STATISTICAL ANALYSIS PLAN (SAP)

BRIDGING TRIAL TO EVALUATE THE INFECTIVITY EQUIVALENCE OF CURRENT AND NEW LOTS OF PLASMODIUM FALCIPARUM STRAIN NF54 (CLONE 3D7) WITHIN THE WRAIR CONTROLLED HUMAN MALARIA INFECTION (CHMI) MODEL

NCT# 03882528

Document Date: 30SEP2019

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WRAIR #2572

Bridging Trial to Evaluate the Infectivity Equivalence of Current and New Lots of Plasmodium falciparum Strain 3D7 within the WRAIR Controlled Human Malaria Infection (CHMI) Model

Short Title: Infectivity and Diagnostics Equivalence Bridging Study

STATISTICAL ANALYSIS PLAN

Version 1.0

September 30, 2019

SIGNATURE PAGE

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Version	Description of Changes
0.1	Initial Version
1.0	Final Approved Version before database lock

Table of Contents

List of Abbreviations and Definitions of Terms

Abbreviation	Explanation
β-HCG	Beta-human chorionic gonadotropin
AE	Adverse event
AI	Associate investigator
AL	Artemether/lumefantrine (Coartem)
ALT	Alanine aminotransferase
AP	Atovaquone/proguanil (Malarone)
AR	Army Regulation
AST	Aspartate aminotransferase
CBC	Complete blood count
CHMI	Controlled human malaria infection
CQ	Chloroquine
CRF	Case Report Form
CTC	Clinical Trials Center
DMP	Data management plan
DoD	Department of Defense
ECG	Electrocardiogram
FDA	United States Food and Drug Administration
GCP	Good clinical practice
HBV	Hepatitis B virus
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIPAA	Health Insurance Portability Accountability Act
HIV	Human immunodeficiency virus
HSPB	Human Subjects Protection Branch
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
MVB	Malaria Vaccine Branch
NHANES I	National Health and Nutrition Examination Survey I
NMRC	Naval Medical Research Command
ORA	Office of Regulated Activities (USAMRMC)
ORP HRPO	Office of Research Protections, Human Research Protection Office
PCR	Polymerase chain reaction
PCT	Parasite clearance time
PI	Principal investigator
PO	By mouth
PSSB	Product Safety Surveillance Branch
SAE	Serious adverse event
SAP	Statistical analysis plan

Abbreviation	Explanation
SAS®	Statistical Analysis Software
SD	Standard deviation
SEM	Standard error of the mean
SID	Screening/Study Identification
SOP	Standard operating procedure
SSP	Study specific procedure
TMF	Trial master file
TSG	The Surgeon General
UAE	Unexpected Adverse Event
UPIRTSOs	Unanticipated Problem Involving Risk to Subjects or Others
USAMRMC	United States Army Medical Research and Materiel Command
WBC	White blood cell
WHO	World Health Organization
WRAIR	Walter Reed Army Institute of Research
WRNMMC	Walter Reed National Military Medical Center

1 INTRODUCTION

1.1 Background

Since the introduction of the Trager-Jensen method of culturing falciparum malaria in 1976, 3D7 and its parental line, NF54, have been used in experimental human malaria infections. From 1989-1995, clinical trials at WRAIR used research grade parasites grown from working stocks used in the laboratory; this included infectivity trials that established the 5-bite challenge model used in Controlled Human Malaria Infection (CHMI) today (Rickman et al-1990). The first isolate of 3D7 to be propagated under cGMP conditions and cryopreserved specifically for use in humans was taken from a blood collection of a single control subject in the NYVAC-Pf7 trial (Ockenhouse et al-1998) who had been infected with research grade 3D7 from the bite of an Anopheles stephensi mosquito. This cryopreserved lot (0285) was first used to test RTS,S in immunologically naïve adults (Stoute et al-1997) and has been used in 53 CHMIs through mid-2018. In early 2010s, it was recognized that Lot 0285 was approaching 20 years in cryopreservation and CHMI demand would exceed the supply of remaining vials; as of April 2018, 13 vials of 0285 remain, 9 of which are reserved for known, near-future CHMI trials.

In 2012, to refresh the cryostock supply, blood draws from 5 control subjects in WRAIR #1853 (S-11-21; CelTOS trial) were cultured to generate a new cGMP lot. Control subject cultures were expanded and each line was screened for mosquito infectivity. Data from 106 mosquito feeds determined which line delivered the most robust and reliable infections in mosquito salivary glands; this line was propagated under cGMP conditions in 2014, tested for sterility and mycoplasma, and submitted to drug sensitivity and whole genome sequencing analyses which showed the new lot of 3D7 (1887) has similar characteristics to lot 0285 and is identifiable as 3D7. Comparative mosquito infections indicated that Lot 1887 was as infectious to mosquitoes as Lot 0285. Lot 1887 was released for human use in 2017 and has been in cryopreservation since lot release. This lot (1887) is awaiting comparative infectivity bridging studies with the current lot, 0285.

1.2 Purpose of the Statistical Analysis Plan

This statistical analysis plan (SAP) provides a more technical and detailed elaboration of the statistical analyses performed to support the objectives of this study as outlined and/or specified in the final study protocol. Specifications of tables, figures, and data listings are contained in a separate document. It should be noted that this document is not meant to represent all the measures assessed or analyses performed in the study as ad hoc and post hoc analyses may be conducted to support regulatory submissions and/or future studies.

Results of the proposed analyses will become the basis of the clinical study report (CSR) for this protocol. This SAP describes the analysis of the primary and secondary endpoints mentioned in the protocol to address the primary and secondary objectives respectively. This will meet requirements for the completion of an initial CSR.

The details outlined in this statistical analysis plan and supplemental documents were produced based off protocol version 3.0 dated January 7, 2019 and the annotated case report forms dated August 8, 2019. Any modifications to these documents would require a review of the statistical analysis plan prior to the start of the final study analysis.

2 STUDY OBJECTIVES

2.1 Primary Objectives

- To characterize the infectivity of the new lot of Plasmodium falciparum strain 3D7 parasites within the standard WRAIR CHMI model

2.2 Secondary Objectives

- To assess the safety of the new lot of *Plasmodium falciparum* parasites
- To assess the kinetics of detecting parasitemia and parasite clearance by quantitative polymerase chain reaction (qPCR) as compared to blood smear
- To obtain plasma samples to restore the testing control pool for malaria serology testing and to acquire samples for future malaria research

3 STUDY ENDPOINTS AND OUTCOMES

3.1 Primary Endpoints

Infective Efficacy:

- Proportion of challenged subjects exposed to the new lot of *Plasmodium falciparum* strain 3D7 developing parasitemia (where parasitemia is defined as the presence of 2 unambiguous parasites on a single smear)

3.2 Secondary Endpoints

Diagnostic Efficacy:

- Time to parasitemia by blood smear after the *P falciparum* challenge
- Time to parasitemia by qPCR after the *P falciparum* challenge
- Quantification of parasite clearance time (PCT) by blood smear after initiation of antimalarial treatment
- Quantification of parasite clearance time (PCT) by qPCR after initiation of antimalarial treatment

Safety:

- Occurrence of related unanticipated problems involving risk to subjects or others, unexpected adverse events, serious adverse events, and/or pregnancies at any time during the study period (enrollment to final follow-up visit)

4 STUDY DESIGN

4.1 General Description

This is a single center, open label CHMI study. The trial design is illustrated in section 4.6.

Up to 12 subjects will be enrolled (defined as having undergone malaria challenge) into this trial. All 12 subjects will be challenged with the new lot of Plasmodium falciparum strain 3D7, lot 1887. Based on logistical considerations, subjects may undergo challenge on multiple sequential days (in groups of 6), or be challenged altogether on the same day. Additional subjects may be recruited and screened as alternates to ensure that 12 subjects undergo

challenge. Any alternates not challenged will be released from the study on the day of challenge.

Challenge will consist of exposure to Plasmodium falciparum sporozoites through the bites of infected mosquitoes. Between Day 5 and Day 19 after the challenge, subjects will be evaluated daily for the development of malaria infection (defined as the presence of 2 unambiguous malaria parasites on a single smear) utilizing blood smear. In addition to smears, beginning on Day of Challenge (Day 0) through approximately Day 19 subjects will also be evaluated for the presence of parasitemia via qPCR. On Day 0, subjects will be evaluated once (prior to challenge, to establish a baseline). From Day 1 through approximately Day 19 subjects will be evaluated once daily. However, only smears will be used for diagnosis during this trial. In the unlikely event a subject is diagnosed with malaria prior to Day 9, they will be treated as an outpatient, with the location of those outpatient visits (WRAIR CTC vs. Hotel) to be determined on a case-by-case basis. Otherwise, subjects that have not been previously diagnosed will be required to stay in a hotel for a maximum of 10 nights starting on or around the evening of Day 9 post challenge.

All subjects diagnosed with malaria infection based on smears will be prescribed a standard treatment regimen consisting of atovaquone-proguanil (AP; Malarone®), artemether/lumefantrine (AL; Coartem®) or chloroquine (CQ), atovaquone-proguanil, to begin on the day that parasitemia is detected. Subjects who do not become parasitemic by Day 19 will be empirically treated using a standard regimen for malaria.

For purposes of confirming treatment effectiveness, daily blood smears will be continued after diagnosis until 3 consecutive negative smears have been documented. Negative smears are those which do not meet the criteria for parasitemia/positive smear defined elsewhere in this protocol. Daily qPCR will be continued until 2 consecutive negative results (separated by approximately 24 hours) have been determined following at least one positive (qPCR) result, or until smears are discontinued, whichever occurs first. If no positive qPCR results occur, then testing will be discontinued when smear efficacy criteria are met. If a treated subject has not met the above criteria for confirming treatment effectiveness by Day 19, they will be followed daily in the CTC for blood draws and continued testing until they meet criteria.

After the hotel phase, all challenged subjects will have a final scheduled follow-up visit on Day 28 (±7 days).

4.2 Study Population

Healthy, malaria-naïve adults (males and non-pregnant, non-lactating females), aged 18 to 50 years old (inclusive) will be recruited from the Washington DC/Baltimore metropolitan area to participate in this Controlled Human Malaria Infection (CHMI) study.

4.3 Sample Size Justification

A maximum of 12 subjects will be enrolled in this study, defined as receiving malaria challenge. This trial is not designed or powered to conduct formal hypothesis testing, but will provide basic descriptive statistics on the infective efficacy of the new malaria lot. A sample size of 12 subjects will provide a start to a formal non-inferiority meta-analysis, while providing preliminary efficacy results to either justify future trials with the new lot or rejection of the new lot. Additionally, in choosing the number of subjects for this trial, the team had to balance the risk to volunteers (from exposure to a potentially life-threatening illness) against the benefits of challenging additional subjects to provide a more significant proof of infectivity.

If it is assumed that the new lot will have the same efficacy as the old lot (95.1%), we can construct a binomial distribution to calculate the probability of how many failures (subjects that do not become positive) we expect to see in this trial. For comparison, the same distribution was constructed using the probability of the 95% lower bound of the historic mean. Additionally, Table 1 displays the infective efficacy rates and the associated 95% confidence intervals from the exact Clopper-Pearson method.

Table 1: Cumulative Probability of Experiencing Failure and Efficacy Rates

Number of Failures	Probability of success is 95.1% (n=12)	Probability of Success is 90.1% (n=12)	Efficacy Rate and 95% Confidence Interval (n=12)
No failures	54.7%	28.6%	100% (73.5% – 100%)
No more than 1 failure	88.6%	66.4%	91.7% (61.5% - 99.8%)
No more than 2 failures	98.1%	89.2%	83.3% (51.6% - 97.9%)
No more than 3 failures	Fails efficacy test	Fails efficacy test	75.0% (42.8% - 94.5%)

From Table 1, there is a probability of 98.1% that there will be no more than 2 failures when assuming that the efficacy is the same as the old lot. This same success probability drops to 89.2% if we assuming an efficacy rate of 90.1%, the lower bound of the 95% confidence interval.

Table 2 displays the width of the confidence intervals for adverse events using the exact Clopper-Pearson method.

Table 2: Width of Confidence Intervals for Frequency of Adverse Events

Number of Events	Probability	95% Confidence Interval
1 event	8.3%	0.2% - 38.5%
2 events	16.7%	2.1% - 48.4%
3 events	25.0%	5.5% - 57.2%
6 events	50.0%	21.1% - 78.9%
12 events	100%	73.5% - 100%

In consideration of all these factors, the team has concluded that 12 subjects (at least 6 per challenge day) is the appropriate sample size for this study.

4.4 Method of Treatment Assignment and Randomization

No randomization will be conducted as all participants will be challenged.

4.5 Study Blinding

This is an open-label study.

4.6 Schedule of Events

Table 1: Study Events Schedule for the Pre-challenge Phase

Pre-challenge Events	**Study Day for All Cohorts[a]**
	-60 to -3
Visit Number	**1**
General Procedures	
Screening, briefing, informed consent documents	●
Comprehension assessment	●
Full medical history and physical examination[b]	●
Check NHANES I criteria[c]	●
Record medications	●
Review inclusion criteria	●
Review exclusion criteria	●
Screening laboratory assays (~35 mL blood)[d]	●
Urine β-HCG pregnancy test (females only)	●
ECG	●
Amount of Blood Volume	
Approximate Blood volume in mL per visit	35
Approximate Cumulative blood volume for study (mL)	35

[a] Some or all of the study dates in this section represent a window of dates.
[b] Vital signs collected at all study visits.
[c] All acronyms defined in the list of abbreviations.
[d] Includes complete blood count, creatinine, glucose, AST, ALT, HIV-1/2, hepatitis B surface antigen, hepatitis C virus antibody, sickle-cell screening and G6PD.

Table 2: Study Event Schedule for Challenge Phase

Phase	Challenge	Post-challenge									Post-challenge Hotel Phase										Final Visit
Study Day[b]	**0**	**1[c]**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9[d]**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19[e]**	**28 ±7**
Visit Number(s)	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
General Procedures [e]																					
Physical exam[f, g]	●																				
Review medical history and medications	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Review inclusion/exclusion criteria	●																				
Review contraindications	●																				
Urine β-HCG pregnancy test (females only) [h]	●																				
Emergency notification card provided	●																				
Sporozoite challenge (CHMI)	●																				
Safety Assessment																					
Safety laboratory assays (~8 mL) [i]	●									●[j]											●
Draw for plasma pools and future use (50mL)[k]								●													●
Assess for AE/SAE[l]	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	● [l]
Diagnostic Assessment																					
Smear (2 mL) [m, n]						●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	
PCR(2 mL)[m, n]	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	
Blood Volume (mL)																					
Approximate blood volume per day	10	2	2	2	2	4	4	54	4	12	4	4	4	4	4	4	4	4	4	4	58

Phase	**Challenge**	**Post-challenge**									**Post-challenge Hotel Phase**										**Final Visit**
Study Day[b]	**0**	**1[c]**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9[d]**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19[e]**	**28 ±7**
Visit Number(s)	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
Approximate total blood volume	45	47	49	51	53	57	61	115	119	131	135	139	143	147	151	155	159	163	167	171	229

[a] Unused alternates will be released from the trial on Day 0.
[b] Some or all of the study dates in this section represent a window of dates. In particular, the final study visit will have a +/- 7 day window.
[c] PCRs will be performed daily on certain study days (Day 1 through potentially Day 21, depending on when treatment is initiated)
[d] Subjects will check in to the hotel on or around the evening of Day 9 post-challenge; the first morning study visit performed in the hotel will be on Day 10.
[e] Subjects treated empirically on Day 19 will be seen daily for additional blood draws for diagnostics, until successful treatment criteria are met. The additional blood draws may increase blood draw volume by up to approximately 20 mL. In this case, the total number of visits may increase up to approximately 25.
[f] Vital signs collected at all study visits.
[g] Challenge site exam on Day 0. Otherwise, directed physical exam as needed per investigator discretion.
[h] All acronyms defined in the list of abbreviations.
[i] Safety labs include CBC, creatinine, AST, and ALT.
[j] Safety labs will be drawn within approximately 24 hours of the diagnosis of parasitemia, preferably at the time of diagnosis.
[k] Future use blood draws will not be collected if subject has not consented to future use of specimens.
[l] SAEs and Pregnancies may be followed to resolution past conclusion of the trial if they meet certain criteria
[m] Total volume will be 4 mL per draw for diagnostics that will be divided and applied to smear and/or qPCR as appropriate.
[n] Blood smears and qPCR assays may be discontinued once the subject has 3 consecutive (daily) negative smears and 2 negative (daily) qPCRs, respectively, following the initiation of treatment. If qPCRs remain positive when smear discontinuation criteria are met, then qPCRs will also be discontinued. If qPCRs never become positive, they will be discontinued when smears are discontinued.

5 PLANNED ANALYSES

5.1 Data Monitoring Committee (DMC)

There is no data monitoring committee for this study.

5.2 Interim Analysis

There is no planned interim analysis for this study.

5.3 Final Analysis

The final analysis will be conducted once all participants have completed the study, the data have undergone quality control measures as detailed in the data management plan, and the database has been locked. The final study data will be transferred to the statistician and the final analysis will be conducted as outlined in this SAP and TLF shells.

6 ANALYSIS SETS

6.1 Intent-to-treat Analysis Set

The intent-to-treat population is defined as all participants that were enrolled into the trial. All analyses regarding subject disposition, demographics, and listings will utilize this analysis set.

6.2 Safety Analysis Set

The safety analysis set is defined as all enrolled participants that started the malaria challenge regardless of infection status. This population will be used for reporting all safety analyses.

6.3 CHMI Analysis Set

The CHMI analysis set is defined as all enrolled subjects who completed the CHMI regardless of infection status. This population will be used for analyses supporting the efficacy objectives.

7 GENERAL CONSIDERATIONS

7.1 Baseline

Baseline results for laboratory measurements and vital signs will be defined as the last recorded value prior to CHMI.

7.2 Retests, Unscheduled Visits and Premature Discontinuation Data

If a measurement value needed to be retested or reassessed due to an unsuccessful first attempt (e.g., a hemolyzed sample, unsuccessful attempt at vital sign, indeterminate result), the reassessment value will be used as long as it is within the visit specific window. If a measurement value was successfully obtained on the first attempt and there is a second unscheduled visit result, the original measurement will be used in summary tables. In the participant listings, all scheduled and unscheduled visits will be displayed.

If a participant discontinued the study prematurely, all data available up to the date of study withdrawal will be used.

7.3 Common Calculations

Any change from baseline values will be calculated as the current observation minus the baseline value.

Proportions will be expressed as percentages (i.e., proportion * 100).

7.4 Software Version

All statistical analysis will be performed using Statistical Analysis Software (SAS® Cary, North Carolina) version 9.4 or greater.

7.5 Coding Dictionaries

All adverse event data and medical history data will be coding using the Medical Dictionary for Regulatory Activities (MedDRA) 22.0. All medications will be coded using the World Health Organization Drug Dictionary (WHODrug) dated September 1, 2019.

7.6 Partial Date Conventions

In the event that partial dates are provided, any listings will display the partial date. In the event a full date is needed for calculations, the following table conventions will be used. Partial dates will be classified using the most specific known portion of the date in order of year then month then day. If a day and year are known but the month is not, the day will not be used and only the year will be used for imputation. If the day is not known, but the month and year are known, then the month and year will be used for imputation.

Partial Date Format (DDMONYYYY)	**Clarifiers**	**Convention**
--MONYYYY	Month and Year are prior to study day 0 or after the last study visit	Set day to the first day of the month. If the partial date is a start date, then set the day to the start of the month. If the partial date is an end date, then set the day to the end of the month.
--MONYYYY	Month and Year are the same as the CHMI	Set day to the same as CHMI date. If the partial date is a start date, then set the day to the CHMI date. If the partial date is an end date, then set the day to the end of the month.
--MONYYYY	Month and Year are between day 0 and last study visit and not in the same month as CHMI	Set day to the first day of the month. If the partial date is the start date, then set the day to the start of the month. If the partial date is an end date, then set the day to the end of the month.

Partial Date Format (DDMONYYYY)	Clarifiers	Convention
-- --- YYYY	Year is prior to CHMI or after the last study visit	Set day and month to the start of the year If the partial date is a start date, then set the month and day to the start of the year. If the partial date is an end date, then set the month and day to the end of the year.
-- --- YYYY	Year is the same as the CHMI	Set the day and month to the CHMI date. If the partial date is a start date, then set the day and month to the same as the CHMI. If the partial date is an end date, then set the month and day to the end of the year.
-- --- YYYY	Year is between the study day 0 and last study visit years and there was no CHMI that year.	Set the day and month to the first of the year. If the partial date is a start date, then set the month and day to the start of the year. If the partial date is an end date, then set the month and day to the end of the year.
-- --- ----	None	No imputations will be done and the date will be treated as missing.

8 STATISTICAL CONSIDERATIONS

8.1 Changes to Analysis from Protocol

There are no planned deviations in analyses from the protocol version reviewed for the creation of this statistical analysis plan.

8.2 Statistical Tests, Point, and Interval Estimation

No formal hypothesis testing will be conducted on the data produced from this study. Infective efficacy will be estimated based upon the proportion of subjects with parasitemia and an interval estimation will be made based upon the exact Clopper-Pearson method for binomial proportions. The same method will be used for interval estimation around the frequency of adverse events.

8.3 Level of Significance to be Used

Statistical significance will be defined with an alpha of 0.05 and/or lack of overlapping values when 95% CIs are calculated. Due to the small sample size of this study, only large

differences in estimates will show statistical significance. Any lack of statistical significance should be interpreted carefully as this trial was not powered to find differences in estimates.

8.4 Adjustments for Covariates and Factors to be Included in Analyses

No adjustments for covariates will be included in this analysis.

8.5 Multicenter Studies

This is a single-center study, therefore, no site specific analyses will be conducted. The study will be performed at the Clinical Trials Center, WRAIR.

8.6 Missing data

All missing data will be documented in the database and treated as missing completely at random. Missing data will not be replaced by the imputed values. Analyses will include all recorded data contributed by subjects included in the relevant analysis population.

8.7 Multiple Comparisons/Multiplicity

No formal hypothesis testing is being conducted in this analysis. Therefore, adjustments for multiple comparisons are not applicable in this study.

8.8 Examination of Subgroups

Subgroup analysis is not applicable in this study.

9 OUTPUT PRESENTATIONS

A full list of table, listing, and figure shells will be prepared outside of this SAP, but in coordination of the development of the SAP. Only one set of output shells will be prepared since only one analysis is being conducted. The output shells will document any standard display properties regarding the look of the output.

Summary tables will be prepared using the safety population for all safety summaries, immunogenicity population for all efficacy and immunogenicity summaries, and intent-to-treat for all other summaries. Listings will display all data that is available using the intent-to-treat population.

10 CALCULATIONS AND DERIVATIONS

10.1 Reference Start Date and Study Day

The reference start date is used as the time point from which the study day is calculated. Study days prior to the reference start date will be negative, while study days after the reference start date are positive. The reference start date is the date of CHMI.

Study day is calculated as the observed date minus the reference start. An observed date on the reference start date will be study day 1.

10.2 Age

Age is calculated from date of birth to date of enrollment.

10.3 Treatment Emergent AEs

Treatment emergent adverse events are defined as having an onset set on or after CHMI (Day 1).

10.4 Binomial Confidence Intervals

The 95% confidence intervals will be calculated using a binomial distribution using the exact Clopper-Pearson method. This is done using the `PROC FREQ` procedure in SAS with the `BINOMIAL(EXACT) ALPHA=0.05` options in the `TABLE` statement.

10.5 Infective Efficacy Rate

The proportion of participants with a parasitemia diagnosis via blood smear.

10.6 Positive Blood Smear

A positive malaria diagnosis via blood smear is defined as the first blood slide with 2 unambiguous parasites on a single blood smear. The date of parasitemia via blood smear will be defined as the date of the first positive blood smear in which this criteria is met.

10.7 Positive qPCR Diagnosis

A qPCR positive result has a parasite load above the lower limit of detection (85 parasites/mL of blood). The date of parasitemia via qPCR will be defined as the date of the first positive qPCR result.

10.8 Time to Parasitemia (blood smear or qPCR)

Time to a positive diagnosis of malaria via a positive blood smear or qPCR is defined as the number of days from CHMI to parasitemia date (e.g., date of first positive smear or qPCR result – CHMI date).

10.9 Parasite Clearance Time (blood smear or qPCR)

Parasite clearance time (PCT) is calculated as the number of days from the start of anti-malaria treatment to the first of three consecutive negative blood smears collected daily or the first of two consecutive negative qPCR results collected daily.

11 DISPOSITION AND PREMATURE DISCONTINUATION

Final subject disposition will be summarized by displaying all participants enrolled into the study, the number of subjects completing CHMI, and their final disposition from the study (e.g. completed they study, terminated early). A listing of all participant dispositions will also be made available.

12 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

Demographic information, to include age, gender, race, and ethnicity, will be summarized.

13 MEDICAL HISTORY

A listing of all abnormal medical history findings will be presented to show the start and end date of all findings. No summary tables will be generated. Medical history data will be coded using the MedDRA Dictionary.

14 PRIOR AND CONCOMITANT MEDICATIONS

A listing of all prior and concomitant medications will be displayed. Medication data will be coded using the WHO Drug Dictionary. Medications starting on the same day as CHMI will be included in the concomitant medication listing.

15 INFECTIVE EFFICACY ANALYSIS

The efficacy of the new lot of parasites will be determined by calculating the infective efficacy rate, which is the proportion of participants with parasitemia, along with the 95% confidence interval using the exact Clopper-Pearson method. The CHMI population will be used for this analysis. A listing of participants and final parasitemia diagnosis will be displayed.

16 SAFETY ANALYSIS

Summary tables of adverse events experienced between CHMI and the final study visit will be displayed by type of event and system organ class as well as relationship to study procedures, severity of adverse event, and serious adverse events. Additionally, descriptive statistics of laboratory measures and vital signs will be presented. Listings of adverse events, serious adverse events, laboratory measures, and vital signs will be generated.

16.1 Adverse Events (AEs)

The number and percentage of subjects experiencing at least one event will be displayed. The frequency of adverse events will be summarized by system organ class and preferred term. Events with the highest severity and relationship to study product will be selected when there are multiple events of a specific term and when appropriate. Additionally, all AE data will be listed individually (including intervention and outcome).

16.1.1 Solicited Adverse Events (AEs)

All adverse events will be categorized in the case report forms as solicited or unsolicited. Solicited events will be classified as systemic or local and will be monitored during the 28 days after CHMI. Solicited local events include pruritus at the challenge site. Solicited systemic events include tachycardia, fever (oral temperature 100.4°F/38°C), feverishness (subjective), headache, fatigue, malaise, chills, sweats, arthralgia, myalgia, gastrointestinal symptoms (nausea/vomiting/diarrhea/abdominal pain).

The intensity of each solicited event is defined in the following table.

Grading of Local Solicited Adverse Events Following Challenge

Local Reaction to Challenge	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pruritus	No interference with activity	Repeated use of topical steroid > 24 hours or some interference with activity	Prevents daily activity	Emergency room (ER) visit or hospitalization

Grading Scale for Systemic Solicited Adverse Events Following Challenge

Systemic AE	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever (measured orally)	100.4 – 101.1 (°F)	101.2 – 102.0 (°F)	>102.1 (°F)	> 40.0 > 104.0
Feverishness	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Malaise	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Chills	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Sweats	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
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
Nausea/Vomiting	No interference with activity or 1-2 episodes/24 hours	Some interference with activity or >2 episodes per 24 hours	Significant; prevents daily activity, and/or requires outpatient IV hydration.	ER visit or hospitalization for hypotensive shock
Diarrhea	2-3 loose stools or <400g/24 hours	4-5 stools or 400-800g/ 24 hours	6 or more waters stools or >800g per 24 hours or requires outpatient iv hydration	ER visit or hospitalization
Myalgias	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hr or some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Arthralgias	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hr or some interference with activity	Significant; prevents daily activity	ER visit or hospitalization

Systemic AE	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Gastrointestinal Symptoms (nausea/vomiting/ diarrhea/ abdominal pain)	No interference with activity or 1–2 episodes of emesis over 24 hr	Some interference with activity or > 2 episodes/24 hr, requiring oral medication	Prevents daily activity, or requires outpatient IV hydration or IV medication	ER visit or hospitalization
Tachycardia – beats per minute	101-115	116-130	>130	ER visit or hospitalization for arrhythmia

16.1.2 Unsolicited Adverse Events (AEs)

Unsolicited events will be monitored from CHMI through the end of the study, which is 28 days after CHMI. Unsolicited events are those that are not listed as solicited in the previous section. Unsolicited adverse events will be graded based on the following table.

Grade	Description
1 (mild)	An AE which is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
2 (moderate)	An AE which is sufficiently discomforting and interferes with normal everyday activities.
3 (severe)	An AE which prevents normal, everyday activities (i.e., prevent attendance at work/school) and would necessitate the administration of corrective therapy

16.1.3 Serious Adverse Events (SAEs)

Serious adverse events will be monitored from CHMI through the final study visit. Summary tables and listings of SAEs will be produced.

16.2 Laboratory Evaluations

For hematology and serum chemistry tests, the mean, mean change, median, and range of all values for each test at baseline and for each visit will be printed in a summary table. A second table (a “shift table”) will be made, showing for each laboratory variable the percentage of subjects whose toxicity grade decreased, stayed the same, or increased between the baseline and each study visit.

16.2.1 Toxicity Grading Criteria

Toxicity Grading Scale for Laboratory Abnormalities

Adverse event	Intensity grade	Intensity [a]
Hemoglobin (males)	Normal range	12.5 - 17.0 g/dl
	1	< 13.5 but ≥ 12.5 g/dl
	2	< 12.5 but ≥ 10.5 g/dl
	3	< 10.5 but ≥ 8.5 g/dl
	4	< 8.5 g/dl

Adverse event	**Intensity grade**	**Intensity [a]**
Hemoglobin (females)	Normal range	11.5 - 15.0 g/dl
	1	< 11.5 but ≥ 10.5 g/dl
	2	< 10.5 but ≥ 9.5 g/dl
	3	<9.5 but ≥8.0 g/dl
	4	< 8.0 g/dl
Increase in leukocytes	Normal range	3200 - 10799 cells/mm^3
(WBC)	1	10800 - 15000 cells/mm^3
	2	15001 - 20000 cells/mm^3
	3	20001 - 25000 cells/mm^3
	4	> 25000 cells/mm^3
Decrease in leukocytes	Normal range	3200 - 10800 cells/mm^3
(WBC)	1	2500 - 3199 cells/mm^3
	2	1500 - 2499 cells/mm^3
	3	1000 - 1499cells/mm^3
		< 1000 cells/mm^3
Decrease in platelets	Normal	140000 - 400000 cells/mm^3
	1	125000 - 139000 cells/mm^3
	2	100000 - 124000 cells/mm^3
	3	25000 - 99999cells/mm^3
	4	< 25000 cells/mm^3
Alanine Aminotransferase	Normal range	Below ULN (60 U/l for males; 40 U/l for females
	1	1.1 - 2.5 x ULN
	2	2.6 – 5 x ULN
	3	> 5 x ULN
	4	> 10 x ULN
Aspartate Aminotransferase	Normal range	Below ULN (40 U/l for males; 35 U/l for females
	1	1.1 - 2.5 x ULN
	2	2.6 – 5 x ULN
	3	> 5 x ULN
	4	> 10 x ULN
Creatinine (males)	Normal range	0.5 - 1.39 mg/dl
	1	1.4 - 1.79 mg/dl
	2	1.8 - 2.0 mg/dl
	3	2.1-2.5 mg/dl
	4	> 2.5 mg/dl or requires dialysis
Creatinine (females)	Normal range	0.5 - 1.29 mg/dl
	1	1.3 – 1.69 mg/dl

Adverse event	Intensity grade	Intensity [a]
	2	1.7 – 1.9 mg/dl
	3	>1.9 mg/dl
	4	> 2.5 mg/dl or requires dialysis
Hypoglycemia	Normal range	70-109 mg/dl
	1	65-69 mg/dl
	2	55-64 mg/dl
	3	45-54 mg/dl
	4	< 45 mg/dl
Hyperglycemia (random)	Normal range	70-109 mg/dl
	1	110-125 mg/dl
	2	126-200 mg/dl
	3	>200 mg/dl
	4	Insulin requirement or hyperosmolar coma

16.1 Vital Signs

Descriptive statistics will be generated for vital sign values and the change from baseline for each study visit. A listing of vital sign values will be presented and will include the toxicity grade for each vital sign value. The toxicity table below if from the US Food and Drug Administration's Guidance document "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials."

16.1.1 Toxicity Grading Criteria

Toxicity Grading Scale for Vital Signs

Vital Sign	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever (°C) (°F)	38.0-38.4 100.4-101.1	38.5-38.9 101.2-102.0	39.0-40 102.1-104	> 40 > 104
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
Tachypnea – breaths per minute	17-20	21-25	>25	Intubation

16.2 Physical Examination

Physical examination findings are not recorded in the case report forms. Any abnormal findings will be reported as adverse events and will be summarized with the adverse event tables.

16.3 Pregnancies

Pregnancy events will be listed in the adverse event listings. No specific details were captured regarding pregnancies in the case report forms.

17 PROTOCOL VIOLATIONS AND DEVIATIONS

The number of protocol deviations will be summarized by the number of participants with a deviation and the total number of deviations by treatment group. A full listing of protocol deviations will be presented.

18 DATA NOT SUMMARIZED OR PRESENTED

All data will be summarized in the final analysis.

19 REFERENCES

ICH. (1995). ICH E3 - Structure and Content of Clinical Study Reports. Guideline, 49. https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM336889.pdf.

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