaining accurate study agent (inoculum and antimalarial drugs) accountability records throughout the study. Dispensing, accountability, and documentation will be in accordance with SOP. All products will be inventoried upon receipt by staff at the NIH CC. The condition of the products at the time of receipt will be documented, as will the time restrictions of use for the syringes containing the malaria challenge agent. The lot numbers and expiry dates of the inoculum and antimalarial drugs will be documented. The investigator, pharmacist, or delegate will ensure that the received products are the specified formulation.

The storage, handling and the disposal of the challenge agents will be in accordance with approved procedures. All dosages prescribed and dispensed to the participants and all dose changes during the study must be documented. All drug supplies are to be used only in accordance with this protocol, and not for any other purpose. All used medications will be fully documented. Used and

unused drug containers must be destroyed at the site once drug accountability is final and has been checked by the sponsor or its delegate, and written permission for destruction has been obtained from the sponsor.

Study products and study accountability logs will be available to the sponsor or their representative as part of the study monitoring procedures. Upon completion of the study, copies of all study drug management records will be provided to the sponsor. Original records will be maintained at the clinical site with the rest of the study records.

6.2.2 Formulation, Appearance, Packaging, and Labeling

The *P. vivax* isolate HMPBS02-Pv is supplied by QIMRB. The NIH DTM prepares the challenge agent for dosing. It has been estimated that each challenge dose will contain approximately $1.5x10^9$ erythrocytes and approximately 564 viable intraerythrocytic wild type *P. vivax*, parasites in a volume of 2 mL of normal injectable saline. The challenge agent will be administered to each volunteer as an intravenous injection. The parasites injected in each volunteer will be quantified retrospectively using qPCR analysis of retained material.

6.2.3 Product Storage and Stability

Transfer, receipt, and maintenance of the *P. vivax* MCB from its storage site to the clinical trial site will follow SOP. During transport and at the study site, the liquid nitrogen vapor phase shipper will be monitored. Receipt of the MCB will be documented on a tracking log by study staff.

The inoculum will be prepared at the clinical trial site on inoculation day (Day 0). The time between preparation of the inoculum and administration to each participant will be a maximum of 115 minutes, during which time the syringes will be kept at the required temperature as recorded on the label.

Artemether/lumefantrine tablets will be maintained in the manufacturer's original packaging, stored below 30°C, and protected from moisture at the CC until prepared for dispensing.

Chloroquine phosphate tablets will be maintained in the manufacturer's original packaging and stored below 30°C at the CC until prepared for dispensing.

IV artesunate is stored at pre-designated quarantine stations by the CDC in preparation for dispensing if needed.

6.2.4 Preparation

The *P. vivax* challenge agent will be prepared in the NIH DTM according to SOP. Briefly, the MCB vial will be thawed and washed, resuspended in normal saline, diluted, and dispensed into syringes. The inoculum will be kept on ice until injected. For preparation of each inoculum, a required volume of the thawed and diluted blood sample will be used, which has been estimated to contain approximately 564 (*P. vivax*) viable parasite-infected erythrocytes. This will be mixed with clinical grade normal saline. Each inoculum dose will contain parasitized and unparasitized RBCs, resuspended in 0.9% sodium chloride IV infusion, in a total volume of 2 mL in syringes. The time between preparation of the challenge agent and inoculation will be maximum 115 minutes, during which time the challenge agent will be stored at 2-15°C until administration (e.g. on ice).

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Not Applicable

6.4 STUDY INTERVENTION COMPLIANCE

Following administration of the challenge agent, adherence to the protocol will be assessed and verified at scheduled study visits, and unscheduled visits where required, including collection of blood samples for qPCR parasitemia monitoring and collection of malaria-related symptom data, including the malaria clinical score. These data will be used to determine the timing of collection for cell banking. The eCRF will be completed following study visits. Results of blood sample testing will be available as electronic reports from the laboratory responsible for testing and entered into the eCRF or printed and stored as hardcopy following review by the investigator.

6.5 CONCOMITANT THERAPY

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Clinical Research Information Management System of the NIAID (CRIMSON) are concomitant prescription medications, over-the-counter medications and supplements.

Concomitant medications, treatments, and procedures are those occurring from administration of the malaria inoculum until the end of the study (last visit). Those occurring prior to administration of the inoculum are classified as prior medications, treatments, and procedures. Medications taken within 28 days before the malaria inoculation will be recorded as prior medication. Prior and concomitant medications, treatments, and procedures permitted in this study are outlined in the inclusion/exclusion criteria (Sections 5 and 5.2).

On inoculation day, participants will be questioned in relation to relevant aspects of compliance with the study protocol, including drug intake since their screening clinic visit. Details of all other drugs taken (prescription and over-the-counter, systemic and topical administration) will be recorded at this time and appropriate action taken. The investigator may permit the use of ibuprofen up to 1.2 g/day or acetaminophen up to 4 g/day for treatment of headache or other pain if required. Any medication taken during the study for treatment of a medical condition or AE is to be recorded in the concomitant medication pages in CRIMSON (exact dose and timing of each dose to be specified).

All concomitant medications other than those routinely used for symptom relief in IBSM trials (i.e., acetaminophen, ibuprofen, ondansetron) or routine medications approved at screening (e.g., oral contraceptive) should be discussed with the investigator before being approved unless deemed medically urgent. If the medication has already been taken, then it should be reviewed by the investigator at the next opportunity and a decision should be made to continue, to stop, to switch to an alternative, or to withdraw the participant from the trial.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

Discontinuation following challenge agent administration does not mean discontinuation fromthe study, and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified after enrollment (including, but not limited to changes from baseline), the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE). Participants are free to withdraw from participation in the study at any time upon request.

7.1.1 Withdrawal Criteria for an Individual Subject

An individual subject will be withdrawn from the study for any of the following:

- An individual subject's decision. The investigator should attempt to determine the reasonfor the subject's decision.
- Non-compliance with study procedures to the extent that it is potentially harmful to thesubject or to the integrity of the study data.
- The subject loses the capacity to provide ongoing informed consent.
- Participants may be withdrawn for any AE that would cause continued participation in the study to not be in the best interest of the participant, as per the investigator's judgment. Any participant who is withdrawn from the study because of an AE related to the investigational product will be followed for safety until at least resolution of that AE and will be encouraged to remain in the safety evaluation for the duration of the study.
- A change in the subject's condition as follows:
    - The participant acquires HIV or viral hepatitis infection during the course of the study.
    - The participant becomes pregnant during the course of the study.
- The investigator determines that continued participation in the study would not be in the best interest of the subject.

7.1.1.1 Re-enrollment and Unplanned Procedure Repetition

Unless otherwise specified within this protocol, each person who is a subject in this study may be enrolled, and may pass through each step and process outlined in the protocol only **ONCE** (i.e., subjects may not "go back" and repeat a protocol step already completed). On a case-by-case basis, a request for re-enrollment, or for repetition of a protocol step or procedure already completed, may be submitted to, reviewed by, and approved by the SMM in writing. The SMM may also recommend or require consultation of the IRB.

7.1.1.2 Replacement of Withdrawn Subjects or Subjects Who Discontinue Study Agent

Participants who have received IBSM and who withdraw or are terminated from the study prior to completion will not be replaced. Participants withdrawn before the receipt of IBSM will be replaced.

All subjects exposed to study agent(s) MUST be included in the safety dataset.

7.2 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if they fail to return for two scheduled visitsand are unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, three telephone calls

and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.

- In the case of a participant failing to attend for visits after inoculation and prior to antimalarial treatment, next of kin and/or cohabitating individual may also be contacted.
- In the case of a participant failing to attend for visits after inoculation and prior to antimalarial treatment, local Public Health services or the relevant Health Department may also be contacted.
- Should the participant continue to be unreachable, they will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 SCREENING PROCEDURES

A screening visit will be scheduled after an initial telephone interview conducted by clinical unit staff has occurred to review background information. During this initial screening visit, the potential participant will read the informed consent form and be encouraged to ask questions. Individuals willing to be considered for inclusion may sign the informed consent form during the screening visit or return to the clinical unit later after further consideration. The participant will be given a copy of the Participant Information Sheet and signed consent form for their records. The signed and dated originals will be held on file. Participation consent must be obtained from all eligible participants prior to performing screening tests.

8.1.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

An IRB approved telephone pre-screening. This screening will be conducted to determine if the subject appears to meet initial eligibility criteria. If the subject does not wish to complete the telephone prescreen, he/she may elect to complete the informed consent process and in-person screening process without first completing a telephone prescreen, as indicated below.

8.1.2 Screening activities performed after a consent for screening has been signed

After providing written consent to participate, the participant will undergo the screening procedures listed below. Some screening tests may be repeated during the Day -3 to Day -1 safety visit (as required) and/or on the day of malaria challenge inoculum administration to determine their continued eligibility. Participants must confirm that they will not be living alone from Day 0 until completion of antimalarial treatment.

Screening will be conducted within 56 days prior to the Day 0 malaria challenge day and will include the following elements:

1. Provide the Participation Information Sheet and informed consent form and allow the participant sufficient time to review the contents of each document.
2. Explain the study via the main Participation Information Sheet and gain informed consentfrom the participant.
3. Ensure the participant has successfully completed the Malaria Comprehension Exam(scoring ≥80% correct and those questions answered in error reviewed).
4. Ensure the participant has signed the informed consent form and received a signed copy.

5. A screening number will be assigned to each participant.
6. Elicit a complete medical history and use of medications.
7. Elicit a social history including alcohol and tobacco use.
8. Undertake a full physical examination.
9. Ask participant to complete the PHQ-2 questionnaire (Appendix F).
10. Assessment of the cardiovascular disease risk based on the NHANES I cardiovascular riskassessment (Appendix A).
11. Record vital signs.
12. Obtain a 12-lead ECG. The ECG should be reviewed, initialed and dated by study study staff and transmitted to the study cardiologist for the final review/read.
13. Collect urine for urinalysis and urine drug screen.
14. Ensure that HIV pre-test counseling has been performed and ensure that the participant has agreed to HIV testing (required by Maryland state law).
15. Collect blood samples for hematology, biochemistry, RBC alloantibodies, Duffy antigen, ethanol level, serum pregnancy testing (if applicable), and bloodborne infection testing.
16. Verify participant meets inclusion/exclusion criteria.

Participants who complete all screening procedures and satisfy all entry criteria will be considered eligible to participate in this study. To be eligible for study entry, laboratory values at screening must not be outside the range of the normal values at a level deemed to be clinically significant. For eligibility parameters, a repeat may be requested to exclude laboratory error.

If screening laboratory results are abnormal, e.g., HIV testing, the volunteer will be referred for appropriate counselling. If any clinically significant abnormalities are detected during screening, the participant will be referred for follow-up tests to a general practitioner or medical specialist as appropriate.

Note: Laboratory studies and ECG completed under a different NIH protocol (such as screening protocol 16-I-0039) can be used for screening purposes as long as they are 56 days prior to study enrollment.

To ensure the participant fully comprehends key concepts related to the study and to highlight areas that may need additional discussion or clarification, a Malaria Comprehension Exam will be administered to the participant before signing the informed consent form. All incorrect responses will be reviewed with the participant, and they must verbalize understanding of all incorrect responses. A score of ≥80% correct is required for enrollment. For participants scoring less than 80%, study staff may choose to review study details again with participant and reassess comprehension with a repeat Malaria Comprehension Exam. At the discretion of the investigator, any participant whose comprehension is questionable, regardless of score, may be excluded from enrollment. Discussions of understanding will be documented in the participant's source documentation.

Participants will also undergo routine DTM donor center screening prior to enrolment to ensure they are considered appropriate to donate blood products by NIH CC DTM service providers. This assessment will include routine screening outlined in Section 8.2.2.1. This visit may occur on the same day as the screening visit or at any time up to and including the Day -3 to Day -1 Eligibility Confirmation Visit. Any concerns regarding eligibility from NIH CC DTM service providers must be discussed with the investigator or delegate.

A maximum of 2 participants will undergo IBSM challenge in this trial. Each participant will be

actively monitored for approximately 3 months.

Definitions for the purpose of this study:

- Screened – participants will receive a study identification (ID) number when the informed consent form is signed and will either be determined as "eligible" or "screen failures" as noted below.
    - Screening may be completed over the course of multiple visits.
    - Screening, in most cases, will occur within 56 days prior to enrollment into the study.
        - If the screening visit is >56 days prior to enrollment, then an updated medical review and laboratory testing will be completed to determine eligibility for enrollment.
- Enrolled – participant will be considered enrolled beginning with the receipt of the IBSM.
- Screen Failures – participants are considered screen failures (Section 5.5) when they meet one of the following criteria after signing the consent form:
    - Screening results reveal that the participant is ineligible.
    - Participant withdraws consent before receiving IBSM.
    - Study completes enrollment prior to the participant being determined eligible for enrollment.
- Discontinued – participants are considered discontinued when they meet 1 or more of the following criteria:
    - Participant withdraws consent after receiving IBSM.
    - Participant is withdrawn by the PI/sponsor.
- Completed – participants are considered completed when they complete the final study visit.

8.2 STUDY EVALUATION & PROCEDURES

8.2.1 Clinical Evaluations

Medical history

Past medical/surgical history includes:

- History of all known allergies
- Current medications, including over-the-counter and herbal preparations
- History of substance abuse and recreational drug use
- History of depression, anxiety, mental illness, emotional problems, use of psychiatricmedications, and previous psychotherapy
- Surgical procedures and results

Physical examination

Full physical examination includes:

- Weight **(screening only)**
- Height **(screening only)**
- Review of systems excluding genitourinary examination and including the following:
    - Head, neck (including thyroid), ears, eyes, nose and throat
    - Heart/circulation

- Chest
- Lungs
- Abdomen
- Skin
- Neurological

Symptom-directed physical examination: physical examinations will be symptom-directed at specified time points (i.e., systems will be reviewed only if clinically indicated at the discretion of the investigator).

The Patient Health Questionnaire-2 (PHQ-2)

All participants will be required to complete the PHQ-2 questionnaire at screening. This is a validated questionnaire used as an objective tool for the assessment of depression (Appendix F).

Vital signs

Vital signs (temperature, heart rate, respiratory rate, and blood pressure) will be measured at screening. Tympanic temperature will be taken at the clinical unit, and sublingual temperature will be taken by participants at home for practical reasons.

Electrocardiograms

A 12-lead ECG will be recorded.Adverse event recording

AEs will be recorded as described in Section 8.3.

Malaria Clinical Score

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). Individual scores for each symptom as well as the total score will be recorded (Appendix B):

Headache	Anorexia
Myalgia (muscle ache)	Nausea
Arthralgia (joint ache)	Vomiting
Fatigue/lethargy	Abdominal discomfort
Malaise (general discomfort/uneasiness)	Fever
Chills/shivering/rigors	Tachycardia
Sweating/hot spells	Hypotension

Photography of rash or injection site reactions

If a participant develops a rash or injection site reaction, photographs may be taken by the investigators. These photographs will not include the participant's face or any identifying scars, marks, or tattoos.

DTM NIH CC donor screening

A routine donor screening assessment by DTM NIH CC service providers to ensure the participant is appropriate for blood donation. At this visit clinical assessments including review of venous access and blood product donation eligibility will be conducted.

8.2.2 Biospecimen Evaluations

Blood sampling

Blood will be collected for clinical laboratory evaluations including hematology, clinical chemistry, serology, and pregnancy testing. Blood samples will also be collected to monitor malaria parasitemia. The estimated blood volume required for these tests is listed in Appendix D. The total volume of whole blood drawn from each adult participant, including the red blood cells collected for the banking procedure, will not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any 8-week period. This volume includes allowance for unscheduled safety laboratory assessments that may be required at the discretion of the PI or the sponsor to ensure participant safety.

Urine sample collection

Urine will be collected for urinalysis, drug screening, and pregnancy testing.

8.2.2.1 Clinical Laboratory Testing

Using standard techniques, the NIH CC laboratory and Department of Transfusion Medicine will perform the following tests:

1. Hematology:
   - Complete blood count (CBC) plus white blood cell differential and platelet count
   - The following CBC parameters will be assessed for safety throughout the trial: WBC, absolute neutrophil count (ANC)/absolute granulocyte count, absolute lymphocyte count (ALC), hemoglobin, and platelet count
   - A manual blood smear should be reviewed if there are immature/abnormal cells detected on the automated differential or if an automated differential was not able to beperformed
   - Reticulocyte count (days -3 to -1 eligibility confirmation visit or at screening if between days -3 to -1, and day 28±3 or early termination visit only)
   - Blood group and Rh(D) (screening visit only)
   - Duffy antigen (required for screening visit)
2. Biochemistry:
   - Acute panel (sodium, potassium, chloride, bicarbonate, creatinine, glucose, urea); hepatic panel (alkaline phosphatase, ALT, AST, total bilirubin, direct bilirubin); mineral panel (albumin, calcium, magnesium, phosphate); uric acid, lactate dehydrogenase (screening visit).
   - Acute panel and hepatic panel (other visits, additional testing at investigator discretion).
3. Bloodborne infection screening
   - DTM Viral Marker (Transplant Donor) Panel: HbsAg, HBV Ab, HCV Ab, HIV1/2 Ab, HTLV1/2 Ab, T.Cruzi Ab, West Nile Virus NAT, HIV/HBC/HCV NAT (screening visit).
   - CMV Ab, EBV Ab, Syphilis (algorithm-based Ab, RPR, TPPA), Babesia Ab, Parvovirus B19 NAT (screening visit). If CMV or EBV IgM positive, further testing may be

warranted.
- CMV, EBV, HHV6 NAT (admission visit). Results not required for banking.

4. RBC alloantibodies (screening and days 28, 90, or early termination visit only)
5. Urine dipstick/urinalysis (screening, eligibility confirmation visit, admission, day 28 visit)
6. Urine and/or serum pregnancy testing (β-hCG)
7. Urine drug screen
8. Serum ethanol level (screening visit only)
9. Serum storage (serum separating tube to hold; days 0 and 90 visit only)
10. Safety storage (EDTA tube hold; admission only)
11. SARS-COV-2 screen (days -3 to -1 eligibility confirmation visit or at screening if between days -3 to -1; as indicated by investigator's discretion/NIH CC policy/Hospital Epidemiology Service recommendations)

    Please note: if a participant tests positive for SARS-COV-2 prior to challenge they will be considered to meet exclusion criterion 13c. A positive SARS-COV-2 test after inoculation will be discussed with hospital epidemiology and investigator discretion will be exercised to determine whether banking may proceed with reasonable safety to hospital staff.

Urinalysis

Urine may be tested by dipstick at the clinical unit. If there are any abnormalities considered clinically significant in blood, leucocytes, or protein, the urine will be sent for formal laboratory urinalysis.

Urine drug screens and serum ethanol tests

If the results of the urine drug screens or serum ethanol tests are positive, participants may be allowed to continue or may be delayed or withdrawn at the investigator's discretion. All participants will be questioned about concomitant medications and use of recreational drugs. The urine drug screen may be repeated if the potential participant denies usage of any of these agents and the test result is believed to be a false positive.

Participants testing positive for acetaminophen at screening and/or inoculation day may still be eligible for study participation at the investigator's discretion. Participants requiring acetaminophen on a daily basis will not be eligible to enroll in the study, as the use of over-the-counter medication during the study is restricted and potential participants should not discontinue their usual medications in order to participate in the study.

Malaria monitoring

Blood will be collected to monitor malaria parasite numbers using qPCR targeting the 18S rRNA gene. Additional blood (up to approximately 2 mL per time point) may be collected for parasite lifecycle stage qRT-PCR at the investigator's discretion. This is to evaluate for the presence of sexual parasite stages (gametocytes) and other parasite lifecycle stages in the blood for research purposes. This blood may also be used for research into various aspects of parasite biology, e.g., gametocytes, parasite lifecycle stages, recrudescence, commitment, etc. Targets for qRT-PCR may include the female gametocyte-specific transcript *pvs25*, the male gametocyte, and the ring-stage as appropriate. This testing will occur between inoculation and Day 28.

Microscopic examination for evidence of parasitemia or gametocytemia may be conducted at the investigator's discretion.

8.2.2.2 Blood Smears

The gold standard for malaria diagnosis and evaluation of efficacy endpoints is the detection of malaria parasites on Giemsa-stained thick blood films. When indicated, blood smears are prepared in duplicate according to standard challenge procedures and evaluated by trained study microscopists, and the results reported to the study investigator. At least 0.5 µL of blood is scanned for the presence of malaria parasites. This method allows for detection of a parasite density as low as 4 parasites/µL and early diagnosis, often before participants become symptomatic for malaria.

Per standard safety guidelines in place for *P. falciparum* and adopted for *P. vivax* challenge studies, symptomatic individuals may undergo more rigorous smear reading whereby 1.5 µL of blood is scanned for the presence of malaria parasites. Slides are considered positive if at least two unambiguous parasites per slide are identified and confirmed by a second microscopist.

8.2.2.3 Malaria PCRs

The Department of Laboratory Medicine at the NIH CC has developed a NIH malaria genus species (4-plex) qPCR with a sensitivity of 10,000 parasites/mL of whole blood (10 parasites/µL), detecting parasitemia approximately 1-2 days before clinical symptoms develop. This assay has been externally validated, is conducted under a CLIA-certified laboratory, and is currently the malaria molecular assay at the NIH CC.

NIH malaria genus species (4-plex) qPCR will be used for malaria diagnosis along with clinical scoring of symptoms per Appendix E.[31] Based on studies completed at QIMRB, timing of treatment typically occurs around day 10 post-inoculation. Once a diagnosis of induced malaria (by qPCR and/or symptoms) has been made, subsequent NIH CC qPCR will be conducted to document decrease in parasites numbers during treatment. Once this is documented, subsequent NIH CC qPCRs will not be done until the end of study visit, but research qPCRs will continue to be completed per the schedule of activities (Section 1.3) or at the investigator's discretion.

Research LMIV qPCR (Section 8.2.3.1) will be completed solely for evaluation of research endpoints, specifically to characterize the presence and kinetics of subpatent parasitemia (sexual and asexual stages) following parasite exposure and following treatment.

8.2.2.4 NIH CC Malaria *P. vivax* PCR

Both assays must be performed to determine the final PCR result. The "Malaria Genus PCR" is a multiplexed PCR that consists of one set of primers and a TaqMan probe that will amplify and detect a 157-bp target region of the 18S rRNA present in the five species of malaria known to commonly infect humans (*P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae,* and *P. knowlesi*). In addition, a separate set of primers and a TaqMan probe specificfor the spiked internal control (IC) DNA is included in the reaction. If a signal is received from both the malaria and IC probes, the patient specimen is considered positive for malaria (unable todetermine the specific species) and there is no indication of PCR inhibition present in the extracted DNA.

The malaria species PCR is performed to determine the *Plasmodium* species present in the patient specimen. This assay is a 4-plex PCR consisting of a combination of four different forward primers and one reverse primer and four different TaqMan probes—each labeled with a different fluorophore—that will amplify and detect varied 18S rRNA regions specific to a particular species

of *Plasmodium*. Detection of the appropriate fluorophore(s) will indicate the presence of *P. falciparum* (104-bp product), *P. vivax* (106-bp product), *P. ovale* (106-bp product), and *P. malariae* (112-bp product). A negative result on this qPCR assay from a specimen that was detected positive on the malaria genus PCR indicates, by default, that the infecting organism is *P. knowlesi*.

8.2.3 Correlative Studies for Research/Pharmacokinetic Studies

8.2.3.1 Research *Pv* (18s) Parasite and *Pv* Gametocyte (Pvs25) qPCR

While detection of parasites on thick blood smears has been the most common primary endpoint in human challenge trials, QIMRB has undertaken 23 clinical trials to date using a validated PCR assay to quantify parasitemia including gametocytemia with consistent results (Figure 2).

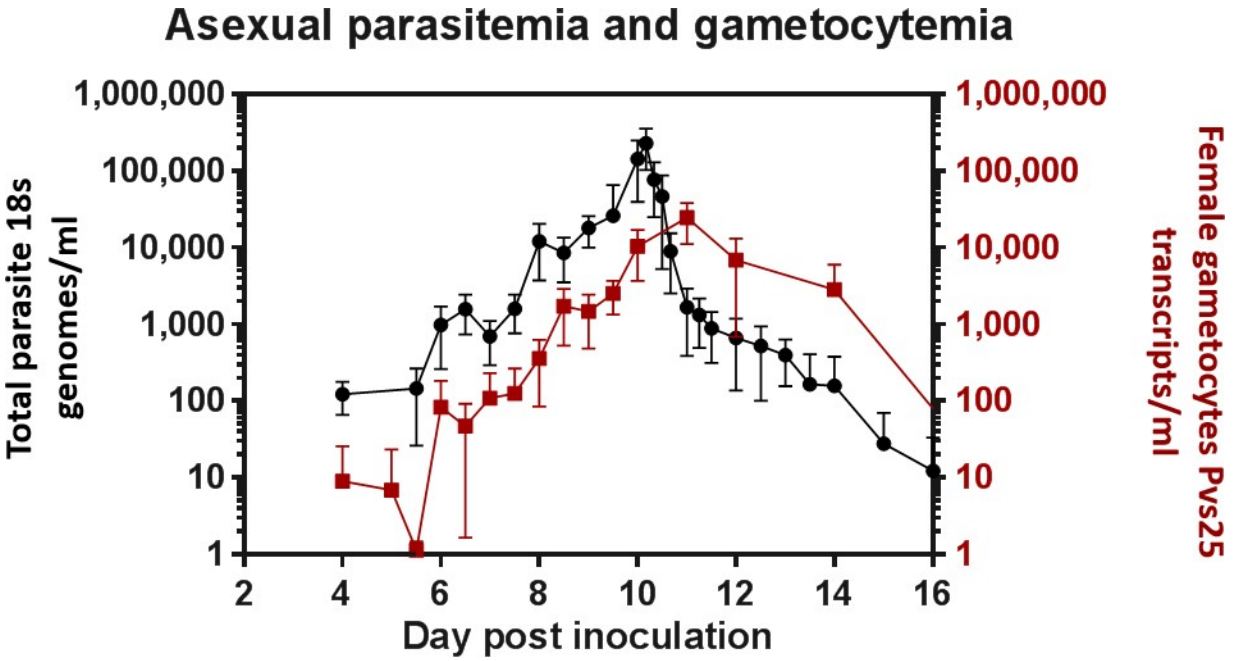


Figure 2. Asexual Parasitemia and Gametocytemia

Asexual parasitemia and gametocytemia following IBSM (HMPBS02-Pv). In a recent study, participants were treated on day 10 post-inoculation. By direct skin feeds (DSF) and direct membrane feeding assays (DMFA), participants were only able to transmit to mosquitoes on day 10 post-inoculation, prior to treatment. On days prior to and after treatment, no mosquitoes were infected by DSF or DMFA.

These molecular assays have significantly increased sensitivity for detection of blood-stage infection approaching 20 parasites/mL, typically 4 days earlier than by paired thick blood smears. Quantification of parasite density by these methods allows evaluation of parasite growth curves, e.g., for assessing the utility of partially effective vaccine candidates. LMIV plans to develop similar Pv qPCR assays to employ for this trial in partnership with QIMRB.

8.2.4 Samples for Genetic/Genomic Analysis

Whole genome transcriptional profiling will be performed to explore possible gene expression profiles or pathways following IBSM. Transcriptional analyses will be performed on whole blood collected as outlined in the schedule of activities.

Blood may be collected via venous puncture and placed in PaxGENE tubes (or equivalent) to preserve RNA integrity until the RNA is extracted. The molecular profiling encompasses the

identification of RNA transcripts present in all humans, which are induced or repressed after each vaccination. This does not represent genetic testing of individuals or their DNA.

8.3 SAFETY AND OTHER ASSESSMENTS

See Sections 8 and 8.2.1 for descriptions of screening assessments.

8.3.1 Day -3 to Day -1 Eligibility Confirmation Visit

Participants (including reserve participants) will report to the NIH CC between Days -3 and -1 for the following baseline assessments, unless screening laboratory assessments were otherwise conducted within this period, in which case repeat sampling will not be required.

1. Collect blood samples for hematology and biochemistry analysis.
2. Collect urine for urinalysis.
3. Collect Sample for SARS-CoV-2 analysis

The timing of these assessments is to ensure that results are available for review by the investigator prior to inoculation on Day 0. Participants with clinically significant laboratory findings at this stage will not be eligible for malaria parasite inoculation.

8.3.2 Enrollment

Participants will be enrolled on a first-eligible-and-available basis. Enrollment may be staggered for safety and based on availability.

On the day of the administration of the challenge agent (IBSM Day 0), up to three previously-screened and eligible back-up participants may be asked to take the place of participants who do not continue to meet eligibility. These alternates will be compensated for their availability on that day even if not inoculated.

8.3.3 Administration of the Challenge Agent (IBSM Day 0)

Each participant (and up to 3 reserve participants) will report to the NIH CC on the morning of Day 0. The investigator will review the participants' screening results prior to their enrollment into the study. The investigator will emphasize the requirement to return for malaria drug treatment after the administration of the challenge agent. Participants will be reviewed by the investigator to confirm their continued eligibility for the study, including confirmation that they will not be living alone from Day 0 until the end of antimalarial treatment by checking housemates' contact details recorded at screening visit.

Participants will be required to repeat screening procedures to determine whether they remain eligible to be enrolled. Participants may have food until a half an hour prior to inoculation. A reserve participant may be asked to replace a participant who does not continue to meet eligibility. These reserves will be compensated for the study visit even if not inoculated.

The procedures that will be undertaken prior to inoculation include:

1. Verify that all applicable eligibility criteria have been met.
2. Elicit information regarding any new medical conditions or illnesses since screening.
3. Confirm three emergency contacts for the volunteer, including one who is aware of participant's study participation and lives in the participant's house.
4. Complete emergency medical release form.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure

that the test isnegative before IBSM; a positive test will exclude the participant from the trial.
6. For females, ensure agreement and compliance with pregnancy prevention before IBSM.
7. Perform urine drug screen.
8. Conduct symptom-directed physical examination.
9. Record vital signs.
10. Obtain a 12-lead ECG.
11. Cannulate participants with an indwelling intravenous cannula for the malaria inoculum,and record which arm is utilized.
12. Collect blood samples for malaria 18S qPCR (parasitemia baseline sample), parasite lifecycle stage qRT-PCR (parasite lifecycle stage baseline sample), and research labs – serum storage, transcriptional analyses.

Administration of the malaria inoculum:

1. IV administer the challenge agent of approximately 564 viable *P. vivax*–infected humanRBCs.
2. Observe for a minimum of 60 minutes after administration of the inoculum to evaluate forimmediate ARs.
3. Educate participant on signs and symptoms of malaria (Appendix E).
4. Emphasize to participant the importance of making all study visits per protocol or asadvised by the clinical staff.
5. Provide participant with thermometers to take temperature readings during the study in theevent of symptoms of fever.
6. Record AEs and concomitant medications.
7. Record vital signs prior to leaving the clinic (approximately 60 minutes after inoculation).
8. Record solicited symptoms for local and systemic IBSM reactogenicity.

8.3.4 Malaria Monitoring

Malaria monitoring via phone (Day 1 to Day 3)

During this period, participants are expected to be asymptomatic. A daily phone call or text message will be made to the participants by clinic staff to monitor participant wellbeing and to solicit any AEs. The solicited symptoms for local and systemic IBSM reactogenicity will be reviewed remotely with participant, and continued pregnancy prevention compliance will be confirmed.

Daily clinic visits for malaria monitoring (Day 4 until admission for rescue treatment)

Daily follow-up will be done from Day 4 until the day of blood collection and rescue treatment initiation at the NIH CC. More frequent visits from Day 9 until treatment may be needed to allow frequent collection of blood samples for parasitemia determination. The study investigator may admit participants earlier than planned for symptom relief and observation prior to blood collection and treatment.

The following procedures will occur during these visits:

1. Perform symptom-directed physical examination when signs and symptoms of malaria are identified or at the investigator's discretion.
2. Record vital signs.

3. Collect blood samples for malaria 18S qPCR and parasite lifecycle stage qRT-PCR (if required). Sample may be collected up to 3 times per day from Day 9 until treatment day (at the discretion of the investigator and if maximum whole blood volume is not exceeded).
4. Review solicited malaria symptoms with participant.
5. Confirm continued pregnancy prevention compliance.
6. Record malaria clinical score.
7. Record AEs and use of concomitant medications.
8. Hematology and biochemistry will be performed on Day 9.
9. Research labs (transcriptional analysis) may be performed on Day 4.
10. SARs-CoV-2 screening may be collected based on investigator discretion, NIH CC policies, or NIH CC Hospital Epidemiology Service recommendations

Inpatient observation, blood collection, and antimalarial treatment phase (estimated Days 9, 10, 11, 12)

Participants will be admitted to the clinical unit for a minimum of 48 hours (extendable at the discretion of the investigator) when the Malaria Clinical Score is >6 (within 24 hours of notification), or parasitemia is >20,000 parasites/mL, or at the investigator's discretion. If a participant remains qPCR-negative through Day 14, they will start treatment on days 15, 16, and 17 and will be considered an infection failure.

Participants will be allowed to leave the inpatient stay 48 hours after initiation of artemether/lumefantrine treatment at the investigator's discretion if the participant is asymptomatic or has only mild (Grade 1) symptoms, has a normal examination, and no clinically significant laboratory abnormalities.

Admission

During the inpatient stay, participants will be seen at least twice daily by study staff and more often if needed.

The following procedures will occur at admission to the clinical unit (or just prior to blood collection):

1. Confirm continued pregnancy prevention compliance.
2. Perform symptom-directed physical examination.
3. Record vital signs.
4. Collect urine for urinalysis, drug screen and pregnancy testing (if applicable).
5. Collect blood samples for hematology, biochemistry, malaria 18S qPCR and parasite lifecycle stage qRT-PCR and malaria blood smear, bloodborne infection screening, research labs - safety storage, transcriptional analyses (optional). In the case where collection of blood for banking is not performed soon after admission but later in the day, blood sample collection can be postponed and performed just prior to the procedure.
6. Record Malaria Clinical Score.
7. Record AEs and use of concomitant medications.
8. Collect an estimated target minimum 200-mL blood sample for cell bank processing with the Department of Transfusion Medicine. The banking procedure should be performed as per the banking SOP. Increased volume may be collected at the discretion of the investigator based on the available maximum whole volume limit.

Rescue treatment and observation

The following procedures will occur during the rescue treatment and observation period:

1. Collect blood samples for hematology, biochemistry (if not collected in prior 24 hours), and malaria 18S qPCR (pre-treatment). A blood sample can also be collected for parasite lifecycle stage qRT-PCR(at the discretion of the investigator and if the maximum whole blood volume is not exceeded).
2. Obtain a 12-lead ECG.
3. Administer artemether/lumefantrine treatment under direct observation.
4. Perform symptom-directed physical examination when signs or symptoms of malaria are identified and it is clinically indicated.
5. Record vital signs 3 times a day while admitted.
6. Record Malaria Clinical Score 3 times a day while admitted.
7. Collect blood samples for malaria 18S qPCR at 12, 24, 36, and 48 hours following artemether/lumefantrine treatment initiation. A peripheral intravenous line may be inserted at any point during the admission to facilitate ease and comfort of frequent lab draws. Additional blood samples can be collected atthe discretion of the investigator if required to ensure the safety of the participant.
8. Record AEs and use of concomitant medications.

Prior to discharge from inpatient unit

Participants will be allowed to leave the unit 48 hours after initiation of artemether/lumefantrine treatment or at the investigator's discretion. Participants may be requested to stay in the unit longer than 48 hours at the investigator's discretion if deemed in their clinical interest.

The following procedures will occur prior to discharge from the clinical unit:

1. Perform symptom-directed physical examination.
2. Record vital signs.
3. Collect blood samples for hematology, biochemistry, and malaria 18S qPCR. A blood sample can also be collected for parasite lifecycle stage qRT-PCR (at the discretion of the investigator and if the maximum whole blood volume is not exceeded).
4. Record AEs and use of concomitant medications.

Chloroquine phosphate rescue treatment (if required)

It is predicted that artemether/lumefantrine treatment will be curative considering that it is a registered drug used for treatment of *P. vivax* malaria infection and all patients to date infected with the challenge agent derived from the HMPBS02-*Pv* MCB have been successfully treated with artemether/lumefantrine alone. However, there is a possibility that resistance to artemether/lumefantrine by the *P. vivax* challenge agent may occur. In case of treatment failure with artemether/lumefantrine, participants will receive a standard course of therapy with chloroquine phosphate within 72 hours of administration of the first dose of artemether/lumefantrine treatment, at the discretion of the investigator. Full details on the decision process to commence chloroquine treatment are presented in Section 6.1.2. If chloroquine dosing is required, safety labs (hematology and biochemistry) will be collected and assessed if not already scheduled prior to first dose and after last dose (or within 1 day of last dose). Monitoring of participants post-chloroquine treatment will occur as presented above for artemether/lumefantrine treatment.

Artesunate rescue treatment (if required)

Treatment of participants with IV artesunate will only occur in the event that participants are unable to complete oral treatment with either artemether/lumefantrine or chloroquine (e.g., the participant is vomiting or otherwise unable to tolerate oral medications). Details on artesunate dosing are presented in Section 6.1.2. Participants will be administered IV artesunate on site and monitored as described above.

Outpatient monitoring post-artemether/lumefantrine treatment (post admission until Day 28 safety visit)

If the participant is discharged before 72 hours post–artemether/lumefantrine treatment, a follow-up visit will be undertaken at approximately 72 hours for clinical evaluation and blood sampling. If the 18S PCR result is positive, the participant will be followed up daily until a minimum of one negative qPCR is detected.

The following procedures will take place during this visit:

1. Confirm continued pregnancy prevention compliance.
2. Collect blood samples for malaria 18S qPCR monitoring. Blood sampling for malaria monitoring will occur at the investigator's discretion if there is concern regarding the possible recrudescence of parasitemia.
3. Collect blood samples for parasite lifecycle stage qRT-PCR at the investigator's discretion.
4. Collect blood samples for hematology and biochemistry.
5. Perform symptom-directed physical examination when signs and symptoms of malaria are identified and it is clinically indicated at the investigator's discretion.
6. Record vital signs.
7. Record Malaria Clinical Score if vital signs are abnormal or at the investigator's discretion.
8. Record AEs and use of concomitant medications.

Day 28±3 (safety visit)

The following procedures will occur on day 28±3:

1. Perform full physical examination.
2. Record vital signs.
3. Collect urine for urinalysis.
4. At the discretion of the investigator and if required for safety reasons, collect blood samples for hematology, biochemistry, malaria 18S qPCR and parasite lifecycle stage qRT-PCR (if required), RBC alloantibodies, research labs - transcriptional analyses (optional).
5. Record AEs and use of concomitant medications.

Follow-up phone call (Day 56±7)

A phone call will be made on day 56±7 to the participants by clinic staff to monitor participant well-being and to solicit any AEs.

End of Study Visit (Day 90±7)

The following procedures will occur at the end of study visit:

1. Perform symptom-directed physical examination.
2. Record vital signs.
3. If not already done on day 28±3, collect blood samples for hematology, biochemistry,

malaria 18S qPCR and parasite lifecycle stage qRT-PCR (if required), RBC alloantibodies
4. Collect blood samples for and safety serum storage.
5. Record AEs and use of concomitant medications.

Early Termination Visit

If withdrawal occurs at any stage of the study, the participant will be asked to complete an end-of-study evaluation. **In addition, participants are informed on the essential requirement to complete the antimalarial drug treatment for their safety.**

Participation in an end-of-study evaluation by each participant is voluntary. Procedures during the early termination visit will include the following if withdrawal occurs prior to Day 28 visit:

1. Perform full physical examination.
2. Record vital signs.
3. Collect urine sample for urinalysis.
4. Obtain blood for hematology, biochemistry, malaria qPCR and parasite lifecycle stageqRT-PCR, RBC alloantibodies, and safety serum storage.
5. Record AEs and use of concomitant medications.

If withdrawal occurs after the Day 28 visit, the procedures outlined for the final study visit will be performed.

Unscheduled Visit

Unscheduled visits for malaria 18S qPCR or safety monitoring may be required at the investigator's discretion based on parasitemia, clinical symptoms, or laboratory results. Participants will be contacted by phone to arrange these visits. Where possible, visits will be arranged at a time that is both convenient for the participant and meets any clinical urgency as determined by the investigator.

Schedule of Activities

The schedule of activities (Section 1.3) details all procedures to be conducted as per this protocol during recruitment and screening, enrollment, inpatient stay, and follow-up. During inpatient hospitalization, participants will be admitted to the NIH CC for observation and clinical management. During outpatient follow-up participants will be seen by study-trained nurses and study staff, including the PI, will be available 24 hours on call for questions and/or concerns.

8.4 ADVERSE EVENTS, AND SERIOUS ADVERSE EVENTS

8.4.1 Definition of an Adverse Event

Adverse Event: An AE is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g. abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

8.4.2 Definition of Serious Adverse Events (SAE)

Serious Adverse Event: An SAE:

- is an AE that results in death.
- is an AE that is life threatening (places the subject at immediate risk of death from the event as it occurred).

- is an AE that requires inpatient hospitalization or prolongs an existing hospitalization.
  NOTE:
  - Hospitalization is considered required if outpatient treatment would generally be considered inappropriate.
  - Same-day surgical procedures that are required to address an AE are considered hospitalizations, even if they do not involve an overnight admission.
  - Hospitalization due to a condition that has not worsened and that pre-dates study participation (e.g., elective correction of an unchanged baseline skin lesion), or due to social circumstance (e.g., prolonged stay to arrange aftercare), or that is planned/required "per protocol" AND that proceeds without prolongation or complication, is NOT considered an SAE by this criterion.
- is, or results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- is a medically important event.
  NOTE: Medical and scientific judgment should be exercised. Events that significantly jeopardize the subject and/or require intervention to prevent one of the SAE outcomes listed above are generally considered medically important, and are thus SAEs.

8.4.2.1 Other Definitions

Adverse Reaction (AR): An AR means any AE caused (see "Causality" below) by a study agent. ARs are a subset of all suspected adverse reactions (SARs; defined below) where there is reason to conclude that the study agent caused the event.

Suspected Adverse Reaction (SAR): SAR means any AE for which there is a reasonable possibility that the study agent caused the AE.

> Per US FDA guidance:
>
> For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal (see "Causality" below) relationship between the study agent and the AE. An SAR implies a lesser degree of certainty about causality than an AR, which means any AE caused by a study agent.
>
> SARs are the subset of all AEs for which there is a reasonable possibility that the study agent caused (see "Causality" below) the event. Inherent in this definition, and in the requirement to report SARs, is the need for the sponsor to evaluate the available evidence and make a judgment about the likelihood that the study agent actually caused the AE.
>
> The sponsor is responsible for making the causality judgment.

Unexpected Adverse Event: An AE is unexpected if it is not listed in the investigator's brochure or package insert (for marketed products) at the frequency, AND specificity, AND severity that has been observed.

NOTE:

- Such events should also be evaluated for possible reporting as unanticipated problems (UPs).
- Unexpected, as used in this definition, also refers to AEs or SARs that are mentioned in the investigator's brochure as occurring with a class of drugs/biologics, or as anticipated from the pharmacological properties of the study agent but are not specifically mentioned

as occurring with the particular study agent under investigation.

Serious and Unexpected Suspected Adverse Reaction (SUSAR): A SUSAR is an SAR (defined above) that is both serious and unexpected.

Protocol Deviation: Any change, divergence, or departure from the IRB-approved research protocol.

1. **Major Deviations:** Deviations from the IRB-approved protocol that have, or may have the potential to, negatively impact the rights, welfare, or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
2. **Minor Deviations:** Deviations that do not have the potential to negatively impact the rights, safety, or welfare of subjects or others, or the scientific integrity or validity of the study.

Non-compliance: Failure of investigator(s) to follow the applicable laws, regulations, or institutional policies governing the protection of human subjects in research, or the requirements or determinations of the IRB, whether intentional or not.

1. **Serious non-compliance:** Non-compliance, whether intentional or not, that results in harm or otherwise materially compromises the rights, welfare and/or safety of the subject. Non-compliance that materially affects the scientific integrity or validity of the research may be considered serious non-compliance, even if it does not result in direct harm to research subjects.
2. **Continuing non-compliance:** A pattern of recurring non-compliance that either has resulted, or, if continued, may result in harm to subjects or otherwise materially compromise the rights, welfare and/or safety of subjects, affect the scientific integrity of the study or validity of the results. The pattern may comprise repetition of the same non-compliant action(s), or different noncompliant events. Such non-compliance may be unintentional (e.g., due to lack of understanding, knowledge, or commitment), or intentional (e.g., due to deliberate choice to ignore or compromise the requirements of any applicable regulation, organizational policy, or determination of the IRB).

8.4.3 Classification of an Adverse Event

All AEs, including all solicited and those that may appear to have a non-study cause (see "Causality" below), will be documented (e.g., on the clinical chart/progress notes/clinical laboratory record), recorded (e.g., in the study-specified case report form [CRF]/research database), and reported (e.g., cumulatively from the research database, or according to protocol-specified expedited reporting mechanism) to the sponsor from the time informed consent is obtained through the timeframe specified below. At each contact with the subject, information regarding AEs will be elicited by open-ended questioning and examinations.

AEs and SAEs, including all solicited AEs will generally be recorded, assessed, and reported according to the timeframes outlined below.

Table 3. Standard Event Recording, Assessment, and Reporting Timeframes

Event type	**Record, assess, and report**
Related SAEs	End of subject participation in study, or if study personnel become aware thereafter
Unrelated SAEs	End of subject participation in study
Related non-seriousAEs of grade 1 to 3	End of subject participation in study
All other relatednon-serious AEs	End of subject participation in study
Unrelated non-serious AEs	End of subject participation in study

The Investigator will assess all AEs with respect to **seriousness** (according to SAE definition above), **severity** (intensity or grade, see below), and **causality** (relationship to study agent and relationship to participation in the research, see below).

If a diagnosis is clinically evident (or subsequently determined), the diagnosis rather than the individual signs and symptoms or lab abnormalities will be recorded as the AE.

The type of AE information (solicited symptoms: direct question of known possible side effects of the product; unsolicited: open-ended questioning such as "do you have any other symptoms"), duration of collected information, and format of information to be captured are summarized below:

IBSM (local reactogenicity related to the injection – solicited questions includes pain/tenderness, redness, swelling/induration, pruritus at the injection site):

- Collected from Day 0 to Day 3.
- Collected from the participant via solicited questions at each phone call/study visit (clinic follow up)

IBSM (systemic reactogenicity related to the injection – solicited questions includes rash, urticaria, pruritus, edema, headache, fever, chills, malaise, myalgia, arthralgia):

- Collected from Day 0 to start of Day 3.
- Collected from the participant via solicited questions at each phone call/study visit (clinicfollow up)

IBSM and Clinical Assessment (systemic symptoms related to parasitemia/malaria infection):

- Malaria clinical score collected from Day 4 to confirmation of successful antimalarial treatment (any open or ongoing symptomswill continue to be followed until conclusion)

Unsolicited AEs will be assessed throughout the study at each study visit (telephone and in-person follow-up) until the final study visit.

All AEs will be graded for severity and assessed for relationship to the study product. Reactions will be graded as described in this protocol.

All local and systemic reactions will be captured on the appropriate source documents and CRIMSON. AEs judged to be possibly, probably, or definitely related to the study product will be followed to adequate resolution. All concomitant medications will be collected through day 28 post-IBSM.

Solicited AEs will be captured by direct questioning during the study. Solicited AEs to be recorded as endpoints for this study are provided below.

Table 4. Solicited Adverse Events

IBSM (local)		
Injection pain/tenderness	Injection swelling/edema	Injection pruritus
Injection erythema/redness	Injection induration	
IBSM (systemic)		
Rash	Headache	Myalgia
Urticaria	Fever	Arthralgias
Generalized pruritus	Chills	Malaise
Generalized edema		
IBSM (malaria)		
Headache	Malaise (general discomfort/uneasiness)	Vomiting
Myalgia (muscle ache)	Chills/shivering/rigors	Abdominal discomfort
Arthralgia (joint ache)	Anorexia	Fever
Fatigue/lethargy	Nausea	Tachycardia
Laboratory Results (specified intervals)		
AST/ALT (increased ALT/AST)	Hgb (decreased Hgb)	WBC (leukopenia, leukocytosis)
Cr (increased Cr)	Platelet (thrombocytopenia, thrombocytosis)	ANC (decreased neutrophil count)
	ALC (decreased lymphocyte count)	

If a clinical diagnosis is associated with an abnormal laboratory finding, the relevant AE should be recorded as the diagnosis rather than the incidental laboratory finding (e.g., "viral hepatitis" should be recorded rather than "elevated transaminases").

Surgical procedures themselves are not AEs; they are therapeutic measures for conditions which may, or may not, be AEs.

All events, both expected/unexpected and related/unrelated will be recorded on a source document. Source documents will include progress notes, laboratory reports, consult notes, and phone call summaries. Source documents will be reviewed in a timely manner by the research team.

AEs that occur following enrollment of the participant are followed until the final outcome is known or until the end of the study follow-up period.

SAEs that have not resolved by the end of the follow-up period will be followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the participantis lost to follow-up), the reason a final outcome could not be obtained will be recorded by the investigator on the AE electronic record.

8.4.3.1 Severity of Event

The investigator will grade the severity of each AE (solicited and unsolicited), including laboratory and testing abnormalitiesand results, according to a modified version of the "Guidance for Industry:

Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" which can be found in Appendix C.

Events that are NOT gradable using the above specified table will be graded as described below.

Table 5. Definitions for Severity of AE Grading

Severity	Definition
Grade 1 (Mild)	No interference with activity, may use one dose of an over-the-counter medication
Grade 2 (Moderate)	Repeated use of non-narcotic pain reliever >24 hours or some interference with activity
Grade 3 (Severe)	Activities of daily living limited to <50% of baseline, medical evaluation/therapy required
Grade 4 (Potentially Life-Threatening)	Extreme limitation in activity, significant assistance required; immediate medical intervention or therapy required to prevent death
Grade 5	Death

8.4.3.1.1 Laboratory Value Assessment and Clinical Significance Criteria

Except as specified below, ALL abnormal lab values of grade 1 or above are REPORTABLE.

Grade 1 and 2 abnormal laboratory values are considered CLINICALLY SIGNIFICANT, and are to be recorded in the research database, and reported, if they meet ONE or more of the following criteria:

- result in a study agent dosage adjustment, interruption, or discontinuation
- are accompanied by clinically abnormal signs or symptoms that are likely related to thelaboratory abnormality (e.g., clinical jaundice)
- indicate a possible organ toxicity (e.g., elevated serum creatinine)
- result in additional/repeat testing or medical intervention (procedures/treatments)(e.g., ECG to evaluate arrhythmia potential with a high serum potassium; one or more ECGs to assess an elevated troponin level; potassium supplementation for hypokalemia)
- indicates possible over-dosage
- are considered clinically significant by the investigator or sponsor medical monitor (SMM)

8.4.3.2 Relationship to Study Intervention

Causality (likelihood that the event is caused by the study agent[s]) will be assessed by the PI considering the factors listed under the following categories:

Definitely Related

- reasonable temporal relationship
- follows a known response pattern
- clear evidence to suggest a causal relationship
- there is no alternative etiology

Probably Related

- reasonable temporal relationship
- follows a suspected response pattern (based on similar agents)
- no evidence of a more likely alternative etiology

Possibly Related

- reasonable temporal relationship
- little evidence for a more likely alternative etiology

Unlikely Related

- does not have a reasonable temporal relationship
  AND/OR
- there is good evidence for a more likely alternative etiology

Not Related

- does not have a temporal relationshipAND/OR
- definitely due to an alternative etiology

Note: Other factors (e.g., dechallenge, rechallenge, if applicable) should also be considered for each causality category when appropriate. Causality assessment is based on available information at the time of the assessment of the AE. The investigator may revise the causality assessment as additional information becomes available.

Causality assessment will be reviewed by the sponsor. The sponsor may make a separate and final determination on the "reasonable possibility" that the event was "related" (comprising definitely, probably, and possibly related) or "unrelated" (comprising unlikely and not related) to the study agent, in keeping with applicable (US FDA) guidance on sponsor IND safety reporting.

8.4.3.3 Expectedness

The investigator or delegate will be responsible for determining whether an adverse event (AE)is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.4.4 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presentingfor medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description,

time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

8.4.5 Adverse Event Reporting

8.4.5.1 Recording of Events

AEs will be promptly recorded in the research database, regardless of possible relationship to study interventions. If a diagnosis is clinically evident (or subsequently determined), the diagnosis rather than the individual signs and symptoms or lab abnormalities will be recorded as the AE. The investigator will review events regularly to ensure they have been captured correctly, and to perform assessment of events individually and cumulatively to assess possible safety trends.

8.4.5.2 Investigator Reporting Responsibilities

The PI and/or equally qualified designee will check daily for events that may require expedited reporting.

The PI and/or equally qualified designee will also monitor all accumulating data no less than weekly, or according to superseding NIH or NIAID policy, whichever is more frequent.

Data will be reviewed by the PI/designee on a regular basis for accuracy and completeness.

Data will be submitted to the sponsor in keeping with all applicable agreements and when requested, such as for periodic safety assessments, review of IND annual reports, review of IND safety reports, and preparation of final study reports.

The PI and/or other study designee will ensure prompt reporting to safety oversight bodies, regulatory entities, and stakeholders as specified below, and per any additional requirements or agreements.

8.4.5.2.1 Adverse Events

Unless otherwise specified above, AE data will be entered into the research database no less than every other week and will include all data through one week prior to database entry.

8.4.6 Serious Adverse Event Reporting

Unless otherwise specified above, all SAEs (regardless of relationship and whether or not they are also UPs) must be reported to the CSO as specified by the CSO (e.g., Research Electronic Data Capture [REDCap] system; use the safety expedited report form [SERF]/email if REDCap is not available). If the preferred/indicated mechanism for reporting is not available, the CSO/SMM should be contacted by telephone, fax, or other reasonable mechanism to avoid delays in reporting.

CSO CONTACT INFORMATION:

Clinical Safety Office5705 Industry Lane
Frederick, MD 21704
Phone 301-846-5301
Fax: 301-846-6224
Email: rchspsafety@mail.nih.gov
https://crimsonredcap.cc.nih.gov/redcap/index.php

Unless otherwise specified above, deaths and immediately life-threatening SAEs must be reported to the CSO promptly, and no later than the **first business day** following the day of study personnel awareness.

All other SAEs must be reported to the CSO no later than the **third business day** following the day of study personnel awareness.

If an individual subject experiences multiple SAEs in a closely timed/overlapping "cause-and-effect" (cascade) sequence, the PI, after careful evaluation, will report ONLY primary/precipitating event(s) individually. SAEs that are determined to be definitely secondary to other SAEs will be detailed in the narrative portion of the report of the relevant primary/precipitating SAE. A clinical rationale and findings to support such reporting should be part of the narrative.

For each SAE report, the research database entry MUST match the corresponding entries on the SAE report (e.g., start and stop dates, event type, relationship, and grade), and **must be updated if necessary** (e.g., if the SAE report was generated after the corresponding AE was entered in the research database).

Unless otherwise specified above, SAEs that have not resolved by the end of the per-protocol follow-up period for the subject are to be followed until final outcome is known (to the degree permitted by the IRB-approved informed consent form). If it is not possible to obtain a final outcome for an SAE (e.g., the subject is lost to follow-up), and to update the CSO, the last known status and the reason a final outcome could not be obtained will be recorded by the investigator on an SAE report update and the CRF.

8.4.6.1 Sponsor's Reporting Responsibilities

Events reported to the sponsor will be promptly evaluated and will be reported as required according to FDA IND safety reporting guidance and regulations. IND safety reports will be sent to other investigators conducting research under the same IND and will be shared with other stakeholders according to applicable agreements (e.g., Cooperative and Development Research Agreements [CRADAs] and Clinical Trial Agreements [CTAs]).

The sponsor will also submit an IND annual report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

All UPs will be evaluated by the sponsor, and a summary of the event, and any necessary (corrective/preventative) actions, will be distributed to investigators conducting research under the same IND as may be relevant and appropriate.

8.4.7 NIH Intramural IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem or is new

information that might affect the willingness of subjects on the NIH study to enroll or remain in the study will need to be reported to the NIH Intramural IRB.

8.4.8 Events of Special Interest

Not applicable.

8.4.9 Reporting of Pregnancy

Unless otherwise specified above, all pregnancies will be reported (by REDCap, or by email and SERF if REDCap is not available) to the CSO no later than the first business day following the day of study personnel awareness.

Pregnancy outcome data (e.g., delivery outcome, spontaneous or elective termination of the pregnancy) will be reported to the CSO no later than the third business day following the day of study personnel awareness (by REDCap, or by email and SERF if REDCap is not available).

Pregnancy itself is not an AE. Events that meet AE or SAE criteria in relation to pregnancy, delivery, or the conceptus/neonate (see Section 8.4.2) are reportable (by REDCap, or by email and SERF if REDCap is not available).

In the event of pregnancy in a study subject exposed to study agent, the following actions will be taken, with the goal of ensuring maternal and fetal well-being:

- Withdraw from the study but continue in follow-up for safety.
- Report to NIH IRB and SMM as an informational item no later than 1 business day after study site awareness.
- Advise research participant to notify the obstetrician of study exposure and provide contact information for the obstetrician to contact the study PI, should this be required, and with the participant's consent.
- Administration of chloroquine daily for 3 days if pregnancy occurs after IBSM.
- Continue to follow for safety for the duration of the pregnancy, and for a period of up to 3 months following delivery for assessment of the neonate.

8.5 UNANTICIPATED PROBLEMS

8.5.1 Definition of Unanticipated Problems (UP)

Unanticipated Problem (UP): A UP is any event, incident, experience, or outcome that is:

1. **unexpected** in terms of nature, severity, or frequency in relation to:
    a. the research (including but not limited to risks) as described in the IRB-approved research protocol and informed consent document, investigator's brochure, or other study documents; **and**
    b. the characteristics of the subject population being studied; **and is**
2. possibly, probably, or definitely related (see "Causality" below) to participation in the research; **and**
3. suggests the research places subjects or others at a **greater risk** of harm (including physical, psychological, economic, or social harm) than was previously known or recognized, per the documents currently approved by the IRB.
    NOTE:

- Per the sponsor, an SAE always meets this "greater risk" criterion.

- An incident, experience, or outcome that meets the definition of a UP generally will warrant consideration of changes to the protocol or informed consent form, or to study procedures (e.g., the manual of procedures for the study), in order to protect the safety, welfare, or rights of participants or others. Some UPs may warrant a corrective and preventive action plan at the discretion of the sponsor or other oversight entities.

Unanticipated Problem that is not an Adverse Event (UPnonAE): A UPnonAE belongs to a subset of UPs that:

- meets the definition of a UP, AND
- does NOT fit the definition of an AE or an SAE
  NOTE: Examples of UPnonAEs include, but are not limited to:
  - a breach of confidentiality
  - prolonged shedding of a vaccine virus beyond the anticipated timeline
  - unexpectedly large number of pregnancies on a study
  - subject departure from an isolation unit prior to meeting all discharge criteria
  - accidental destruction of study records
  - unaccounted-for study agent
  - overdosage, underdosage, or other significant error in administration or use of study agent or intervention, even if there is no AE/SAE
  - development of an actual or possible concern for study agent purity, sterility, potency, dosage, etc.
    NOTE: A decision to temporarily quarantine, or to permanently not use all or part of study agent supply due to an unexpected finding or event (e.g., particulate, cloudiness, temperature excursion), even if there is no known or proven issue (i.e., out of an "abundance of caution"), is considered a UPnonAE.

8.5.2 Unanticipated Problem Reporting

The investigator will report UPs to the NIH IRB as per Policy 801.

Unless otherwise specified above, UPs (as defined in this protocol, or as defined by the IRB of record, whichever definition is more conservative) that are also AEs or SAEs, must be reported to the CSO (by REDCap, or by email and SERF if REDCap is not available) no later than when they are due to be reported to the IRB.

UPnonAEs are NOT reported to the CSO but must be reported to the Clinical Trials Management (CTM) group and the IRB according to their requirements and preferred methods. If the UPnonAE raises a significant potential subject safety concern, the SMM should be consulted by email or phone no later than when reports are made to the IRB and/or CTM

8.6 ADDITIONAL REPORTING REQUIREMENTS

8.6.1 Safety Oversight and Reporting

A safety review and communication plan (SRCP) is required for this protocol. The SRCP is an internal communications document between the PI and the CSO, as sponsor representative, which delineates key safety oversight responsibilities of the PI, the CSO, and other stakeholders. The SRCP includes a plan for conducting periodic safety surveillance assessments by the CSO.

A SMM, representing the sponsor, has been appointed for oversight of safety in this clinical study. The SMM will be responsible for performing safety assessments as outlined in the SRCP.

As this study has a limited sample size of two participants and all interventions have been previously well characterized, safety oversight will be limited to the CSO and SMM.

8.6.2 Reporting to the NIH IRB

Non-compliance and other reportable events will be reported to the NIH IRB according toHuman Research Protections Program (HRPP) Policy 801.

8.6.3 Reporting to the NIAIAD Clinical Director

The PI will report UPs, major protocol deviations, and deaths to the NIAID clinical director according to institutional timelines.

8.7 HALTING RULES FOR THE PROTOCOL

"Halting" is discontinuation of study intervention/treatment/dosing (agent/placebo/procedure, etc.) for all subjects in a study and suspension of enrollment until a decision is made to either resume or permanently discontinue such activity. Subjects continue to be followed for safety during a halt.

The halting rules are:

- One or more participants experience an SAE that is determined to be possibly, probably or definitely related to IBSM or
- One or more participants experience a hypersensitivity reaction that is probably or definitely related to IBSM or
- Any severe clinical illness occurs that is not explained by a diagnosis that is unrelated or unlikely related to study product or
- Two participants experience a solicited or unsolicited Grade 3 event (excluding laboratory abnormalities) or a Grade 4 event that is determined to be possibly, probably or definitely related to the study product or
- Any safety issue that the PI or the CSO determines should halt the study.

In addition, the FDA or any regulatory body having oversight authority may halt the study at any time.

8.7.1 Reporting a study halt

If a halting criterion is met, a description of the AE(s) or safety issue must be reported by the PI, within 1 business day to the CSO and the IRB according to their requirements.

8.7.2 Resumption of a halted study

The CSO, in collaboration with the PI, will determine if study activities, including enrollment, study agent administration, and/or other study interventions, may be resumed and any additional modifications or requirements that may apply.

The CSO or sponsor designee will notify the PI of the decision. The PI will notify the IRB of the decision according to the IRB's process.

8.7.3 Discontinuation of study agent/intervention

Subjects who do not resume study agent/study intervention will continue to be followed for protocol-specified safety assessments or as clinically indicated, whichever is more conservative.

9 STATISTICAL CONSIDERATIONS

This study involves collecting blood from donors experimentally infected with malaria to generate blood stage parasite banks. As such, no formal statistical analysis plan will be generated.

The safety analysis dataset will include all participants who receive the malaria inoculum. This population will be used to analyze all safety data as well as demographic and baseline data.

All measured variables and derived values will be listed. As the sample size for this study is two participants, with an accrual ceiling of 50 participants, individual data will be presented in lieu of descriptive statistics. Categorical data will be presented using N and % (using the number of participants without missing data in the calculation).

9.1 SAFETY ANALYSES

The overall number and percentage of participants with at least one AE (and SAE) will be tabulated over the entire study period. All AE data will be summarized by Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term, and maximum severity. Vital signs, routine safety laboratory data, and ECG parameters will be summarized descriptively by time point. Both absolute values and change from baseline (inoculation) will be presented.

9.2 BASELINE DESCRIPTIVE STATISTICS

Demographic data, including the total number of observations, will be presented. The participant disposition will be summarized. Study completion, study withdrawals, exclusions, and violations will be summarized and the reasons for withdrawal, exclusions, and violations will be listed. Medical history, current medical conditions, previous and concomitant medications, results of laboratory screening tests, drug tests and any other relevant baseline information will be listed by participant.

10 REGULATORY AND OPERATIONAL CONSIDERATIONS

10.1 INFORMED CONSENT PROCESS

10.1.1 Consent/Assent Procedures and Documentation

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research participant. It is an ongoing conversation between the human research participant and the researchers, which begins before consent is given and continues until the end of the participant's involvement in the research. Discussions about the research will provide essential information about the study and include purpose, duration, experimental procedures, alternatives, risks, and benefits. Coercion and undue influence will be minimized by informing participants that their decision to join the study will not affect any medical care they are currently receiving at the NIH, or their eligibility to participate in other research studies at the NIH. Participants will be given as much time as they need to read the consent form and ask questions of the investigators. Participants will also be given time to discuss their participation with family members, friends, and other healthcare providers. Informed consent will be obtained in person in a private setting by a study team member authorized to obtain consent.

An IRB approved telephone pre-screening will first be conducted to determine if the subject appears to meet initial eligibility criteria. A waiver of consent is not required for telephone pre-screening per 45CFR46.116(g) §46.116. In this telephone pre-screening, general demographic information is requested as well as a series of yes/no medical questions and a list of current

medications; this information will be used to determine the general health and potential eligibility of the subject. If the subject does not wish to complete the telephone prescreen, he/she may elect to complete the informed consent process and in-person screening process without first completing a telephone prescreen, as indicated below.

If the volunteer meets initial eligibility criteria or opts out of the telephone prescreen, then they will be scheduled to appear in person at the National Institutes of Health (NIH) Clinical Center (CC) for evaluation by a healthcare provider.

Participants will be fully informed of the nature of the study, the properties and adverse effects of the inoculum and potential rescue treatments and all relevant aspects of study procedures in the 'Participant Information Sheet'. The Participant Information Sheet and informed consent form describe in detail the study agents, study procedures, and risks.

The participants will sign the informed consent document prior to undergoing any research procedures. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The researcher will document the signing of the consent form in the participant's medical record. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 Participation of Subjects Who Are/Become Decisionally Impaired

Participants who become decisionally impaired during their participation in this study, and can therefore no longer provide their ongoing informed consent, will be withdrawn from the study.

10.1.3 Considerations for Consent of NIH Staff

Even though NIH staff members are not targeted, if they are incidentally enrolled, informed consent will be obtained as detailed above with following additional protections:

Consent from staff members will be obtained by an individual independent of the individual's team whenever possible. Otherwise, the consent procedure will be independently monitored as described in Policy 404 to minimize the risk of undue pressure on the staff member.

10.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. If the study is prematurely terminated or suspended, the PI will promptly inform study participants, the IRB, the IND sponsor, and regulatory authorities, and will provide the reason(s) for the termination or suspension. Study participants will be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

The study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the IND sponsor, IRB, and/or FDA.

10.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB, and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, institutional policies, or sponsor requirements.

All records will be kept confidential to the extent provided by federal, state and local law. The study monitors and other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records. Records will be kept locked and all computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by IRB, the FDA, the NIH, the Office of Human Research Protections (OHRP), or the sponsor's designee.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.4 FUTURE USE OF STORED SPECIMENS AND DATA

Intended Use: Samples and data collected under this protocol will be used to study malaria and related diseases and possible ARs to IBSM. Genetic testing will not be performed.

Storage: Access to stored research samples will be limited using either a locked room or a locked freezer. Samples will be stored at the LMIV in Bethesda, MD or at LMIV's designated repository, Thermo Scientific, Rockville, MD. Samples and data will be stored using codes assigned by the investigators or their designees. Data will be kept in password-protected computers. Only investigators or their designees will have access to the samples and data.

Tracking: Samples will be tracked using a sample-tracking software program, e.g., Freezerworks.

Disposition: In the future, other investigators (both at the NIH and outside) may wish to study these samples and/or data. If the planned research falls within the category of "human subjects research" on the part of the NIH researchers, IRB review and approval will be obtained. This includes the NIH researchers sending out coded and linked specimens or data and getting results that they can link back to their participants.

Reporting the Loss or Destruction of Samples/Specimens/Data to the IRB: Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) that meets the definition of a reportable event will be reported to the NIH IRB according to Policy 801.

Consent to allow long-term storage of study samples is a part of the inclusion criteria for this study. However, if a participant decides following enrollment not to have their samples stored, then the PI or designee will destroy all known remaining samples and report this destruction to the participant and the NIH IRB. This decision will not affect the participant's continued participation in this protocol, or any other protocols supported by the NIH.

10.5 SAFETY OVERSIGHT

Safety oversight for this study is described in Section 8.6.1: Safety Oversight and reporting.

10.6 CLINICAL MONITORING

According to the ICH E6(R2) GCP guidelines, section 5.18, and FDA 21 CFR 312.50, clinical protocols are required to be adequately monitored by the study sponsor. This study monitoring will be conducted according to the "NIAID Intramural Clinical Monitoring Guidelines." Monitors under contract to the NIAID/OCRPRO will visit the clinical research site to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the consent process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points and prompt reporting of all SAEs; 3) to compare abstracted information with individual subjects' records and source documents (subjects' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (OHRP, FDA) and applicable guidelines (ICH GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms) and pertinent hospital data abstracts or clinical records readily available for inspection by the IRB, FDA, the site monitors, and the NIAID staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the site and study principal investigators and study staff prior to enrollment. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status, and regulatory obligations.

10.7 QUALITY ASSURANCE AND QUALITY CONTROL

During the study, the PI and study team will be responsible for implementing a quality management plan. Additionally, the study team will be responsible for completing and submitting

a summary report on the quality plan to the NIAID clinical director or designee at least annually as detailed in the quality management plan. A courtesy copy will also be sent to CTM.

10.8 DATA HANDLING AND RECORD KEEPING

10.8.1 Data Collection and Management Responsibilities

Study data will be maintained in CRIMSON and/or REDCap and collected directly from participants during study visits and telephone calls or will be abstracted from participants' medical records. Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary to confirm the data abstracted for this study. Data entry into CRIMSON will be performed by authorized individuals. The investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner. Study data, including cumulative subject accrual numbers, should be generated via the chosen data capture method and submitted to the IRB as needed.

10.8.2 Study Records Retention

The investigator is responsible for retaining all essential documents listed in the ICH GCP guidelines. Study records will be maintained by the PI according to the timelines specified in 21 CFR 312.62 or a minimum of 7 years, and in compliance with institutional, IRB, state, and federal medical records retention requirements, whichever is longest. All stored records will be kept confidential to the extent required by federal, state, and local law.

Should the investigator wish to assign the study records to another party and/or move them to another location, the investigator will provide written notification of such intent to OCRPRO/NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location. NIAID will be notified in writing and written OCRPRO/NIAID permission shall be obtained by the site prior to destruction or relocation of research records.

10.9 PROTOCOL DEVIATIONS

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations to the NIH IRB as per Policy 801. All deviations must be addressed in study source documents and reported to CTM. The investigator isresponsible for knowing and adhering to the reviewing IRB requirements.

10.9.1 NIH Definition of Protocol Deviation

The definition of a protocol deviation is provided in Section 8.4.2.1.

10.10 PUBLICATION AND DATA SHARING POLICY

10.10.1.1 Human Data Sharing Plan

We will comply with NIH policies on data access, sharing, and dissemination, and clinical trials registration, as applicable. Human data generated in this study will be shared for future research as follows:

- De-identified data in an NIH-funded or approved public repository.
- De-identified data in another public repository.
- Identified data in the Biomedical Translational Research Information System (automatic for activities in the CC).
- De-identified or identified data with approved outside collaborators under appropriate agreements.

- De-identified data in publications and/or public presentations.

Data will be shared at the time of or shortly after publication.

10.10.1.2 Genomic Data Sharing Plan

Not applicable.

10.11 COLLABORATIVE AGREEMENTS

Amendment #1 to the Research Collaboration Agreement (RCA) dated 20 December 2017 between QIMRB and LMIV specifies the transfer of up to 6 vials of blood stage *P. vivax* cell bank (HMPBS02-Pv) isolates from QIMRB to LMIV.

10.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with NIAID has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11 ABBREVIATIONS

AE	adverse event
ALC	absolute lymphocyte count
ALT	alanine transaminase
ANC	absolute neutrophil count
AR	adverse reaction
AST	aspartate transaminase
β-hCG	human choriogonadotropin
CBC	complete blood count
CC	Clinical Center
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CHMI	controlled human malaria infection
CK	creatine kinase
CMV	cytomegalovirus
CRF	case report form
CRIMSON	Clinical Research Information Management System of theNIAID
CSO	Clinical Safety Office
CTM	Clinical Trials Management
CYP	cytochrome p450 isozyme
DMFA	direct membrane feeding assay
DSF	direct skin feed
DTM	Department of Transfusion Medicine
EBV	Epstein-Barr Virus
ECG	electrocardiogram
ELISA	enzyme-linked immunosorbent assay
EOS	End of Study
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HHV	human herpes virus
HIV	human immunodeficiency virus
HMP	human malaria parasite
HMPBS-*Pv*	human malaria parasite blood stage *Plasmodium vivax*
IBSM	induced blood stage malaria
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ID	identification
IND	Investigational New Drug application
IRB	institutional review board

IV	intravenous(ly)
LDH	lactate dehydrogenase
LFT	liver function test
LLN	lower limit of normal
LMIV	Laboratory of Immunology and Vaccinology
MCB	master cell bank
MedDRA	Medical Dictionary for Regulatory Activities
NAT	nucleic acid test
NHANES	National Health and Nutrition Examination Survey
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
OHRP	Office of Human Research Protections
PHQ-2	Patient Health Questionnaire-2
PI	Principal Investigator
QIMRB	Queensland Institute of Medical Research, Berghofer
qPCR	quantitative polymerase chain reaction
qRT-PCR	quantitative reverse transcriptase polymerase chain reaction
QTc	corrected QT interval
RBC	red blood cell
REDCap	Research Electronic Data Capture
Rh	Rhesus
SAE	serious adverse event
SAR	suspected adverse reaction
SERF	safety expedited report form
SMM	sponsor medical monitor
SOP	standard operating procedure
SRCP	safety review and communications plan
SUSAR	suspected unexpected serious adverse reaction
ULN	upper limit of normal
UP	unanticipated problem
UPnonAE	unanticipated problem that is not an adverse event
US	United States
WHO	World Health Organization

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Appendix A: Cardiovascular Risk Assessment

As part of the eligibility determination, participants will be screened for cardiac risk based on the NHANES I study criteria (Gaziano, Young et al. 2008), and screening electrocardiogram. Results will be documented in the participant source documentation and Cardiac Risk Assessment CRF.

NHANES cardiovascular risk assessment includes the following assessments:

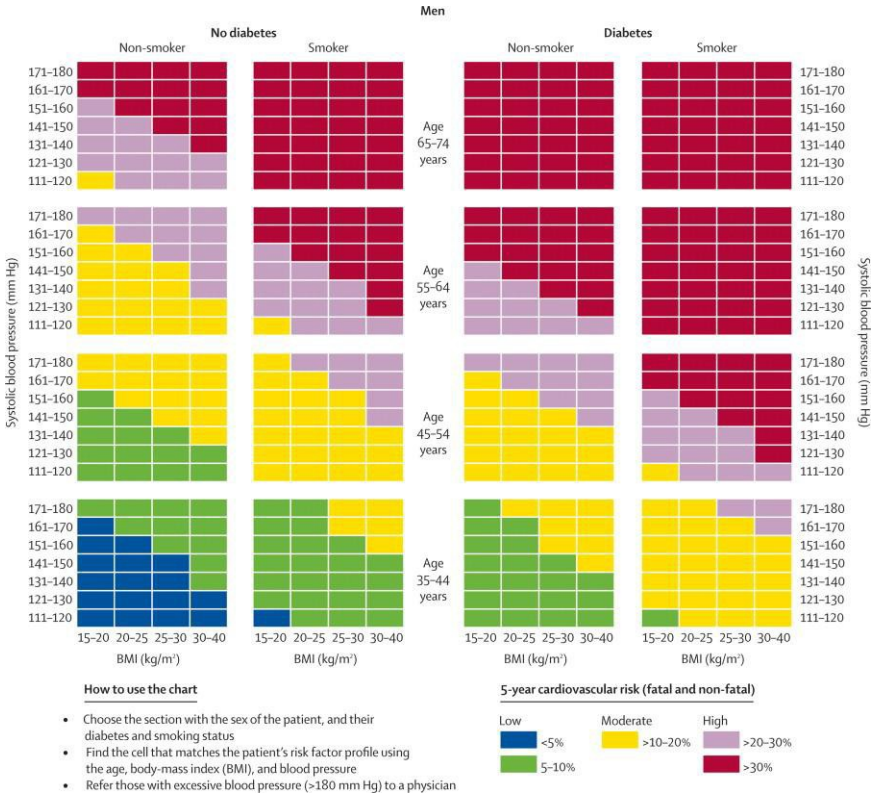
- Evaluation of **risk factors:** Calculated BMI [weight (kg)/height (m2)], measured Systolic
- Blood Pressure, smoking status and known diabetes status as reported by the participant on review of medical history
- Evaluation of **5-year cardiovascular risk** using: Low, Moderate, High

Note: participants under the age of 35 are considered low risk by the NHANES I risk assessment

Only participants classified as low risk by the NHANES I criteria (green and blue categories below) who have a non-significant ECG, as determined by the study cardiologist, are eligible for the study.

NHANES I Classification for Women

NHANES I Classification for Men



Appendix B: Clinical Score for Malaria

[31]

Participant ID:				
Study Day:	Date:			
Symptoms	**Clinical Score**			
	Absent(0)	Mild(1)	Moderate(2)	Severe(3)
Headache				
Myalgia (muscle aches)				
Arthralgia (joint aches)				
Fatigue/lethargy				
Malaise (general discomfort/uneasiness)				
Chills/Shivering/Rigors				
Sweating/hot spells				
Anorexia				
Nausea				
Vomiting				
Abdominal discomfort				
Fever				
Tachycardia				
Hypotension				
Total Score:				

Maximum Score = 42 Threshold for treatment > 6

Appendix C: Toxicity Grading Scales

These tables are modified versions of the FDA Toxicity Grading Scale for healthy adult and adolescent participants enrolled in Preventive Vaccine Clinical Trials to be used to grade adverse events.

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Erythema/Redness *	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative
Induration/Swelling **	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Pruritus	No interference with activity	Requiring PO or topical treatment > 24 hours or IV medications or steroids for ≤24 hours	Requiring IV medication or steroids for >24 hours	ER visit or hospitalization

* In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
** Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement

Vital Signs * /Systemic adverse	**Mild (Grade 1)**	**Moderate(Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening**
Fever (°C) **	38.0 – 38.4	38.5 – 38.9	39.0 – 40	> 40
Tachycardia – beats per minute	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia – beats per minute***	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Rash	Localized rash with no medical intervention indicated	Localized rash with medical intervention indicated	Generalized rash with medical intervention indicated	ER visit or hospitalization
Abdominal Pain	No or minimal interference with usual activities	Greater than minimal interference with usual activities	Inability to perform usual activities	ER visit or hospitalization
Anorexia	Transient (< 24 hours) or intermittent anorexia with no or minimal interference with oral intake	Persistent anorexia resulting in decreased oral intake for 24 – 48 hours	Persistent anorexia resulting in minimal oral intake for > 48 hours OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Back Pain	No or minimal interference with usual activities	Greater than minimal interference with usual activities	Inability to perform usual activities	ER visit or hospitalization
Chills/Sweats	No or minimal interference with usual activities	Greater than minimal interference with usual activities	Inability to perform usual activities	ER visit or hospitalization
Generalized Pruritus	Itching causing no or minimal interference with usual activities	Greater than minimal interference with usual activities	Inability to perform usual activities	ER visit or hospitalization

Generalized Edema	Localized edema causing no or minimal interference with usual activities	Greater than minimal interference with usual activities	Inability to perform usual activities	ER visit or hospitalization
Vital Signs * /Systemic adverse events	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Dizziness	Dizziness causing minimal interference and above baseline	Dizziness causing greater than minimal interference with usual social & functional activities	Dizziness causing inability to perform usual social & functional activities	Disabling dizziness causing inabilityto perform basicself-care functions
Change in Vision	Change in vision causing minimal interference and above baseline	Difficulty seeing causing greater than minimal interference with usual social & functional activities	Difficulty seeing (diplopia, blurriness) causing inability to perform usual social & functional activities	Disabling change in vision(visual field loss, blindness) causing inabilityto perform basicself-care functions
Insomnia or Difficulty Sleeping	Difficulty sleeping causing minimal interference and above baseline	Difficulty sleeping causing greater than minimal interference with usual social & functional activities	Difficulty sleeping causing inability to perform usual social & functional activities	Disabling insomnia causing inabilityto perform basicself-care functions
Photosensitivity	Sensitivity to light causing no or minimal interference with usual activities	Greater than minimal interference with usual activities secondary to sensitivity to light	Inability to perform usual activities secondary to sensitivity of light	ER visit or hospitalization
Nausea/vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensiveshock
Diarrhea	2 – 3 loose stools or < 400 gms/24 hours	4 – 5 stools or 400 – 800 gms/24 hours	6 or more watery stools or > 800gms/24 hours or requires outpatient IV hydration	ER visit or hospitalization

Vital Signs * /Systemic	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening**
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue/Malaise	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Arthralgia	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Urticaria	No interference with activity	Requiring PO or topical treatment > 24 hours or IV medications or steroids for ≤24 hours	Requiring IV medication or steroids for >24 hours	ER visit or hospitalization
Tinnitus	Ringing in your ears causing no or minimal interferencewith usual activities	Greater than minimal interference with usual activities secondary to ringing in your ears	Inability to perform usual activities secondary to ringing in your ears.	ER visit or hospitalization
Peripheral Neuropathy	Tingling in your hands or feet causingno or minimal interference with usual activities	Tingling in your hands or feet causing greater than minimal interference with usual activities	Inability to perform usual activities due to tingling or pain in your hands or feet	ER visit or hospitalization

Chest Pain (non-musculoskeletal)	Transient (< 24 hours) or intermittent chest pain with no or minimal interference	Persistent chest pain resulting in greater than minimal interference with usual activities	Persistent chest pain resulting in inability to perform usual activities secondary to chest pain	ER visit or hospitalization

* Participant should be at rest for all vital sign measurements.
** Oral temperature; no recent hot or cold beverages or smoking.
*** When resting heart rate is between 60 – 100 beats per minute. Use clinical judgment when characterizing bradycardia among some healthy participant populations, for example, conditioned athletes.

Serum*	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)****
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires
Liver Function Tests – ALT, AST	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN

Hematology *	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Hemoglobin	10.0 -11.0	8.5 - 9.9	7.5 – 8.4	< 7.5
Hemoglobin (Male)	12.0 – 13.0	10.0 - 11.9	8.0 - 9.9	< 8.0
WBC Increase - cell/mm^3	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
WBC Decrease - cell/mm^3	2,500-3500	1,500 – 2499	1,000 – 1,499	< 1,000
Lymphocytes	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils	1000-1499	750-999	500 – 749	< 500
Platelets Decreased - cell/mm^3	125000-140000	100000-124000	25,000 – 99000	< 25,000

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normalparameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.
** The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities
as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within a grade 3 parameter (125-127 mE/L) should be recorded as a grade 4 hyponatremia event if the participant had a new seizure associated with the low sodium value.
***ULN is the upper limit of the normal range.
^Values adjusted according to the NIH clinical laboratory normal values

Urine*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Protein	Trace	1 +	2+	Hospitalization or Dialysis
Glucose	Trace	1 +	2+	Hospitalization for Hyperglycemia
Blood (microscopic) – red blood cells per microliter (rbc/µL)	17 – 30	31– 60	> 60 and/or gross blood	Hospitalization or packed red blood cells (PRBC) transfusion

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.
^Values adjusted according to the NIH clinical laboratory normal values

Appendix D: Total Whole Blood Volume for Collection During Study

Procedure	**Sample**	**Volume per sample (mL)**	**No. samples per participant for the first 30 days**	**Total volume per participant (mL) for the first 30 days**
Laboratory Safety Assessment	Hematology	2	4	8
		4	1	4
	Biochemistry (including LFT)	5	5	25
	Safety Serum storage	5	1	5
Cannulation	Discard	2	8 (+2 if required)	16 (+4 if required)
Malaria Monitoring	Malaria qPCR (18S)	2	15 (+5 if required)	30 (+10 if required)
	Microscopy	2	0 (as needed)	0
	Parasite life-cycle stage qRT-PCR	2	5 (+3 if required)	10 (+6 if required)
HMP bank blood borne infection testing		55	Up to 1	55
Study volume (mL) not including banking volume				153 (+20 if required)
Blood Collection for Banking		200	1	200 (+ discretionary volume)
Total study volume (mL)				353 (+ discretionary volume)

Appendix E: Symptoms and Signs of Malaria

Following administration of the intravenous malaria challenge agent and during the post-challenge period, the following signs and symptoms of malaria will be monitored:

Signs of Malaria

- Fever (≥38°C)
- Chills/Shivering/Rigors
- Tachycardia
- Hypotension

Symptoms of Malaria

- Headache
- Myalgia (muscle ache)
- Arthralgia (joint ache)
- Fatigue/lethargy
- Malaise (general discomfort/uneasiness)
- Sweating/hot spells
- Anorexia
- Nausea
- Vomiting
- Abdominal discomfort

Appendix F: The Patient Health Questionnaire-2 (PHQ-2)

The PHQ-2 inquires about the frequency of depressed mood and anhedonia over the past two weeks. The PHQ-2 includes the first two items of the PHQ-9. The purpose of the PHQ-2 is not to establish a final diagnosis or to monitor depression severity, but rather to screen for depression in a "first step" approach.

The Patient Health Questionnaire-2 (PHQ-2)

Patient Name _____ Date of Visit _____

Over the past 2 weeks, how often have you been bothered by any of the following problems?	**Not At all**	**Several Days**	**More Than Half the Days**	**Nearly Every Day**
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed or hopeless	0	1	2	3

Scoring

A PHQ-2 score ranges from 0-6. A PHQ-2 score of 3 or higher will be the cut off point for exclusion for screening purposes.