**STUDY PROTOCOL**

# 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

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| **Sponsor** | The Surgeon General, Department of the Army |
| **Sponsor's Representative (Acting):** | Mark S. Paxton, JD, MS<br>US Army Medical Research and Materiel Command (USAMRMC)<br>1430 Veterans Drive, Ft. Detrick, MD 21702-5009<br>301-619-0317; DSN: 343-0317<br>Fax: 301-619-0197<br>Email: Mark.s.paxton.civ@mail.mil |
| **Source of Funding:** | MIDRP |
| **Sponsor's Medical Expert for the Trial:** | James E. Moon, COL, MC, USA<br>Malaria Vaccine Branch<br>Walter Reed Army Institute of Research<br>503 Robert Grant Avenue<br>Silver Spring, MD 20910<br>Tel: (301) 319-9176<br>Fax: (301) 319-9449<br>Email: James.e.moon.mil@mail.mil |
| **Research Monitor:** | Ramiro Gutierrez, MD CAPT, MC, USN<br>NMRC<br>503 Robert Grant Avenue<br>Silver Spring, MD 20910<br>Tel: 301-319-3193<br>Email: Ramiro.l.gutierrez.mil@mail.mil |
| **Principal Investigator:** | James E. Moon, COL, MC, USA<br>Malaria Vaccine Branch<br>Walter Reed Army Institute of Research<br>503 Robert Grant Avenue<br>Silver Spring, MD 20910<br>Tel: (301) 319-9176<br>Fax: (301) 319-7358<br>Email: James.e.moon.mil@mail.mil |

**Subinvestigators:**

Paige Waterman, MD
COL, MC, USA
WRAIR (Main Campus)
Phone: 301-319-9312
Email: Paige.e.waterman.mil@mail.mil

Melinda Hamer, MD
LTC, MC, USA
WRAIR (Main Campus)
Phone: 301-319-3136
Email: Melinda.j.hamer.mil@mail.mil

Christine Lee, MD
WRAIR (Main Campus)
Phone: 301-319-9850
Email: Christine.e.lee12.ctr@mail.mil

Michael Koren, MD
MAJ, MC, USA
WRAIR (Main Campus)
Phone: (301) 319-9904/(240) 274-0158
Email: Michael.a.koren2.mil@mail.mil

Julie Ake, MD
LTC, MC, USA
WRAIR Military HIV Research Program
6720-A Rockledge Drive
Bethesda, MD 20817
Phone: (301) 500-3621
Email: Jake@hivresearch.org

Justin Curley, MD
LTC, MC, USA
WRAIR (Main Campus)
Phone: 301-319-9687
Email: Justin.m.curley.mil@mail.mil

Jesse DeLuca, DO
LTC, MC, USA
WRAIR (Main Campus)
Phone: 301-319-9418
Email: Jesse.p.deluca.mil@mail.mil

Ilin Chuang, MD
CAPT, MC, USN
Naval Medical Research Center
Phone: (301) 319-9786
Email: Ilin.chuang.mil@mail.mil

Judith Epstein, MD
CAPT, MC, USN
Naval Medical Research Center
Phone: (301) 319-7686
Email: Judith.e.epstein.mil@mail.mil

Daniel Selig, MD
CPT, MC, USA
Phone: 240-475-6111
Email: daniel.j.selig.mil@mail.mil

Viseth Ngauy, COL, MC, USA
Director, Malaria Vaccine Branch
Walter Reed Army Institute of Research
503 Robert Grant Avenue
Silver Spring, MD 20910
Tel: (301) 319-9389
Fax: (301) 319-7358
Email: Viseth.ngauy.mil@mail.mil

Jack N. Hutter MAJ, MC, USA
Clinical Trials Center
Walter Reed Army Institute of Research
503 Robert Grant Avenue
Silver Spring, MD 20910
Telephone: 301-319-3095
Email: Jack.n.hutter.mil@mail.mil

**Research Associates:**

Edwin Kamau, LTC, MS, USA
Malaria Vaccine Branch
Walter Reed Army Institute of Research

Lindsey Garver, PhD
Entomology Branch
Walter Reed Army Institute of Research

Jack Komisar, PhD
Malaria Vaccine Branch
Walter Reed Army Institute of Research

Martha Sedegah, PhD
Naval Medical Research Center

Elizabeth Duncan, MS
Malaria Vaccine Branch
Walter Reed Army Institute of Research

| | |
|---|---|
| | Jangwoo Lee, CPT, MS, USA<br>Malaria Vaccine Branch<br>Walter Reed Army Institute of Research |
| | Sheila Peel, MSPH, PhD, DAC<br>Department of Laboratory Diagnostics and Monitoring Military HIV Research Program<br>Walter Reed Army Institute of Research |
| | Linda Jagodzinski, PhD<br>Department of Laboratory Diagnostics and Monitoring<br>Military HIV Research Program<br>Walter Reed Army Institute of Research |
| **Clinical Trial Site** | Clinical Trials Center, Room 2w78<br>Center for Enabling Capabilities<br>Walter Reed Army Institute of Research<br>503 Robert Grant Ave.<br>Silver Spring, MD 20910<br>Te1: (301) 319-9660 |

**Clinical Laboratories and Other Departments/Institutions Involved in the Trial**

| | |
|---|---|
| **Site Investigational Product Accountability** | Lindsey Garver, PhD<br>Entomology Branch<br>503 Robert Grant Ave<br>Silver Spring, MD 20910<br>Tel: (301)-319-9824<br>Fax: (301) 319-9290<br>Email: Lindsey.s.garverbaldwin.civ@mail.mil |
| **Clinical Laboratory:** | Quest Diagnostics Inc.<br>1901 Sulphur Spring Road<br>Baltimore, MD 21227<br>Tel: (410) 247-9100 |
| **Research Laboratories:** | Malaria Serology Laboratory, Malaria Vaccine Branch, Walter Reed Army Institute of Research<br>Department of Molecular Parasitology, Malaria Vaccine Branch, Walter Reed Army Institute of Research<br>Department of Laboratory Diagnostics and Monitoring, Military HIV Research Program, Walter Reed Army Institute of Research |
| **Ombudsman:** | Michael Hrycyk<br>Quality Assurance Office<br>Walter Reed Army Institute of Research |

| | |
|---|---|
| **Statistician** | Devin J. Hunt, MS<br>Clinical Trials Center<br>Walter Reed Army Institute of Research<br>Silver Spring, MD 20910<br>301-319-9571 (direct)<br>301-452-0813 (mobile)<br>Email: Devin.j.hunt.ctr@mail.mil |
| **Clinical Monitoring and Safety Surveillance** | US Army Medical Research and Materiel Command<br>Office of Regulated Activities<br>1430 Veterans Drive Fort Detrick,<br>MD 21702-5009<br>Fax: 301-619-7790<br>Telephone: 301-619-1106 |
| **Institutional Review Boards** | Walter Reed Army Institute of Research Institutional Review Board<br>503 Robert Grant Avenue<br>Silver Spring, MD 20910<br>Tel: (301) 319-9940<br>Fax: (301) 319-9961<br>Email: usarmy.detrick.medcom-wrair.mbx.hspb@mail.mil |
| **USAMRMC Office of Research Protections** | Human Research Protection Office<br>US Army Medical Research and Materiel Command<br>ATTN: MCMR-RPH<br>504 Scott Street<br>Fort Detrick, MD 21702-5012<br>Fax: 301-619-7803<br>Telephone: 301-619-2165<br>Email: usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil |

# INVESTIGATOR'S AGREEMENT

**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**

"I have read this protocol and agree to conduct the study as outlined herein in accordance with International Conference on Harmonization Good Clinical Practice Guideline and FDA, DoD, and United States Army Regulations."

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| James E. Moon, MD, COL, MC, USA<br>Principal Investigator<br>Malaria Vaccine Branch<br>Walter Reed Army Institute of Research | Date |

# EMERGENCY CONTACT INFORMATION

**Table 1: Emergency Contact Information**

| Role in Study | Name | Address and Telephone Number |
|---|---|---|
| Principal Investigator | James E. Moon, COL, MC, USA | Malaria Vaccine Branch (MVB)<br>Walter Reed Army Institute of Research (WRAIR)<br>503 Robert Grant Avenue<br>Silver Spring, MD 20910<br>Tel: (301) 319-9176<br>Fax: (301) 319-9449<br>Email: James.e.moon.mil@mail.mil |
| Subinvestigators: | Paige Waterman COL, MC, USA | Telephone: 301-319-9312<br>Email:<br>Paige.e.waterman.mil@mail.mil |
| | Judith Epstein CAPT, MC, USN | Telephone: 301-319-7686<br>Email: Judith.e.epstein.mil@mail.mil |
| | Melinda Hamer LTC, MC, USA | Telephone: 301-319-3136<br>Email: Melinda.j.hamer.mil@mail.mil |
| | Christine Lee, MD | Telephone: 301-319-9650<br>Email: Christine.e.lee12.ctr@mail.mil |
| | Julie Ake LTC, MC, USA | Telephone: 301-500-3621<br>Email: jake@hivresearch.org |
| | Justin Curley LTC, MC, USA | Telephone: 301-319-9687<br>Email:Justin.m.curley.mil@mail.mil |
| | Ilin Chuang CAPT, MC, USN | Telephone: 301-319-9786<br>Email: Ilin.chuang.mil@mail.mil |
| | Michael Koren MAJ, MC, USA | Telephone: 321-319-9904<br>Email:<br>Michael.a.koren2.mil@mail.mil |
| | Jesse DeLuca LTC, MC, USA | Telephone: 301-319-9418<br>Email: Jesse.p.deluca.mil@mail.mil |
| | Daniel J. Selig CPT, MC, USA | Telephone: 301-319-9931<br>Email: Daniel.j.selig.mil@mail.mil |
| | Jack N. Hutter MAJ, MC, USA | Telephone: 301-319-3095<br>Email: Jack.n.hutter.mil@mail.mil |
| | Viseth Ngauy, COL, MC, USA | Tel: (301) 319-9389<br>Email: viseth.ngauy.mil@mail.mil |

| Role in Study | Name | Address and Telephone Number |
|---|---|---|
| Research Monitor | Ramiro Gutierrez, MD<br>CAPT, MC, USN | NMRC<br>503 Robert Grant Avenue<br>Silver Spring, MD 20910<br>Tel: 301-319-3193<br>Email:<br>Ramiro.l.gutierrez.mil@mail.mil |
| Ombudsman | Michael Hrycyk | Walter Reed Army Institute of Research<br>Telephone: 301-319-2002 |
| Office of Regulated Activities Product Safety and Surveillance Branch | Teresa Deal, RN | USAMRMC<br>1430 Veterans Drive<br>Ft. Detrick, MD 21702-5009<br>Fax: 301-619-7790<br>Telephone: 301-619-1005 |
| Sponsor's Representative | Mark Paxton | USAMRMC<br>1430 Veterans Drive<br>Ft. Detrick, MD 21702-5009<br>301-619-0317<br>Email: Mark.s.paxton.civ@mail.mil |
| Institutional Review Board | WRAIR IRB | WRAIR IRB<br>503 Robert Grant Avenue<br>Silver Spring, MD 20910<br>Tel: (301) 319-9940<br>Fax: (301) 319-9961<br>Email: usarmy.detrick.medcom-wrair.mbx.hspb@mail.mil |

# 2. SYNOPSIS

**Name of Sponsor:**
The Surgeon General, Department of the Army

**Name of Investigational Product:**
Mosquitoes (female *Anopheles*) infected with *Plasmodium falciparum* strain NF54 (clone 3D7) (lot 1887)

**Title of Study:**
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

**Study Center(s): WRAIR** Clinical Trials Center

**Principal Investigator:**
James E. Moon, COL, MC, USA

| **Study Period (years):** | **Phase of development:** |
|---|---|
| Estimated date first subject enrolled: February 2019<br>Estimated date last subject completed: May 2019 | Not applicable |

**Objectives:**

**Primary:**

- To characterize the infectivity of the new lot (1887) of *Plasmodium falciparum* strain NF54 (clone 3D7)within the standard WRAIR CHMI model

**Secondary:**

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

**Methodology:**

This is a single center, open label CHMI study. CHMI will consist of exposure to *Plasmodium falciparum* sporozoites through the bites of infected mosquitoes. Beginning 5 days after the challenge, subjects will be evaluated daily for the development of malaria infection using a blood smear. Unless previously diagnosed and fully treated, subjects 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 malaria treatment regimen to begin on the day that parasitemia is detected. Subjects who do not become parasitemic (via smear) by Day 19 will be empirically treated for malaria.

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

**Estimated Number of Subjects Screened:**
Up to 50

**Maximum Number of Subjects Enrolled:**

| |
|---|
| 12 (Enrollment defined as receipt of malaria challenge) |
| **Main Criteria for Inclusion/Exclusion:**<br>Healthy adults, aged 18-50, military or civilian, males or non-pregnant, non-lactating females, who are able to give informed consent, understand the risks and benefits of the study and understand and are willing to comply with all protocol procedures and time commitments |
| **Investigational Product Dosage, Schedule, and Mode of Administration:**<br>Laboratory cultured *Plasmodium falciparum* strain 3D7 delivered via the bite of 5 infected mosquitoes with salivary gland scores of at least 2+ (11-100 sporozoites observed). |
| **Duration of Treatment:**<br>Up to approximately 3 months for all subjects: up to 2 months for recruitment and screening, and 1 month for challenge and post-challenge follow-up. |
| **Criteria for Evaluation:**<br>Infective Efficacy:<br>• Proportion of challenged subjects exposed to the new lot of *Plasmodium falciparum* strain 3D7 parasites developing parasitemia (defined as 2 unambiguous malaria parasites on a single smear).<br>Diagnostic Efficacy:<br>• Time to parasitemia by blood smear after the *P falciparum* challenge<br>• Time to parasitemia by qPCR after the *P falciparum* challenge<br>• Quantification of parasite clearance time (PCT) by blood smear after initiation of antimalarial treatment<br>• Quantification of parasite clearance time (PCT) by qPCR after initiation of antimalarial treatment<br>Safety:<br>• Occurrence of related unanticipated problems involving risk to subjects or others, adverse events, serious adverse events, and/or pregnancies at any time during the study period (enrollment to final follow-up visit) |
| **Statistical Methods:**<br>The proportion of subjects with parasitemia will be compared against the historical efficacy rate. No formal hypothesis testing will be conducted and all statistics will be descriptive in nature. This data will be the start of a series of trials used to conduct a more formal non-inferiority meta-analysis. The frequency of Adverse Events will be presented. Secondary Objectives will be evaluated by describing the time to parasitemia and the parasite clearance times. A formal statistical analysis plan will be developed prior to database lock or any analysis of data. |

# 3. TABLE OF CONTENTS, LIST OF TABLES, AND LIST OF FIGURES

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# 4. 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 |

| Abbreviation | Explanation |
|---|---|
| PCT | Parasite clearance time |
| PI | Principal investigator |
| PO | By mouth |
| PSSB | Product Safety Surveillance Branch |
| SAE | Serious adverse event |
| SAP | Statistical analysis plan |
| 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 |

# 5. INTRODUCTION

## 5.1. Background

Since the introduction of the Trager-Jensen method of culturing *Plasmodium falciparum* malaria in 1976, 3D7 and its parental line, NF54, have been used in experimental human malaria infections. From 1989 to 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 in 1995 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 (from a single donor) 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 NF54 (clone 3D7) (1887) has similar characteristics to lot 0285 and is identifiable as NF54 (clone 3D7). Based on prevalence and intensity of oocyst and sporozoite 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.

## 5.2. Rationale for Study

The current lot (0285) of *P falciparum* infected mosquitoes, strain NF54 (clone 3D7)is 23 years old (as of 2018) and is only anticipated to last through 2019. A new lot (1887) of strain NF54 (clone 3D7), derived from subjects infected with the original lot of infected mosquitoes was prepared under GMP conditions in 2014 and has been released for use in humans. The proposed study is necessary to bridge the 2 lots and demonstrate comparative infectivity in humans. This study is required to establish the baseline infectivity for future studies, ensuring continuity and comparability of CHMI for vaccine and drug development worldwide.

If the proposed study is not performed, then once the current parasite lot (0285) is expended (currently estimated to occur at some point in 2019), the DoD will lose the use of this CHMI model, and all future DoD clinical malaria research efforts will be significantly hindered as a result.

## 5.3. Military Relevance

As there is no known correlate of protection for malaria, all potential prophylactic or therapeutic agents must be evaluated in either CHMI or field settings to assess their efficacy. The Military population is generally non-immune to malaria, so field trials must use a non-immune population. Endemic regions with non-immune populations are difficult to identify or are unavailable making field trials potentially unfeasible. Therefore, the WRAIR CHMI model is a critical tool in continuing DoD research efforts to protect the Warfighter from malaria.

## 5.4. Name and Description of the Investigational Product

The investigational product for this study is *Anopheles stephensi* mosquitoes infected with laboratory cultured *P falciparum* strain NF54 (clone 3D7), lot 1887. The 3D7 clone of *P falciparum* strain NF54 is a human malaria isolate that has never been passed through monkeys and is well adapted to culture. It is a good producer of gametocytes that can infect mosquitoes, it is susceptible to FDA-approved antimalarial treatment readily available in the United States, and has previously been used in various CHMI clinical trials to successfully infect human subjects under a Biologics Master File (BMF-5855) submitted to the FDA. Master seed lots of these parasites have been developed under GMP conditions and stored at WRAIR. All blood products used for malaria and mosquito culturing will be commercially tested for HIV, HBV, HCV, HTLV1/2, West Nile Virus, Trypanosoma cruzi (Chagas disease), and syphilis. The mosquitoes used will be laboratory born and reared *Anopheles stephensi*. This species is relatively easy to maintain in the laboratory for up to 21 days after infection with malaria and they feed readily on humans and are able to transmit malaria to subjects. Over 1,000 subjects have been challenged at WRAIR and NMRC using the current lot (0285) of strain NF54 (clone 3D7) without requiring a single inpatient admission as a result of malaria infection.

The original seed lot (0285) and the replacement seed lot (1887) were shown in parallel standard membrane feeding assays to equivalently infect laboratory reared *A stephensi*. Whole genome sequencing of both lots compared against the reference 3D7 genome database reported the lots to be genetically identical. The drug sensitivity profiles of both lots are the same.

The NF54 strain (clone 3D7) and its use is described in BMF 5855 held by The Surgeon General, Department of the Army. Information on the provenance and testing of both lots are contained within the master file.

## 5.5. Summary of Nonclinical and Clinical Trials

### 5.5.1. Nonclinical Studies

NF54 (clone 3D7) is one of the most widely used laboratory-cultured *P falciparum* strains in the world. It is considered a canonical reference strain for all fields of *P falciparum* research. Lot 1887 in particular was tested for viability upon thaw from cryostock, growth in culture (increased parasitemia, appearance of gametocyte forms, exflagellation) and for *A stephensi* infectivity (oocyst infection prevalence and intensity in midguts and sporozoite prevalence and rating in salivary glands). For all measures tested, Lot 1887 met standards and was comparable to Lot 0285. Specific data from these studies are contained within BMF 5855.

### 5.5.2. Clinical Studies

The WRAIR CHMI model has been used for over 30 years, and more than 1,500 subjects have been safely and successfully challenged with this model at WRAIR and NMRC during this time. Prior to 1996, NF54 (clone 3D7) CHMIs, including initial infectivity assessments, R32 and NYVAC-Pf7 vaccine efficacy studies, and delivery of irradiated parasites by mosquito bite were performed with mosquitoes infected with research-grade NF54 (clone 3D7) *P falciparum*. Lot 0285 was derived from a control subject in the NYVAC-Pf7 trial, produced under cGMP conditions and used in WRAIR CHMIs until present day. Recent clinical studies using NF54 (clone 3D7) CHMI are summarized in Table 2. Current methods of infected mosquito production have been in place since 2012. Since then, historical transmission rate to subjects receiving bites from 5 mosquitoes infected with NF54 (clone3D7) lot 0285 without antimalarial prophylaxis is 95.1% (range 83.3-100%).

**Table 2: Summary of Infectivity Controls in Clinical Trials using NF54 (clone 3D7) lot 0285**

| Study identifier(s) | Challenge Date | Total Subjects Challenged | Control Subjects | Malaria Positive Control Subjects |
|---|---|---|---|---|
| CelTOS 2012 (WRAIR #1853; S-11-21) | Aug 2012 | 35 | 6 | 5/6 |
| CelTOS 2015 (WRAIR #2113; S-14-02) | Mar 2015 | 29 | 6 | 6/6 |
| VRC312 (NIH 11-1-0257) | Oct 2012 | 22 | 6 | 5/6 |
| | Feb 2013 | 22 | 6 | 6/6 |
| VRC314 (WRAIR #2101; NIH 14-1-0035; BB-IND-14826) | Jun 2014 | 15 | 6 | 6/6 |
| | Sep 2014 | 38 | 6 | 5/6 |
| | Oct 2014 | 15 | 6 | 5/6 |
| | Feb 2015 | 38 | 6 | 6/6 |
| | Jul 2015 | 18 | 6 | 5/6 |
| | Oct 2015 | 20 | 6 | 6/6 |
| Warfighter I (WRAIR #2080; NMRC2014.004; BB-IND-14826) | Nov 2014 | 35 | 6 | 6/6 |
| | Apr 2015 | 31 | 6 | 6/6 |
| MAL034 (with Oxford University) | Jul 2012 | 36 | 12 | 12/12 |
| MAL071 (WRAIR #2007; BB-IND-12937) | Feb 2014 | 58 | 12 | 12/12 |
| | Sep 2014 | 37 | 6 | 6/6 |
| MAL092 (WRAIR #2345; BB-IND-12937) | Jun 2018 | 128 | 24 | 22/24 (6/6, 5/6, 6/6, 5/6) |

| Study identifier(s) | Challenge Date | Total Subjects Challenged | Control Subjects | Malaria Positive Control Subjects |
|---|---|---|---|---|
| VAC045 (with Oxford University) | Jun 2013 | 30 | 6 | 6/6 |
| VAC055 (WRAIR #2114; with Oxford University) | Nov 2013 | 45 | 6 | 6/6 |
| | May 2014 | 24 | 6 | 6/6 |
| VAC059 (WRAIR #2201; with Oxford University) | Mar 2015 | 52 | 6 | 6/6 |
| VAC065 (WRAIR #2388; with Oxford University) | Jan 2017 | 50 | 6 | 6/6 |
| | Sep 2017 | 40 | 6 | 5/6 |

## 5.6. Known and Potential Risks and Benefits to Human Subjects

### 5.6.1. Risks/Discomfort to Subjects and Precautions to Minimize Risk

Outlined below are anticipated and unexpected adverse reactions, and a brief description of procedures to ameliorate risks and symptoms. All known risks and precautions described here are explained in detail in the informed consent.

#### 5.6.1.1. Risks Associated with the Challenge

Risks associated with malaria challenge include local inflammatory reactions to mosquito bites and the development of malaria infection.

The adverse events that might reasonably be expected to occur in subjects who are exposed to malaria infection via the WRAIR CHMI model include, but are not necessarily limited to:

- Pruritus at the challenge site
- 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)

Three cardiovascular events (1 myocardial infarction, 1 myocardial infarction vs. myocarditis, 1 myocarditis) in 3 different subjects have been described at Radboud University Nijmegen Medical Center (RUNMC) in subjects participating in clinical trials that include CHMI. While it is not possible to definitively rule out a contributory role of the *P falciparum* CHMI, a causal association has never been established. Rather, the cardiovascular episodes that have occurred were felt to be coincidental to the CHMI in all 3 cases. Of note, all three of these individuals made a complete recovery following treatment. (Verhage et al-2005, Nieman et al-2009, van Meer et al-2014)

There is a possibility of complications resulting from *P falciparum* infection in subjects who acquire the infection naturally; however, these complications are seen in high grade parasitemia following delays in diagnosis and treatment. Under the carefully controlled conditions of this study and frequent laboratory evaluations to detect active infection, the chance of serious illness or death from malaria infection is minimal. During previous studies that included CHMI studies at WRAIR and NMRC, involving *P falciparum* and more than 1,500 subjects, there has never been a mortality or episode in which a subject required hospitalization. In addition, no individual undergoing a NF54 (Clone 3D7) *P falciparum* challenge has ever had complicated, severe, or recurrent malaria. Recently, a potential recurrence, still under evaluation, did occur in a challenge utilizing a different strain of malaria. However, based on three decades of use, this is still not an expected result from challenge with NF54(Clone 3D7). In the unlikely event that malaria were to recur in a trial subject, they would be evaluated and treated as described elsewhere in this protocol.

Transient abnormalities such as fever, headache, mild anemia, thrombocytopenia, leukopenia, splenomegaly, hepatic tenderness, and fatigue are known consequences of malaria. In untreated cases, malaria infections can lead to kidney, liver, or brain damage, and death. Other risks, although highly unlikely, include a systemic allergic reaction to mosquito bites and the chance that the mosquitoes may transmit another infectious agent. No previous subject that has undergone CHMI using this model/protocol has developed a complication of malaria or has been unable to take the prescribed course of oral antimalarial treatment. There are no known cases of these mosquitoes transmitting other infectious agents in the context of a trial with CHMI.

#### 5.6.1.2. Risks Associated with Malaria Treatment

Additional risks include side effects from antimalarial treatment.

Subjects who develop malaria infection will be treated with a standard dose of atovaquone/proguanil (Malarone; AP); artemether/lumefantrine (Coartem: AL) or chloroquine (CQ). AP may cause nausea, vomiting, abdominal pain, upset stomach, headache, diarrhea, weakness, loss of appetite, itching and dizziness. AL may cause headache, dizziness, loss of appetite, weakness, fever, chills, tiredness, muscle or joint pain, nausea, vomiting, abdominal pain, cough, and trouble sleeping. CQ may cause nausea, vomiting, stomach upset, cramps, loss of appetite, diarrhea, fatigue, weakness, tinnitus, dizziness, pruritus, rash, or headache.

#### 5.6.1.2. Pregnancy

Malaria infection during pregnancy is dangerous to both the pregnant woman and the unborn baby; therefore, pregnant females will be excluded from this study. Study subjects should not become pregnant during the study and for at least 3 months after the challenge.

#### 5.6.1.3. Lactation

Malaria is not known to be transmitted via breastfeeding. However, antimalarial agents (like most drugs) may transmit to infants via breastfeeding, and some of the agents planned for this trial are not recommended for use in infants. As such, lactating females will be excluded from participation in the study.

#### 5.6.1.4. Venipuncture

Blood sampling carries a minimal risk of minor discomfort and the possibility of minor bruising at the site of the needle puncture and, rarely, the possibility of infection at the needle puncture site.

Phlebotomy will be done by trained staff using aseptic technique to reduce the risk of complications. The amount of blood loss will be monitored closely to meet the guideline from the American Association of Blood Bank that no more than 450 mL will be collected in any 8-week period.

#### 5.6.1.5. Allergic Reaction

Antimalarial drugs will be utilized in this trial, and are unlikely to have ever been used by subjects previously. As with any drug administration and no matter what precautions are taken, there is always the risk of a serious, or even life-threatening, allergic reaction. Trained medical personnel and emergency equipment will be available at the trial site to address medical emergencies such as anaphylaxis, angioedema, bronchospasm, and laryngospasm.

#### 5.6.1.6. Unknown Risks

Furthermore, as with all research there is the remote possibility of risks that are unknown or that cannot be foreseen based on current information.

### 5.6.2. Alternatives to this IND Product or Study

At this time, there is no alternate method to ensure this new lot of parasites is safe and predictably infectious in humans. Subjects may choose not to participate in the study.

### 5.6.3. Intended Benefit for Subjects

No direct benefit is intended for study subjects; however, subjects may indirectly benefit from general medical evaluation and health screening procedures including testing for HIV, Hepatitis B, and Hepatitis C viruses. If they are found to be positive for any infectious diseases other than malaria from *P falciparum*, including HIV, they will be referred to their primary physician where they will receive counseling and further medical attention earlier than if they did not know of their disease status. Society as a whole may benefit from information learned through this study, and in particular from future malaria research studies enabled by this new lot of parasites.

### 5.6.4. Risks to the Study Personnel and the Environment

The principal risk in the clinical setting is the handling of needles that may be contaminated with blood or body fluids and the associated risk of acquiring a blood-borne pathogen including HBV,

hepatitis C virus (HCV), and HIV. Adherence to standard operating procedures (SOP) for working with infectious agents and universal precautions will reduce the risk of exposure.

There are no known risks to the environment other than those associated with the generation of biohazardous waste. All biohazardous waste will be disposed of as stipulated by local, state, and Federal regulations and in accordance with study site SOPs.

The Investigative staff members who are in the WRAIR insectary during the malaria challenge have a small risk of exposure to malaria. This small risk is greatly minimized by following stringent insectary SOPs regarding the handling of mosquitoes, the execution of the mosquito challenge, and physical movement inside the insectary.

The risk of accidentally transmitting malaria to a person in the community will be negligible because of the following factors/measures:

- The infected mosquitoes will be raised in the secure insectary at WRAIR
- All malaria challenges occur in the secure insectary
- The infected mosquitoes never leave the secured insectary area at any time
- Malaria infections in subjects will be treated promptly, before gametocytes can develop (generally 10 days after the development of patent malaria), further decreasing any risk of transmission to local mosquitoes
- Malaria cannot be transmitted person-to-person (ie, it is not contagious)

## 5.7. Route of Administration, Dosage Regimen, Treatment Period, and Justification

The malaria challenge will be conducted according to Walter Reed Army Institute of Research (WRAIR) standard operating procedures. Briefly, mosquitoes infected via membrane feeds prior to challenge and containing sporozoites in their salivary glands will be allowed to feed on the subjects. For each subject, 5 mosquitoes will be initially allowed to feed. After 5 minutes of exposure to the subject's arm, the midgut and salivary gland of each of the 5 mosquitoes will be immediately dissected to confirm the presence of a blood meal and to determine the infectivity rate and the salivary gland score. The salivary gland score is a measure of the number of sporozoites observed under a microscope in media after crushing the mosquito salivary glands. Glands are scored according to a logarithmic scale: 0 = no sporozoites; 1 = 1-10 sporozoites; 2 = 11-100 sporozoites; 3 = 101-1000 sporozoites; 4 = >1000 sporozoites. A mosquito must pass two criteria to be accepted as a qualifying bite: visual confirmation of blood in the midgut and a gland score of 2 or greater. Dissectors will document the gland score or "not fed" status of each dissected mosquito, count the number of qualifying bites and determine the number of mosquito bites still needed to achieve 5 qualifying bites. Another group of mosquitoes of this number will then be allowed to feed on the subject. For example, if a subject has infective bites from 3 mosquitoes in the first feeding (out of the 5 mosquitoes used), then only 2 fresh mosquitoes will be used in the second feeding. This cycle will continue until the subject receives a total of 5 infected mosquito bites from mosquitoes with a minimum 2+ salivary gland score (2+ = 11-100 sporozoites observed), the supply of prepared mosquitoes is exhausted, or the volunteer requests to withdraw from the trial. In the unlikely event that one of the last two options occurs, the subject will be treated empirically for malaria and withdrawn from the trial as discussed in

Section 8.5.4 below. Dissectors will review the documentation, verify 5 qualifying bites were received and notify the study investigator. The study investigator reviews the documentation to confirm before releasing the subject from the challenge room. (Rickman et al-1990).

## 5.8. Compliance Statement

The study will be conducted according to the protocol and in compliance with International Conference on Harmonization (ICH) Good Clinical Practice (GCP), Belmont Principles, and other applicable regulatory and Department of Defense (DoD) requirements. All identified study personnel will be trained to perform their roles and will carry out their responsibilities in accordance with ICH GCP guidelines and the clinic site's SOPs.

## 5.9. Study Population

Up to 12 healthy, malaria-naïve adults, aged 18 to 50 years (inclusive) from the diverse local community of the Greater Metropolitan Washington, DC/ Baltimore area will be enrolled (defined as receiving malaria challenge) in the study. Additional subjects may be recruited and screened as alternates to ensure 12 subjects are challenged. Based on previous enrollments for malaria vaccine trials at the WRAIR CTC, it is estimated that at least 3 times the number of subjects to be enrolled will need to be screened in order to reach the target number of 12.

## 5.10. Study Site

The majority of subject visits will occur at the Walter Reed Army Institute of Research (WRAIR) Clinical Trials Center (Silver Spring, MD) to include: recruitment, screening, enrollment, and certain follow-up visits. Malaria Challenge will occur in the Insectary and associated rooms at WRAIR. Post-challenge, subjects will spend some time and have some of their follow-up visits in a local hotel.

# 6. TRIAL OBJECTIVES AND ENDPOINTS

## 6.1. Objectives

### 6.1.1. Primary Objective

- To characterize the infectivity of the new lot (1887) of *Plasmodium falciparum* strain NF54 (clone 3D7), parasites within the standard WRAIR CHMI model

### 6.1.2. 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 as part of the process of validating the assay for future use
- To obtain plasma samples to restore the testing control pool for malaria serology testing and to acquire samples for future malaria research

## 6.2. Endpoints

### 6.2.1. Primary Endpoints

**Infective Efficacy:**

- Proportion of challenged subjects exposed to the new lot of *Plasmodium falciparum* strain NF54 (clone 3D7) developing parasitemia (where parasitemia is defined as the presence of 2 unambiguous parasites on a single smear)

### 6.2.2. 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)

# 7. TRIAL DESIGN

## 7.1. Overall Study Design

This is a single center, open label CHMI study. The trial design is illustrated in Table 3 and Table 4 and is detailed below.

Healthy, malaria-naïve adults (males and non-pregnant, non-lactating females), aged 18 to 50 years old (inclusive) will be recruited from the Washington/ Baltimore DC metropolitan area to participate in this Controlled Human Malaria Infection (CHMI) study. Up to 12 subjects will enrolled (defined as having undergone malaria challenge) into this trial. All 12 subjects will be challenged with the new lot of female *Anopheles* mosquitoes infected with *Plasmodium falciparum* strain NF54 (clone 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. Beginning on Day 5 and going through 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,

subjects will also be evaluated for the presence of parasitemia via qPCR. However, only blood smears will be used for diagnosis during this trial. Testing by qPCR will be performed beginning on Day of Challenge (Day 0) through approximately Day 19 after challenge.. On Day 0, subjects will be evaluated by qPCR once prior to challenge, to establish a baseline. From Day 1 through approximately Day 19 subjects will be evaluated by qPCR once daily. 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 blood 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).

**Table 3: 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) | ● |

| **Pre-challenge Events** | **Study Day for All Cohorts[a]** |
|---|---|
| | **-60 to -3** |
| **Visit Number** | **1** |
| 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 4: 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] |

| **Phase** | **Challenge** | **Post-challenge** | | | | | | | | | **Post-challenge Hotel Phase** | | | | | | | | | | **Final Visit** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study Day[b]** | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9[d]** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19[e]** | **28 (±7 days)** |
| **Visit Number(s)** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** |
| **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 |
| 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.

## 7.2. Measures Taken to Minimize/Avoid Bias

### 7.2.1. Randomization

Randomization will not be used in this study, as all subjects will undergo the same procedures.

### 7.2.2. Blinding

Blinding is not applicable and will not be used in this protocol.

## 7.3. Investigational Product

Table 5 presents a summary description of the investigational product.

**Table 5: Investigational Product**

| | |
|---|---|
| **Product Name** | P falciparum infected mosquitoes (Strain NF54 (clone 3D7), lot 1887) |
| **Unit Dose** | Qualifying Mosquito bites (n=5) |
| **Route of Administration** | Mosquito bites |
| **Manufacturer** | WRAIR Entomology |
| **Lot Number** | 25Mar19-LotBridge(1887)-3D7 |
| **Product Indication** | For evaluation of controlled human malaria infection (CHMI) model |

### 7.3.1. Investigational Product Storage

The infected mosquitoes are stored in mesh-topped cardboard feeding cartons in environmentally controlled chambers at 26±1ºC and 80±5% relative humidity.

### 7.3.2. Investigational Product Preparation

The *A stephensi* female mosquitoes infected with *P falciparum* strain NF54 (clone 3D7) parasites used for the challenge will be reared in the WRAIR insectary. The mosquitoes are fed on human washed packed erythrocytes and serum via a glass membrane feeding system that is warmed by a circulating water bath. Once the mosquito eggs enter the phase of adult emergence, approximately 250 to 300 females are transferred to mesh-topped cardboard feeding carton with 10% sucrose.

### 7.3.3. Investigational Product Accountability

The Sponsor's representative maintains ultimate responsibility for product accountability, but has delegated site product accountability responsibility to the principal investigator at WRAIR Insectary. The principal investigator is responsible for and will maintain logs of investigational product receipt, storage, accountability by subject in controlled access document storage space at the study site. The principal investigator may delegate, in writing, this responsibility to another individual, but at the study site, the principal investigator is ultimately responsible for the investigational product and its proper storage upon receipt at the study site until it is destroyed, as directed by the Sponsor's representative.

All unused or partially used investigational product (in this case, infected mosquitoes prepared as described above) will be destroyed by the PI or designee by being incinerated and/or disposed of as biohazardous waste according to local SOPs, as directed by the Sponsor's representative and as stipulated by local, state, and Federal regulations. Unused or partially used investigational product may only be destroyed or transferred after authorization has been received from the Sponsor's representative.

## 7.4. Duration of Subject Participation

Approximately 3 months for all subjects (Up to 2 months for recruitment and screening and 1 month for challenge and post-challenge follow-up).

## 7.5. Study Termination Criteria

The PI, research monitor, sponsor's representative, the IRB, and/or the USAMRMC ORP, or the FDA may stop or suspend the use of this product at any time.

Any suspensions to include continuing review lapses, voluntary or involuntary clinical holds, or terminations of this research by an IRB, the Sponsor, or regulatory agencies will be reported within 48 hours to the IRB of record and the Sponsor's representative by phone, email, or facsimile.

## 7.6. Identification of Data to be Recorded on the Case Report Forms

The electronic case report form (eCRF) data will be transcribed from source documentation. No source data will be recorded directly in the eCRF (ie, without prior written or electronic record of data). The transcribed data will be consistent with the source documents or the discrepancies will be explained.

For more information on data handling, refer to Section 12.2.

# 8. SELECTION AND WITHDRAWAL OF SUBJECTS

## 8.1. Recruitment of Subjects

Up to 12 healthy, malaria-naïve adults, aged 18-50 years (inclusive) will be enrolled (defined as receiving malaria challenge) in the study. Based on previous enrollments for CHMI studies, it is estimated that at least 3 times the number of subjects to be enrolled will need to be screened (approximately 36) in order to reach the target number of 12. Additional subjects may be recruited and screened as alternates to ensure that the target number of 12 challenged subjects is met.

Recruiting will take place through the WRAIR CTC according to applicable regulations and policies (eg, DoD Common Rule, 32 CFR 219 and AR 40-38). The recruitment process will be the same for both military and civilian personnel.

Subjects will not be replaced if they withdraw or are eliminated from the study after having undergone CHMI.

Healthy, malaria-naive adult subjects from the locally diverse community of the Greater Metropolitan Washington DC/ Baltimore area will be recruited under this protocol when approved by the IRB of record. Recruitment materials are presented in Appendix A. Contact will be made with potential subjects by advertising mechanisms such as newspapers, flyers, emails, websites, direct emails to all WRAIR staff, postings on public electronic bulletin boards, other social media (such as Facebook and Geofencing), and posters aimed at adults living in the greater Washington, DC metro region. Email announcements will include information on posters excluding any photos unless attached as complete flyer. A version of the recruiting script may also be emailed to interested individuals. The CTC also may place generic television advertisements recruiting for subject participation in the CTC studies (without mentioning specific studies).

Recruitment may include oral presentations, with approved recruiting material available, at events, meetings, and briefings where the desired population may be reasonably expected to attend. In accordance with DoD Instruction 3216.02, an ombudsman not directly associated with the greater than minimal risk research to be performed will be present during recruitment activities involving Service members in a group setting (morning formations, etc.). The ombudsman will ensure that "the voluntary involvement or recruitment of the Service members is clearly and adequately stressed and that the information provided about the research is clear, adequate, and accurate." Active duty military subjects will require approval from their supervisor through their branch director using a signed Statement of Supervisor's Approval.

## 8.2. Informed Consent Process

The informed consent process will adhere with applicable regulations and SOP. One of the investigators and/or delegated study staff will provide an oral explanation of the study in lay terms. Alternatively, an IRB-approved power point presentation or video may be used to instruct potential subjects about the study. Questions on the purpose of the protocol, protocol procedures, and risks to the subjects will then be solicited. Any question that cannot be answered will be referred to the PI or AI. The PI or designee shall give the subjects ample opportunity to inquire about details of the study, discuss with other people and ask any questions before dating and signing the consent form. Subject information and consent form language will be at a reading level fully comprehensible to the potential subjects. No subject should grant consent until he or she has had the opportunity to read the informed consent document and questions have been answered to his/her satisfaction. Informed consent includes the principle that it is critical the subject be informed about the principal potential risks and benefits prior to study participation. This information will allow the subject to make a personal risk versus benefit decision and understand the following:

- Participation is entirely voluntary
- Subjects may withdraw from participation at any time
- Refusal to participate involves no penalty
- The subject is free to ask any questions that will allow him/her to understand the nature of the protocol
- A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by US law. The web site will not include information that can identify the

volunteer. At most, the web site will include a summary of the results and can be searched at any time.

An additional informed consent document for HIV testing will be signed by all subjects. Informed consent will be obtained in accordance with 21 CFR 50, 32 CFR 219, DoD Common Rule, and the principles of the Belmont Report prior to initiating any study procedures.

- Information will be given in both oral and written form whenever possible and deemed appropriate by the IRB. The written consent document contains the required and all applicable optional elements of informed consent as described in 21 CFR 50.25 (including a full explanation of the possible risks, advantages, and alternate treatment options, and availability of treatment in the case of injury). In addition, the consent document will comply with DoD policy and regulations.
- Each subject's signed informed consent document must be kept on file by the PI for possible inspection by regulatory authorities and/or regulatory compliance persons. The subjects will receive a copy of the signed and dated written informed consent document and any other written information provided to the subjects.
- The signed consent document indicates that the subject permits access to research and relevant medical records by the sponsor's representative and by representatives of the FDA. The sponsor's representative will submit a copy of the initial IRB- and sponsor's representative-approved consent form to the FDA and will maintain copies of revised consent documents that have been reviewed and approved by the IRB/ethics committee

Subjects will also be required to pass a short multiple-choice quiz that assesses their understanding of this study prior to enrollment (Appendix B). Subjects must achieve a score of at least 80% correct on a multiple choice quiz that assesses their understanding of the study; subjects will be allowed 2 attempts to pass the quiz of understanding.

Should the protocol be modified, the consent document will be revised to reflect any necessary changes made in the protocol with regard to the content of the subject consent document. If a previously enrolled subject is directly affected by the change, the subject will receive a copy of the revised informed consent document. The approved revision will be read, signed, and dated by the subject. Because the informed consent process is ongoing the PI or AI will be available to answer subjects' questions at any time during the study period. Subjects will be periodically reminded about the purpose of the study and the study procedures that will take place during future visits.

## 8.3. Eligibility Screening

Subjects who have signed an informed consent document will provide a medical history, undergo a physical examination and routine laboratory screening tests and be screened for cardiac risk factors using family and personal medical history, blood pressure screening, National Health and Nutrition Examination Survey I (NHANES I) criteria (Appendix C), and an electrocardiogram (ECG). (Gaziano et al-2008) Female subjects will provide a urine specimen for a urine pregnancy test.

Subjects found to be seropositive for HIV-1/2, HBV, or HCV will be counseled by the PI and referred to a health care provider for further evaluation. Positive HIV, HBV, or HCV results will also be reported by the study team to the Maryland AIDS Administration, Department of Health and Mental Hygiene within 24 hours of being confirmed as per Health General Article §18-205. If a subject is in the military, positive HIV results will be reported by the study team to the military preventive medicine service.

Subjects must meet all inclusion criteria and will be excluded from participation if they meet any of the exclusion criteria.

All screening tests will be performed prior to entry into the study and safety labs may be repeated during the course of the trial as deemed necessary by the investigators to include measurement of plasma concentrations of anti-malarial drugs in the event it becomes necessary to validate the challenge model. The medical history, physical examination, and laboratory findings for subjects will be recorded in the source screening data documents. Screening labs may be repeated at the discretion of the clinical investigator in order to properly evaluate a subject as routine labs may be outside normal parameters at a single blood draw due to physiologic variability or laboratory error/artifact. Non-clinically significant Grade 1 laboratory abnormalities which persist on repeat testing may, at the discretion of the principal investigator, be deemed non-exclusionary if they are felt to represent the subject's normal baseline for that test. Vital signs (temperature, pulse, blood pressure) are recorded at screening and all other scheduled study visits unless indicated otherwise. Concomitant medications are also recorded at all study visits.

### 8.3.1. Subject Inclusion Criteria

Subjects must meet all of the following criteria to be included in the study:

- Healthy adults (male or non-pregnant, non-lactating female) 18 to 50 years of age (inclusive) at the time of screening
- If the subject is female, she must be of non-childbearing potential (ie, either surgically sterilized or one year post-menopausal), abstinent or using adequate contraceptive precautions (eg, intrauterine contraceptive device; oral contraceptives; diaphragm or condom in combination with contraceptive jelly, cream or foam; Norplant® or Depo-Provera®) during this study and must agree to continue such precautions until three months after challenge
- Free of significant health problems (defined as active or chronic health problems negatively impacting normal daily functioning) as established by medical history, laboratory, and clinical examination before entering the study
- Available and willing to participate in all planned study visits for the duration of study (approximately 3 months)
- Willing to comply with all study procedures, to include but not limited to taking anti-malarial treatment, and staying in a pre-determined hotel during the designated post-CHMI follow-up period, if indicated
- No plans to travel to a malaria endemic area (http://www.cdc.gov/malaria/map/) during the course of the study

- Willing to refrain from blood donation during the study and for at least 3 years following CHMI.
- Written informed consent must be obtained from the subject before screening procedures are performed
- Subjects must score at least 80% correct on a multiple-choice quiz that assesses their understanding of this study
  - If they do not score 80% on the initial quiz, the protocol information will be reviewed with them, and they will have the opportunity to retest
  - If a subject fails to correctly answer 80% of the questions after 2 attempts, he or she will be excluded from the study
- If a subject is active duty military, he or she must obtain approval from his or her supervisor to participate

### 8.3.2. Subject Exclusion Criteria

Subjects meeting any of the following criteria will be excluded from the study:

- Any history of malaria infection.
- History of travel to *P falciparum* endemic areas in the 6 months (168 days) prior to day of challenge, or planned travel to such areas during the course of the study.
- History of long-term residence (>5 years) in an area known to have significant transmission of *P falciparum* (http://www.cdc.gov/malaria/map/)
- Any history of receiving a malaria vaccine
- History of receipt of malaria prophylaxis or treatment during 1 month (28 days) prior to day of challenge, or planned use during the study period (outside of the drugs provided by the study team).
- History of use of any antimicrobials or other agents with significant antimalarial activity (examples include tetracycline, doxycycline, clindamycin, azithromycin, and sulfa drugs) during the 1 month (28 days) prior to day of challenge, or planned use during the study period.
- Use of any investigational or non-registered drug or vaccine within 1 month (28 days) preceding the day of challenge or planned use during the study period.
- Current or chronic use of systemic immunosuppressant pharmacotherapy or immunomodulators; however, subjects may be allowed to use inhaled steroids or topical steroids.
- Pregnant (positive β-human chorionic gonadotropin test, β-HCG) or lactating female at screening or plans to become pregnant or breastfeed from the time of enrollment until three months after challenge

- Allergy to or use of medications known to interact with all the antimalarial drugs (CQ, AP, AL) which may be utilized in this trial. Subjects are not excluded if able to safely take at least one of the proposed antimalarial drugs for the trial.
- Significant (eg, systemic) hypersensitivity reactions to mosquito bites (local hypersensitivity reactions at the site of mosquito bites are not an exclusion criterion)
- Positive sickle cell screening test including evidence of sickle cell trait or sickle cell anemia (due to its effect on susceptibility to malaria)
- History of thalassemia or thalassemia trait (due to its effect on susceptibility to malaria), or any other clinically significant (defined as negatively impacting subject health and/or normal daily functioning) blood disorder
- Acute or chronic, clinically significant (defined as negatively impacting subject health and/or normal daily functioning), pulmonary, cardiovascular, endocrine, hepatic, immunological or renal functional abnormality, as determined by history, physical examination, or laboratory evaluation
- Chronic or active neurologic disease including seizure disorder (excepting a single febrile seizure of childhood) and chronic migraine headaches
- History of cancer (except for basal cell carcinoma of the skin)
- Abnormal (clinically significant, defined as suggesting an active medical condition or pathology that could negatively impact subject health and/or normal daily functioning, as determined by physician investigator) baseline laboratory screening tests
- Seropositive for HIV or HCV or hepatitis B surface antigen (HBsAg) positive
- Evidence of increased cardiovascular disease risk, "moderate" or "high", according to the National Health And Nutrition Examination Survey I (NHANES I) criteria. Note: NHANES I criteria will be applied for all subjects including subjects aged 20-35 years old.
- An abnormal baseline screening electrocardiogram, defined as one showing pathologic Q waves and significant ST-T wave changes; left ventricular hypertrophy; any non-sinus rhythm excluding isolated premature atrial contractions; right or left bundle branch block; or advanced (secondary or tertiary) A-V heart block.
- History of splenectomy
- Hepatomegaly, right upper quadrant abdominal pain or tenderness
- History of psoriasis or porphyria
- Plan for surgery during the period from enrollment until 3 months post-CHMI, with the exception of minor cutaneous procedures
- Suspected or known current alcohol or drug abuse as determined from the medical history or by physical examination

- Any other significant finding that in the opinion of the PI would increase the risk of having an adverse outcome from participating in this study

## 8.4. Contraindications to Challenge

If a subject has a minor illness (eg, a mild upper respiratory infection) but does not have a fever during the pre-challenge assessment, the subject may be challenged at the discretion of the PI (in consultation with the research monitor). If the subject is moderately to severely ill with a fever (temperature greater than or equal to 38.0°C (100.4°F) the subject will not be challenged and will be withdrawn from the study.

Pregnant women cannot be challenged.

## 8.5. Subject Withdrawal Criteria

A subject withdrawal is anyone who has not completed the final study visit. A subject may be withdrawn for an AE or SAE resulting in a safety concern, for noncompliance with protocol requirements, or for other reasons, such as:

- Consent withdrawal, not due to an AE
- Migrated/moved from the study area
- Lost to follow-up
- Subject unable to continue with all scheduled visits
- Death
- Other (specify)

A subject may end his or her participation in the study at any time. If a subject withdraws, the PI or designee will make a reasonable effort to determine the reason for the withdrawal from the study.

### 8.5.1. When and How to Withdraw Subjects

A subject may end his or her participation in the study at any time. If a subject withdraws, the investigator will make a reasonable effort to determine the reason for the withdrawal from the study and to complete termination procedures. Telephone calls, registered letters, and email correspondence are considered reasonable effort. For subjects leaving the study, a targeted examination may be performed, if medically indicated and if permitted by the subject.

A subject may be withdrawn for an adverse event (AE) or serious adverse event (SAE) resulting in a safety concern or for noncompliance with protocol requirements. When a subject withdraws due to an AE or is withdrawn by the principal investigator due to an AE, the sponsor's safety office, the USAMRMC Office of Regulated Activities (ORA) Product Safety Surveillance Branch (PSSB), must be notified within 24 hours (usarmy.detrick.medcom-usamrmc.mbx.sae-reporting@mail.mil). Investigators must follow specific policy regarding the timely reporting of AEs and SAEs to the IRB (Section 10.5.1.2). In all cases, the PI will make a reasonable effort to complete study termination procedures.

If a subject meets withdrawal conditions for a concomitant medication violation or noncompliance, this should clearly be stated in the source document and the study termination electronic CRF.

### 8.5.2. Data Collected for Withdrawn Subjects

All samples and data collected up to the time of withdrawal may be utilized in evaluations and analysis for the protocol. The study termination eCRF will be completed with the reason for withdrawal specified.

### 8.5.3. Replacement of Subjects

Subjects will not be replaced after enrollment.

### 8.5.4. Follow-up for Withdrawn Subjects

If a subject ends his or her participation in the study prior to challenge, the investigator will make a reasonable effort to determine the reason for the subject's withdrawal and record the information appropriately. If deemed necessary, the investigator may request the subject come in for a final visit to ensure subject safety.

Clinical follow-up after a challenge is important to ensure the safety of the subject. Subjects who withdraw from the study are required to complete clinical follow-up visits and antimalarial treatment for safety purposes. Since untreated malaria infection could have severe consequences including death, subjects will be sufficiently counseled for this requirement as indicated in the informed consent document and advised not to participate if they do not think they can meet this requirement. Withdrawal from the study for any reason will not impact the subject's medical care.

# 9. TREATMENT OF SUBJECTS

## 9.1. Malaria Challenge

Visit time and event schedules for the primary malaria sporozoite challenge and follow-up are presented in Table 4.

The challenge will be performed according to standard operating procedures (SOPs) maintained in the WRAIR Entomology Branch regarding this procedure. The *A stephensi* female mosquitoes infected with strain NF54 (clone 3D7) *P falciparum* parasites used for the challenge will be reared in the WRAIR insectary. Briefly, each subject will have 5 "infected" mosquitoes feed on their forearm for 5 minutes. These same mosquitoes will then be dissected to confirm that they were infected and the salivary glands will be scored. If all 5 have not adequately fed and/or are not infected with a +2 salivary gland score or better, additional mosquitoes will be allowed to feed in 5 minute intervals until a total of 5 infected mosquitoes have fed on the subject.

## 9.2. Determination of Parasitemia

For this trial parasitemia will be determined by utilizing 2 methods: microscopy of Giemsa-stained thick blood films (smear) and through qPCR. However, only blood smear results will be

utilized for diagnosis and evaluation of treatment success. Both procedures will be performed according to draft SOPs by the applicable supporting laboratory identified above. For the purposes of this trial, parasitemia will be defined as the presence of 2 unambiguous malaria parasites on a single smear.

All blood films (positive and negative) will be archived for later reexamination and confirmation, until the protocol is closed.

## 9.3. Management of Challenged Subjects

The pre-patent period (time between exposure and development of demonstrable parasitemia) for *P falciparum* in man normally averages 9 to 12 days. In previous studies, the pre-patent period in controls varied from 7-18 days. The shortest reported pre-patent period in man is 5 days, and the longest is 25 days (Ballou-1987).

Beginning on Day 1 after their challenge, subjects will be seen and evaluated daily by a study investigator and blood will be drawn for determination of parasitemia by qPCR. Beginning on Day 5 after their challenge, blood from these daily draws will also be utilized to generate blood smears. As above, only smear results will be utilized for formal diagnosis and evaluation of treatment success.

If fever or symptoms develop at any time, the subject will be evaluated by the study investigator and smears may be done more frequently (every 6 to 12 hours) at the discretion of the investigator. Likewise, qPCR testing may be performed more frequently than planned (up to twice a day) at investigator discretion to evaluate symptomatic volunteers or as part of a safety evaluation. A confirmed positive result will be relayed immediately to the on-call investigator/study personnel by the microscopists. The infection will be treated early (ie, as soon as parasites can be identified on thick smear) according to one of the treatment regimens outlined below.

Beginning on Day 9 post-challenge, a group of hotel rooms in the local area will be reserved for malaria-challenged subjects. The subjects will be required to spend their nights there (or, in the case of subjects who work at night, spend sleeping hours there) to allow for more rapid assessment of any potential symptoms of malaria during the most likely period of patency. There will be an investigator present on-site and available for subject assessment. There will also be qualified study personnel on site 24 hours per day during the hotel phase of the study.

During the hotel phase, all challenged subjects will be assessed on a daily basis in an identical manner. An evaluation will be done each morning (headache, muscle aches, etc) and blood will be drawn for smear and qPCR. All challenged subjects will be instructed to check in with clinical staff by telephone call or in-person each evening during the hotel stay until they are positive for malaria. They will be asked if they feel any different since they were seen in the morning. At any time required, the on-duty investigator will arrange for the timely production of blood smears, along with their examination and interpretation, in order to treat subjects for malaria. Once a positive smear is identified, daily blood films will continue to be obtained until 3 consecutive films are negative (separated by more than 12 hours).

The maximum hotel stay for malaria-challenged subjects should be approximately 10 nights. A subject who develops malaria, is treated, and has three consecutive negative malaria smears and 2 consecutive negative qPCRs (separated by more than 12 hours), will not need to remain in the

hotel. The investigators will be responsible for accounting for any subjects who do not arrive in the hotel during the challenge phase. If required, the investigators will physically locate and treat any malaria-infected subject who is unable to maintain the follow-up dictated by this study.

If infection does not develop by Day 19, the subject will be started on empiric treatment for malaria, and will be released from the hotel. In such cases, subjects will then be required to follow-up at the clinic daily for completion of treatment and blood collection (for smears and qPCR) until treatment efficacy criteria (3 consecutive negative smears) have been met.

Following diagnosis, qPCR will be performed daily until negative results from 2 consecutive days (tests separated by more than 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. As above, treatment efficacy will be based on smear results, not qPCR results.

All subjects will have a final visit at the clinic Day 28 after challenge.

## 9.4. Malaria Treatment

As soon as a malaria infection is documented in a subject, or a subject reaches Day 19 without demonstration of parasitemia, he/she will be treated with standard doses of oral Malarone (1 g/400 mg, 4 adult tablets per day for 3 consecutive days) under direct observation. This regimen has been 100% effective in previous WRAIR and NMRC malaria vaccine studies using the same malaria strain as will be used in this challenge model. Such early treatment minimizes the risk of developing a complicated malaria infection. Alternatively, a standard oral dosage of artemether/lumefantrine (Coartem) of 80 mg/480 mg (4 tablets initial dose, 4 tablets 8 hours later, and then 4 tablets twice daily for the following 2 days) or chloroquine (a total of 1500 mg chloroquine base: 600 mg base initially, followed by 300 mg base given approximately 6, 24, and 48 hours later) can be used to treat subjects.

The investigators will have available approved antipyretics, such as acetaminophen and ibuprofen, for subjects experiencing fever and myalgias. In addition, other approved medications will be available to the investigators, which may include, but are not limited to: ondansetron and loperamide (Imodium) to treat other signs/symptoms as necessary. Investigators will always assure that subjects do not have underlying allergies or other contraindications to any of these medications prior to their use. An alternative antipyretic will be provided if the subject is allergic to a prescribed drug.

It is anticipated that treatment of malaria will be curative, since relapses do not occur after adequate treatment of *P falciparum* infections. No previous subject infected with NF54 (lot 3D7) *P falciparum* and treated by WRAIR or NMRC has had a malaria recrudescence or relapse. Subjects will be advised to contact the principal investigator, and to advise their personal physician of their participation in this malaria study, if fever, headache, or other symptoms possibly related to malaria develop at any time within 6 months r after completion of the study. If contacted and in their medical judgement deemed appropriate, the principal investigator may arrange for diagnostic evaluation for malaria. In the unlikely event that malaria recurs, the subject will be retreated one of the study medications as above, or with an alternate anti-malarial as determined by the principal investigator.

## 9.5. Study Completion

When all the follow-up visits for the study are completed, subjects will be notified that they have completed the study. In addition, subjects who are treated for malaria following challenge will not be able to donate blood for 3 years, per the American Red Cross guidelines.

## 9.6. Concomitant Medications

At each study visit, the investigator will document any medications the subject has taken since the preceding visit. All medications taken by the subject beginning at enrollment until the end of Day 28 will be recorded.

## 9.7. Procedures for Monitoring Subject Compliance

The malaria challenge will be administered under direct supervision of investigational staff; therefore, no procedures for monitoring subject compliance are necessary. Subjects will receive telephone (verbal or text) reminders prior to their scheduled appointments and subjects will be contacted to schedule make-up visits for missed appointments.

# 10. SAFETY ASSESSMENT

Safety monitoring will be conducted throughout the study; therefore safety concerns will be identified by continuous review of the data by the PI, clinic staff, clinical monitor, research monitor, and United States Army Medical Research and Materiel Command (USAMRMC) Office of Regulated Activities (ORA) Product Safety Surveillance Branch (PSSB).

**Study Safety Management:** The IRB, research monitor, and principal investigator will review any safety concern. A data safety monitoring board (DSMB) is not required for this study.

**Research Monitor:** The Department of Defense research monitor is responsible for overseeing the safety of the research and report observations/findings to the IRB or a designated institutional official. The research monitor will review all unanticipated problems involving risks to subjects or others associated with the protocol and provide an independent report of the event to the IRB. The research monitor may discuss the research protocol with the investigators; shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor’s report; and shall have the responsibility to promptly report their observations and findings to the IRB or other designated official and the HRPO.

In addition to the responsibilities above the research monitor is required to review and provide an unbiased written report for all SAEs and subject deaths to the ORA PSSB (Safety Office) within 24 hours of their awareness of the event using the Research Monitor Report form. The report provided must include, at a minimum, a brief summary of the research monitor's review of the event and event outcome, relationship of the event to the investigational product and whether or not the research monitor concurs with the details of the study investigator's report. For this protocol the secondary research monitor will provide input in the event of a discrepancy between the investigator’s report and the primary research monitor’s report.

The ORA Safety Pharmacovigilance (PVG) physician, as delegated by the sponsor, evaluates all safety cases and provides the final determination on relatedness to the product, and whether expedited reporting is warranted, per current FDA regulation and guidance.

**USARMRC ORA PSSB:** PSSB is responsible for coordinating and integrating the review of safety data regarding The Surgeon General (TSG)-sponsored products. The PSSB reviews each SAE report for medical consistency, accuracy, and completeness and follows each event until it is satisfactorily resolved.

## 10.1. Adverse Event Definitions

The following terms, as defined by 21 CFR 312.32, apply to IND safety reporting.

### 10.1.1. Adverse Event or Suspected Adverse Reaction

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

### 10.1.2. Solicited Adverse Event

For this study, a solicited AE is an event expected as part of malaria infection. The solicited AEs for this study include:

- Pruritus at the challenge site
- 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)

### 10.1.3. Serious Adverse Event or Serious Suspected Adverse Reaction

An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- Life-threatening adverse event: an adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

### 10.1.4. Unexpected Adverse Event or Unexpected Suspected Adverse Reaction

An adverse event or suspected adverse reaction is considered “unexpected” if it is not consistent with malaria infection.

### 10.1.5. Unanticipated Problems Involving Risks To Subjects Or Others

Federal regulations require that unanticipated problems involving risks to subjects or others be promptly reported to the IRB. These events encompass a broader category of events than SAEs and may include issues such as problems with loss of control of subject data or the investigational product; adverse psychological reactions; or breach of confidentiality. Risks to others (eg, program personnel) must also be reported.

Unanticipated problems involving risks to subjects or others are any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given (a) the procedures that are described in the protocol, investigators brochure or informed consent document; and (b) the characteristics of the subject population;
- Related or possibly related to a subject’s participation in the study; and
- Suggests that the study places subjects or others at a greater risk of harm than was previously known or recognized.

The IRB and/or the ORP will evaluate reports from the PI and research monitor to determine whether a given incident, experience or outcome constitutes an unanticipated problem involving risk to subjects or others and, in coordination with the sponsor, ensure upward reporting of the unanticipated problems involving risk to subjects or others to the appropriate regulatory offices.

## 10.2. Adverse Event Relationship to Investigational Product

The investigator must assign a relationship of each AE to the receipt of the investigational product (in this case, malaria challenge). The investigator will use clinical judgment in conjunction with the assessment of a plausible biologic mechanism, a temporal relationship between the onset of the event in relation to receipt of the investigational product, and identification of possible alternate etiologies including underlying disease, concurrent illness or concomitant medications. The guidelines in Table 6 should be used by investigators to assess the relationship of an AE to study product administration. Only a physician can make this determination.

**Table 6: Adverse Event Relationship to the Investigational Product Categories**

| Relationship | Description |
|---|---|
| Not related | No reasonable causal relationship between the investigational product administered and the SAE. Applies to those events for which evidence exists that there is an alternate etiology. |
| Unlikely | Likely unrelated to the investigational product. Likely to be related to factors other than investigational product, but cannot be ruled out with certainty. |
| Possibly | An association between the event and the administration of investigational product cannot be ruled out. There is a reasonable temporal association, but there may also be an alternative etiology such as the subject's clinical status or underlying factors including other therapy. |
| Probably | There is a high degree of certainty that a relationship to the investigational product exists. There is a reasonable temporal association, and the event cannot be explained by known characteristics of the subject's clinical state or factors including other therapy. |
| Definite | An association exists between the receipt of investigational product and the event. An association to other factors has been ruled out. |

## 10.3. Adverse Event Severity Assessment

All AEs will be assessed for severity by the investigator. Inherent in this assessment is the medical and clinical consideration of all information surrounding the event including any medical intervention required. Each event will be assigned one of the following categories: mild, moderate, severe, potentially life-threatening or fatal. Refer to the grading scale in Appendix D for further guidance in the assignment of severity. The criteria in Table 7 may be used for any symptom not included in the grading scale. Any Grade 4 (life-threatening) or Grade 5 (fatal) AE must be reported as an SAE.

The CRF for AEs will reflect only the highest severity for continuous days an event occurred.

**Table 7: Adverse Event Severity Grading Scale**

| Severity | Grade | Description |
|---|---|---|
| Mild | 1 | Does not interfere with routine activities<br>Minimal level of discomfort |
| Moderate | 2 | Interferes with routine activities<br>Moderate level of discomfort |
| Severe | 3 | Unable to perform routine activities<br>Significant level of discomfort |
| Potentially life-threatening | 4 | Hospitalization, ER, or urgent intervention indicated |
| Fatal | 5 | Results in death |

FDA guidelines for toxicity will be followed; however, if a subject is evaluated in an emergency room for nonlife threatening illness or symptoms (ie, visits emergency department on weekend for mild problems because the physician's office is closed), the information from that visit will be reviewed and severity of the adverse event will be assessed according to the subject's clinical signs and symptoms.

As defined by the ICH guideline for GCP, the term "severe" is often used to describe intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious", which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

## 10.4. Recording Adverse Events

### 10.4.1. Methods/Timing for Assessing, Recording, and Analyzing Safety Endpoints

AEs and SAEs will be assessed at all study visits, documented in the source records, and recorded on the CRFs using accepted medical terms and/or the diagnoses that accurately characterize the event. It should be noted that the form for collection of SAE information is not the same as the AE CRF. Where the same data are collected, the forms must be completed in a consistent manner. The same AE terms, for example, should be used on both forms. When a diagnosis is known, the AE term recorded on the CRF will be the diagnosis rather than a constellation of symptoms. The investigator will assess all AEs for seriousness, relationship to investigational product, severity, and other possible etiologies and record the following on the AE CRF: a description of the event ( if the event consists of a cluster of signs and symptoms, a diagnosis should be recorded rather than each sign and symptom); onset date and time; stop date and time; intensity (recorded as mild, moderate, or severe, potentially life threatening, or fatal); seriousness (an SAE Report Form must also be completed for SAEs); causality (relationship to study drug); outcome (eg, not recovered/not resolved (ongoing), recovering/resolving, recovered/resolved, recovered/resolved with sequelae, death, or unknown (if applicable); and action taken with the study agent (eg, no action, study agent discontinued, or other action taken. When an event has not resolved by study closure, it will be documented on the AE CRF as "ongoing".

The timeframe for the collection of AEs and SAEs begins on the day of the challenge through 28 days post challenge.

### 10.4.2. Duration of Follow-Up of Subjects after Adverse Events

Investigators will follow AEs until resolution or to the end of the study (if not previously resolved), and SAEs to resolution, even if this extends beyond the prescribed reporting period. Resolution is the return to baseline status or stabilization of the condition with the probability that it will become chronic. The SAE outcomes will be reported to the sponsor's representative using the Serious Adverse Event Report Form the USAMRMC Office of Regulated Activities, Product Safety Surveillance Branch, SAE mailbox: usarmy.detrick.medcom-usamrmc.mbx.sae-reporting@mail.mil using the Serious Adverse Event Report Form.

Investigators are not obligated to actively seek SAEs in former subjects; however, if a SAE, considered to be related to the investigational product is brought to the attention of the investigator *at any time* following completion of the study, the event will be reported to the sponsor's safety office as defined in Section 10.5.

## 10.5. Reporting Adverse Events

The PI will report all AEs to the USAMRMC, and the IRB of record in the appropriate safety, annual, and/or final reports. After appropriate data cleaning and query resolution between the clinical site, sponsor's clinical monitor, and clinical data manager, SAEs from the clinical database will be reconciled with the sponsor's SAE database

### 10.5.1. Reporting Serious and Unexpected Adverse Events

Contact information for reporting SAEs is provided in Table 8.

#### 10.5.1.1. Reporting to the Sponsor

All SAEs must, at a minimum, be reported by emailing or faxing the initial SAE Report Form promptly (within 24 hours) to the sponsor's safety office, the Office of Regulated Activities, Product Safety Surveillance Branch as per 21 CFR 312.64, whether or not the event is considered related to study product. Further, the investigator should comply with relevant study site SOPs on reporting SAEs.

The minimum information that the investigator will provide to the USAMRMC PSSB is specified in Table 9. The sponsor's representative may request additional information for purposes of the study.

**Table 8: Study Contacts for Reporting Serious Adverse Events and Unanticipated Problems Involving Risk to Patients or Others**

| | |
|---|---|
| **Sponsor's Safety Office** | US Army Medical Research & Materiel Command<br>ATTN: MCMR-UMR<br>1430 Veterans Drive<br>Fort Detrick, MD 21702-5009<br>Fax: 301-619-7790<br>Telephone: 301-619-1005<br>Email: usarmy.detrick.medcom-usamrmc.mbx.sae-reporting@mail.mil |
| **Institutional Review Board** | WRAIR IRB<br>503 Robert Grant Avenue<br>Silver Spring, MD 20910<br>Telephone: (301) 319-9940<br>Fax: (301) 319-9961<br>Email: usarmy.detrick.medcom-wrair.mbx.hspb@mail.mil |
| **USAMRMC Office of Research Protections** | Human Research Protection Office<br>US Army Medical Research and Materiel Command<br>ATTN: MCMR-RPH<br>504 Scott Street<br>Fort Detrick, MD 21702-5012<br>Fax: 301-619-7803<br>Telephone: 301-619-2165<br>Email: usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil |
| **Research Monitors** | Ramiro Gutierrez, MD, CAPT, MC, USN<br>NMRC<br>503 Robert Grant Avenue<br>Silver Spring, MD 20910<br>Tel: 301-319-3193<br>Email: Ramiro.l.gutierrez.mil@mail.mil |

**Table 9: SAE Information to be Reported to the Sponsor's Safety Office**

| Notification Method | Information to be Provided |
|---|---|
| **Email or Telephone (within 24 hours)** | IND number, sponsor study number, name of the investigational product, and investigator name and contact number |
| | Subject identification number |
| | SAE term, description, onset date, date(s) of investigational product administration, severity, relationship to the investigational product, and subject's current status |
| **AND** | |
| **Email or Fax** | Sponsor-approved Serious adverse event report form |
| | Medical record progress notes including pertinent laboratory/diagnostic test results |

NOTE: When submitting SAE reports via email, the subject line of each email notification will read as follows:

**SAFETY REPORT – IND # _____, Sponsor Study #_____, Subject# _____, Event term: _____**

In order to comply with regulations mandating sponsor notification of specified SAEs to the FDA within 7 calendar days, investigators must submit additional information as soon as it is available. The sponsor's representative will report unexpected SAEs associated with the use of the drug to the FDA as specified at 21 CFR 312.32 (c).

Investigators must follow all relevant regulatory requirements as well as specific policy regarding the timely reporting of SAEs to the research monitor and the IRB and/or the USAMRMC ORP.

Reporting to the sponsor's safety office does not fulfill the investigator's duty to report all unanticipated problems involving risk to human subjects or others to the IRB. The PI will notify the IRB and/or the USAMRMC ORP, and the research monitor.

#### 10.5.1.2. Reporting to the IRB

Unanticipated problems involving risk to subjects or others, SAEs related to participation in the study, and all subject deaths related to participation in the study should be promptly reported by telephone, email, or fax to the IRB and/or the USAMRMC ORP. A complete written report should follow the initial notification.

Investigators are required to forward safety information provided by the sponsor's representative to the IRB.

### 10.5.2. Reporting Additional Immediately Reportable Events to the Sponsor's Safety Office, the IRB, and/or the USAMRMC ORP

#### 10.5.2.1. Pregnancy

Each pregnancy must be reported immediately (within 24 hours of identification) by completing and submitting the Pregnancy Report Form by email or fax to the sponsor's safety office

(USAMRMC Product Safety Surveillance Branch). Report the incident to local IRB and/or the USAMRMC ORP in accordance with IRB policy.

Subjects who become pregnant after Day 0 will be followed to term, and the following information will be gathered and documented on the Pregnancy Report Form:, for outcome, type, date of delivery, Apgar scores, and health status of the mother and child including the child's gender, head circumference, gestational age at delivery, height, and weight. Complications and or abnormalities should be reported including any premature terminations. A pregnancy is reported as an AE or SAE only when there is suspicion that the investigational product may have interfered with the effectiveness of contraception or there was a serious complication in the pregnancy including a spontaneous abortion or an elective termination for medical rationale. Any subject determined to be pregnant after malaria challenge, but before diagnosis and/or treatment for malaria, will be treated empirically for malaria using a protocol antimalarial that is safe for use in pregnancy.

#### 10.5.2.2. AE-related Withdrawal of Consent

Any AE-related withdrawal of consent during the study must be reported immediately (within 24 hours of identification) by email or fax to the sponsor's representative. Report the withdrawal to local IRB and/or the USAMRMC ORP in accordance with IRB policy.

#### 10.5.2.3. Pending Inspections/Issuance of Reports

The knowledge of any pending compliance inspection/visit by the FDA, Office for Human Research Protections (Department of Health and Human Services), or other government agency concerning clinical investigation or research, the issuance of Inspection Reports, FDA Form 483, warning letters, or actions taken by any regulatory agency including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to the local IRB and/or the USAMRMC ORP and the sponsor's representative (USAMRMC ORA).

### 10.5.3. Annual and Final Reports

SAEs and AEs for inclusion in annual and final reports to the FDA will be provided from the clinical database by the clinical data manager in USAMRMC ORA.

### 10.5.4. IND Annual Report to the FDA

The PI will be responsible for the preparation of a detailed annual synopsis of clinical activity, including adverse events, for submission to the sponsor's representative (USAMRMC ORA). Each annual report will summarize IND activity for 1 year beginning approximately 3 months before the IND FDA anniversary date. The sponsor's representative will notify the PI of the due date with sufficient time for the PI to assemble the required information.

### 10.5.5. Final Report

A final study report will be prepared in accordance with "Guidance for Industry: Submission of Abbreviated Reports and Synopses in Support of Marketing Applications", ICH E3 Guideline "Structure and Content of Clinical Study Reports", and electronic Common Technical Document requirements and standards and provided to the sponsor's representative for review and approval.

The sponsor's representative will use this report to prepare the final clinical study report for submission to the FDA.

# 11. STATISTICS

## 11.1. Description of Statistical Methods

All data will be collected and verified prior to analysis. No interim analyses are planned. The statistical analyses for the trial objectives will be executed as outlined below, unless modified via a protocol amendment or justified in the final report, as applicable.

A formal statistical analysis plan (SAP), as a separate document, will contain detailed information regarding data analyses to be performed and will be finalized before database lock or data analysis occurs.

### 11.1.1. Analysis Addressing the Primary Study Objective

The primary objective of this trial is to evaluate the infective efficacy of the new lot of NF54 (clone 3D7) parasites within the standard WRAIR CHMI model.

This trial will evaluate the primary objective by informally comparing the efficacy of the new lot, defined as the proportion of subjects with parasitemia confirmed by blood smear, to the proportion of historically positive controls from previous studies. Since 2012 (the time of the last significant update to challenge procedures) infectivity control subjects in WRAIR CHMI trials have demonstrated an overall efficacy of 95.1% (range 83.3-100%), as can be calculated from Table 2 in this protocol. A meta-analysis using a mixed model with random intercepts and study effects provides a 95% confidence interval of the mean to be 90.1% to 97.6%.

No formal hypothesis testing of the primary objective will be conducted as the sample size needed to conduct a true non-inferiority study is far too great. Following FDA guidance on non-inferiority trials, a trial of 220 subjects would be required to detect a difference with the same mean efficacy (95.1%), 80% power, a one-sided alpha of 0.025, and a non-inferiority margin set at the 95% lower bound of the historic data (90.1%). This sample size could be reduced to 36 subjects if the efficacy of the new lot is 100%, of which there is no empirical evidence (calculated using PASS 16). Such a trial size would represent one of the largest CHMI trials ever performed, and be logistically and financially untenable under current resources. In addition, there are potential safety and ethical concerns with exposing such a large number of persons to a potentially fatal disease using an untested lot of parasites, whose characteristics, though unlikely, may prove different (ie, less safe) than the old lot.

Every CHMI at WRAIR since 2012 has utilized 6 infectivity control subjects per challenge day. In all of these trials, at least 5 of the 6 control subjects developed malaria. If the new lot's initial infective efficacy is lower than the minimum historical infective efficacy of the old lot (5/6, 83.3%), then the new lot will be considered insufficiently infective, and not be used for further human trials without revision, replacement, and/or further bridging trials. However, if the infective efficacy meets the minimum historical level (at least 10 of the 12 subjects are positive), then the new lot will be considered acceptable for possible use in trials with experimental product. If efficacy is acceptable, it is intended that these 12 subjects will provide the nucleus of a new lot data set that will eventually grow large enough, with the addition of control data from

future studies, to perform a true non-inferiority analysis using standardized meta-analysis techniques to compare the two lots.

### 11.1.2. Analysis Addressing the Secondary Study Objective

#### 11.1.2.1. Time to Parasitemia

Time to parasitemia will be determined for all subjects for both smear and qPCR. Time to parasitemia will be defined as the time (in Days) from malaria challenge to confirmed parasitemia. Confirmed parasitemia by malaria smear will be defined as the observation of 2 confirmed parasites on a single smear. Confirmed parasitemia by qPCR will be defined as a single positive qPCR assay result.

Time to parasitemia for individual subjects, the median and range time-to-parasitemia, and 95% confidence intervals using a *t* distribution for each diagnostic method will be determined.

#### 11.1.2.2. Time to Clearance

Time to Clearance will be determined for all subjects for both smear and qPCR. Time to Clearance will be defined as the time (in Days) from malaria diagnosis to confirmed clearance of parasitemia. Confirmed clearance of parasitemia by malaria smear will be defined as the observation of 3 sequential negative (no parasites observed) daily smears. Confirmed clearance of parasitemia by qPCR will be defined as 2 sequential daily (approximately 24 hours apart) negative qPCR assay results. For clarity, the day of the final test in each sequence (smear, PCR) will be used as the day of confirmed clearance for calculation purposes (ie, the clearance will not be 'backdated' to the first negative result).

Time to clearance for individual subjects, the median and range time-to-clearance, and 95% confidence intervals using a t distribution for each diagnostic method will be determined.

### 11.1.3. Safety Analysis

Safety analysis will include data collected from all subjects. Adverse event data will be listed individually (including intervention and outcome) and summarized. Serious and/or unexpected AEs will also be discussed on a case-by-case basis. The frequency of each adverse event type will be presented along with a 95% confidence interval using an exact method for binomial distributions.

## 11.2. Planned Enrollment and Reason for Sample Size

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 10 displays the infective efficacy rates and the associated 95% confidence intervals.

**Table 10: 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 10 , 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 11 displays the width of the confidence intervals for adverse events using an exact binomial calculation.

**Table 11: 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.

## 11.3. Level of Significance to be Used

No formal statistical testing is planned in this analysis. Confidence intervals will be presented when appropriate and will use a 95% confidence interval.

## 11.4. Statistical Criteria for the Termination of the Trial

There are no statistical criteria for study termination in this trial.

## 11.5. Accounting for Missing, Unused, and Spurious Data

The SAP will be followed even if data are missing. Subjects with missing data will not be replaced nor will missing values within any subject's record be imputed. Non analyzable data will be documented in the deviations.

## 11.6. Procedures for Reporting Deviations from the Original Statistical Plan

Any deviation(s) from the original SAP as indicated in the protocol will be described in an amendment to the protocol and the SAP. Deviations from the SAP will be documented in accordance with study site SOPs.

## 11.7. Selection of Subjects to be Included in Analyses

For analysis of the primary objective, as well as for the secondary objectives regarding time to parasitemia and clearance of parasitemia, data from all subjects who are challenged will be included.

# 12. ADMINISTRATIVE

## 12.1. Ethics

### 12.1.1. Ethics Review/Institutional Review Board

This study will be conducted in accordance with all applicable Federal and DoD human research protections requirements and the Belmont Principles of respect for persons, beneficence, and justice.

The procedures set out in this study are designed to ensure that the sponsor's representative and all study personnel abide by the principles of the ICH GCP Guideline and the CFR. The PI confirms this by signing this study protocol and FDA Form 1572.

The study will be conducted under a protocol reviewed by the IRB of record. The study is to be conducted by scientifically and medically qualified persons. The IRB will determine whether the benefits of the study are in proportion to the risks. The rights and welfare of the subjects will be respected; the physicians conducting the study will ensure that the hazards do not outweigh the potential benefits; the results to be reported will be accurate; subjects will give their informed consent and will be competent to do so and not under duress; and all study staff will comply with the ethical principles in 21 CFR Part 50 and the Belmont Principles.

Enrollment in this protocol will not begin until all required approvals have been obtained and the formal authorization letter is received by the PI from the sponsor's representative.

### 12.1.2. Confidentiality

In this research, the subject's health information will be collected and used to conduct the study; to monitor the subject's health status; to measure effects of the malaria challenge; to determine research results, and possibly to develop new tests, procedures, and commercial products. Health

information is used to report results of research to the sponsor's representative and Federal regulators and may be reviewed during study audits for compliance with study plans, regulations, and research policies. After the study ends, each subject has the right to see and receive a copy of his/her information.

Representatives of the TSG as the IND sponsor, USAMRMC ORA as the sponsor's representative, the local IRB and/or the ORP, the DoD, and the FDA are eligible to photocopy and review records related to this protocol as a part of their responsibility to protect the participants of this protocol. In addition, these representatives are eligible to witness the applicable study procedures to assure the safety of subjects.

No personal identifier will be used in any publication or communication used to support this research study. The subject's identification number will be used in the event it becomes necessary to identify data specific to a single subject.

Although every measure will be taken to assure the confidentiality of subjects in this study, there is the theoretical risk associated with breach of confidentiality by participating in this study. In a few select circumstances, complete confidentiality will be unable to be guaranteed for subjects. These will include required reporting of transfusion-transmissible infectious diseases (such as HIV or hepatitis) to applicable military and public health agencies, and required reporting of criminal offenses under the Uniform Code of Military Justice (UCMJ) to appropriate military authorities. All subjects will be informed of these exceptions to confidentiality during the consent process.

### 12.1.3. Compensation for Participation

A subject will be compensated for the time and effort required for his or her participation in this study and for specific study procedures (such as blood collection). A breakdown of specific compensation for each visit is provided in the consent documentation.

Compensation for screening will be $50 for all potential subjects, regardless of whether that potential subject is eligible for or interested in enrollment in the study. Compensation for each study visit will range from $50 to $350 (including bonus) for civilian subjects who are not Federal employees. For subjects who are Federally employed (military or civilian), compensation is limited to $50 per visit and can only be given for visits in which blood is drawn, unless the subject is on approved leave, or the visit occurs outside of normal duty hours. In these cases, Federally-employed subjects will be eligible for the same compensation as civilian subjects who are not Federal employees.

For unscheduled visits which involve a laboratory draw, subjects will be compensated $50. Assuming no unplanned visits, civilian (non-Federal employee) subjects who participate will be receive up to approximately $2875 in compensation. Federally-employed subjects who participate will receive up to approximately $1250 in compensation, unless they are on approved leave or participating outside of normal duty hours.

Subjects who choose to stop participation in the study will retain all of the compensation to which they are entitled up to the time they leave the study. Subjects who are removed from the study by the investigator for failure to comply with study procedures will retain all of the compensation to which they were entitled up to the time of the action.

Subjects will not be compensated for travel expenses incurred as a result of their participation in this study nor will transportation be provided for them.

Compensation payments will be distributed after completion of the pertinent study visit or phase. Payment will be by cash, check, or pre-paid debit/ATM card or by direct deposit. Payment will generally either be provided by direct hand-to-hand distribution to the subject, or through electronic allotment to a previously distributed pre-paid debit/ATM card. However, in rare cases checks may be mailed to subjects. Payments for several visits may be bundled for efficiency and convenience of study staff. Subjects will be informed in the consent process that if they receive more than $600 for their participation in the study, study personnel will be required to report subject compensation as income to the IRS via a Form 1099.

The payments described above are the only financial compensation available to subjects for their participation in this research.

### 12.1.4. Medical Care for Research-Related Injury

All non-exempt research involving human subjects shall, at a minimum, meet the requirement of 32 CFR 219.116(a)(6).

If a subject is injured because of participation in this research and is a DoD healthcare beneficiary (eg, active duty in the military, military spouse or dependent), the subject is entitled to medical care for that injury within the DoD healthcare system, as long as the subject remains a DoD healthcare beneficiary. This care includes, but is not limited to, free medical care at Army hospitals or clinics.

If a subject is injured because of participation in this research and is not a DoD healthcare beneficiary, the subject entitled to medical care for that injury at an Army hospital or clinic; medical care charges for care at an Army hospital or clinic will be waived. The subject is also entitled to care for that injury, but such care for that injury at other DoD (non-Army) hospitals or clinics may be limited by time, and the subject's insurance may be billed. It cannot be determined in advance which Army or DoD hospital or clinic will provide care. If the subject obtains care for research-related injuries outside of an Army or DoD hospital or clinic, the subject or the subject's insurance will be responsible for medical expenses.

### 12.1.5. Written Informed Consent

The informed consent process and document will be reviewed and approved by the local IRB and/or the ORP and sponsor's representative prior to initiation of the study. The consent document contains a full explanation of the possible risks, advantages, and alternate treatment options, and availability of treatment in the case of injury, in accordance with 21 CFR 50. The consent document indicates that by signature, the subject permits witnessing of applicable study procedures by the sponsor's representative, as well as access to relevant medical records by the sponsor's representative and by representatives of the FDA. The sponsor's representative will submit a copy of the initial IRB- and sponsor's representative-approved consent form to the FDA and will maintain copies of revised consent documents that have been reviewed and approved by the local IRB and/or the ORP.

A written informed consent document will be signed by the subject before any study-related procedures are initiated for that subject. This consent document must be retained by the

investigator as part of the study records. Each subject will receive a copy of the signed informed consent document. The investigators or their designees will present the protocol in lay terms to individual subjects. Questions on the purpose of the protocol, protocol procedures, and risks to the subjects will then be solicited. Any question that cannot be answered will be referred to the PI. No subject should grant consent until questions have been answered to his/her satisfaction. The subject should understand that the study product is an investigational drug and is not licensed by the FDA for commercial use, but is permitted to be used in this clinical research. Informed consent includes the principle that it is critical the subject be informed about the principal potential risks and benefits. This information will allow the subject to make a personal risk versus benefit decision and understand the following:

- Participation is entirely voluntary,
- Subjects may withdraw from participation at any time,
- Refusal to participate involves no penalty, and
- The individual is free to ask any questions that will allow him/her to understand the nature of the protocol.

Should the protocol be modified, the subject consent document must be revised to reflect the changes to the protocol. If a previously enrolled subject is directly affected by the change, the subject will receive a copy of the revised informed consent document. The approved revision will be read, signed, and dated by the subject.

The subject will be informed that a description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by US law.

## 12.2. Protocol Approval and Modification

### 12.2.1. Review/Approval of Study Protocol

Before a clinical study can be initiated, the study protocol and other required documents will be submitted to the following departments in the order listed for review and/or approval, with the final review by the FDA:

- Integrated Product Team
- Sponsor's Representative Team (USAMRMC ORA)
- Sponsor Protocol Review Board
- Local IRB and/or the ORP
- Sponsor's Representative (acting for The Surgeon General of the Army)

Enrollment in this protocol will not begin until all approvals have been obtained and the formal authorization letter is received by the PI from the sponsor's representative.

### 12.2.2. Protocol Modifications

All modifications to the protocol and supporting documents (informed consent, study-specific procedures, SOPs, recruitment materials, etc) will be reviewed and approved prior to implementation. Any protocol amendment will be agreed upon and approved by the sponsor's

representative prior to submission to the local IRB and/or the ORP and prior to implementation of said change or modification. Any modification that could potentially increase risk to patients will be submitted to the FDA prior to implementation.

The informed consent document will be revised to concur with any amendment as appropriate and must be reviewed and approved with the amendment. Any subject already enrolled in the program will be informed of the revision and asked to sign the revised informed consent document if the modification directly affects the individual's participation in the program. A copy of the revised, signed, and dated informed consent document will be given to the patient. All original versions of the informed consent document will be retained in the protocol regulatory file, and a copy will be retained in the protocol regulatory file.

### 12.2.3. Protocol Deviation Procedures

All subject-specific deviations from the protocol (eg, failure to return for follow-up visits or blood collection within the time indicated in the protocol) will be documented. The PI or designee will be responsible for identifying and reporting all deviations, which are defined as isolated occurrences involving a procedure that did not follow the study protocol or study-specific procedure. Deviations will be reported annually in the continuing review report to the local IRB and/or the ORP and, if appropriate, in the final study report. Action taken in response to the deviation, and the impact of the deviation will be assessed by the PI or subinvestigator and recorded as significant or nonsignificant.

Significant or major deviations are departures from protocol that have a potential to affect the rights of the research participant, to increase the risk to the participant, to change the willingness of the subject to continue participation, or to compromise the integrity of the study data in such a way that the study objectives may not be achieved. Major deviations that occur in greater than minimal risk protocols will be reported to the IRB of record within 48 hours of identification and recorded in the deviation study log, and a written report submitted within 10 working days of becoming aware of the deviation. All reports will be submitted with a cover memo naming the protocol, description of the event, summary of any harm to study participant(s) and steps to prevent further deviations. A summary of the major deviations occurring within the reporting period should also be included in the continuing review reports.

Insignificant or minor deviations are routine departures that typically involve a subject's failure to comply with the protocol (Example: missing scheduled visits). Minor deviations that occur in greater than minimal risk protocols will be reported to the IRB of record in the Continuing Review Report(s) or Closeout Report.

Any protocol deviation that adversely affects the safety or rights of a subject or scientific integrity of the study or any instances of serious or continuing non-compliance with the regulations or requirements will be reported immediately to the sponsor's representative (through USAMRMC ORA), the local IRB, and/or the ORP.

## 12.3. Sample and Data Handling

The primary source document for this study will be the subject's medical record. If separate research records are maintained by the investigator(s), the medical record and the research records will be considered the source documents for the purposes of auditing the study. All

source documents and subject study files will be retained at the site and stored in a locked records room with key access only given to WRAIR CTC key personnel.

For this study, an EDC database system will be used for the collection of the study data in an electronic format. The EDC database system will be designed based on the protocol requirements, the approved eCRF layouts and specifications, and in accordance with 21 CFR Part 11. The eCRF layouts and specifications define and identify the applicable source data that will be collected and captured into the EDC database system. The applicable source data will be electronically entered by the study site designee onto the eCRF (data entry screens) in the EDC database system. The investigator is ultimately responsible for the accuracy of the data transcribed on the eCRF. Data monitoring and management will be performed in the EDC database system by the study clinical monitor and the designated data management group.

A detailed data management plan will be written and approved by the designated data management group and the Sponsor's oversight data management

### 12.3.1. Sample Handling and Management

Samples collected under this protocol will be used to conduct screening, protocol-related safety evaluations and to generate de-identified control pools for malaria-research assays and future use. The amounts of blood to be drawn are provided in Table 3 and Table 4. All samples will be collected using standard techniques and safety precautions. To maintain clarity and confidentiality, specimens will be labeled with at least the collection date or visit number, study number, and the applicable subject identification (ID) code (either screening or study) for the subject. Labels may be in barcode or text format.

If necessary, biological samples will be stored temporarily at the CTC to await transport to appropriate laboratories for processing and analysis. Transport and storage of these biological samples will be handled according to applicable SOPs.

Samples collected for safety assessments will be transported to the applicable local commercial laboratory for testing. Storage and destruction of these samples will be as per the applicable laboratory SOPs. It is expected that these samples will be destroyed by the testing facility shortly after successful completion of testing (as per their SOPs), and no long term storage of these samples is planned.

Samples collected for de-identified control pool generation and future use, will likewise be transported to the Malaria Serology Laboratory and Flow Cytometry Center at WRAIR for processing, use, and storage.

Storage and destruction of these samples will be as per the applicable facility SOPs. These samples, either individually, or in pool form, will be stored for a maximum of 20 years (counting from when the last subject performed the last study visit), unless local rules, regulations or guidelines arise in the interim which require different timeframes or different procedures.

Any study involving the future use of biological specimens obtained under this study will require the appropriate IRB approval. Specimens will not be stored for future use without written consent from the study subjects.

### 12.3.2. Data Management

The laboratory data will be either be uploaded as a batch file from the laboratories to the study database and/ or the site will transcribe the data from the source documents and then enter it into a validated, password-protected data management system. Source documents will be stored at the clinical site in an access-controlled, locked storage space.

Subjects will be identified on CRFs by a unique subject identification number and on source documents by name and date of birth. No personal identifier will be used in any publication or communication used to support this research study. The subject identification number will be used if it becomes necessary to identify data specific to a single subject. Representatives of USAMRMC, the sponsor's representative, the local IRB and/or the USAMRMC ORP, and the FDA are eligible to review medical and research records related to this study as a part of their responsibility to protect human subjects in clinical research. Personal identifiers will be removed from photocopied medical and research records.

### 12.3.3. Retention of Records

The PI must maintain all documentation relating to the study for a period of at least 2 years after the completion of the investigation. If it becomes necessary for the sponsor's representative or designee or the FDA to review any documentation relating to the study, the investigator must permit access to such records.

The PI will be responsible for retaining sufficient information about each subject, so that the sponsor's representative, the local IRB, the FDA, employees of USAMRMC, or other regulatory authorities may have access to this information should the need arise.

It is the policy of the USAMRMC that data sheets are to be completed for all subjects participating in research (Form 60-R, Volunteer Registry Data Sheet). The data sheets will be entered into this Command's Volunteer Registry Database. The information to be entered into this confidential data base includes the subject's name, address, and Social Security Number; study title; and dates of participation. The intent of this data base is twofold: first, to readily answer questions concerning an individual's participation in research sponsored by USAMRMC; and second, to ensure that USAMRMC can exercise its obligation to ensure research subjects are adequately warned (duty to warn) of risks and to provide new information as it becomes available. The information will be stored at USAMRMC for a minimum of 75 years. The Volunteer Registry Database is a separate entity and is not linked to the study database.

## 12.4. Study Monitoring

Study monitoring will be the responsibility of the USAMRMC ORA. Upon successful approval of the protocol and establishment of the regulatory file, the clinical monitor will establish a clinical monitoring plan. To ensure that the investigator and the study staff understand and accept their defined responsibilities, the clinical monitor will maintain regular correspondence with the site and may be present during the course of the study to verify the acceptability of the facilities, compliance with the investigational plan and relevant regulations, and the maintenance of complete records. As needed, the clinical monitor may witness the informed consent process or other applicable study procedures to assure the safety of subjects and the investigators' compliance with the protocol and GCPs.

Monitoring visits by a sponsor's representative-designated clinical monitor will be scheduled to take place at the initiation of the study, during the study at appropriate intervals, and after the last subject has completed the study. A report of monitoring observations will be provided to the PI (for corrective actions) and the product manager.

If appropriate, the monitoring plan will be provided to the IRB for review.

## 12.5. Audits and Inspections

Authorized representatives of the sponsor, the FDA, the independent ethics committee or institutional review board may visit the site to perform audits or inspections, including source data verification. The purpose of the audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guideline of the ICH, and any applicable regulatory requirements.

Knowledge of any pending compliance inspections/visits by OHRP, FDA, or other government agencies concerning clinical investigation or research, the issuance of inspection reports, warning letters or actions taken by any regulatory agencies including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to the IRB of record and USAMRMC ORA by phone, email, or by facsimile.

The HSPB will report knowledge of any pending inspections/audits by regulatory agencies to the US Army Medical Research and Materiel Command (USAMRMC), Office of Research Protections (ORP), Human Research Protection Office (HRPO) as per SOP UWZ-C-636.

## 12.6. Access to Source Data/Documents

The investigators and study staff, the DoD research monitor, members of the IRB of record, representatives of the USAMRMC including ORA, DoD, and other government agencies are authorized access to the study data as part of their duties and part of their responsibility to protect human subjects in research.

## 12.7. Reports

### 12.7.1. Annual and Final Reports

The principal investigator will report all AEs to USAMRMC and the local IRB in the appropriate safety, annual, and/or final reports. After appropriate data cleaning and query resolution between the clinical site, sponsor's clinical monitor, and clinical data manager, SAEs from the clinical database will be reconciled with the sponsor's SAE database. SAEs and AEs for inclusion in annual and final reports to the FDA will be provided from the clinical database by the clinical data manager in USAMRMC ORA.

#### 12.7.1.1. IND Annual Report to the FDA

The PI will be responsible for the preparation of a detailed annual synopsis of clinical activity, including adverse events, for submission to the sponsor's representative (USAMRMC ORA). Each annual report will summarize IND activity for 1 year beginning approximately 3 months

before the IND FDA anniversary date. The sponsor's representative will notify the PI of the due date with sufficient time for the PI to assemble the required information.

#### 12.7.1.2. Final Report

A final study report will be prepared in accordance with "Guidance for Industry: Submission of Abbreviated Reports and Synopses in Support of Marketing Applications", ICH E3 Guideline "Structure and Content of Clinical Study Reports", and electronic Common Technical Document requirements and standards and provided to the sponsor's representative for review and approval. The sponsor's representative will use this report to prepare the final clinical study report for submission to the FDA.

### 12.7.2. IRB Reports

#### 12.7.2.1. Continuing Review Reports

The PI will prepare and submit continuing review reports and comply with other in accordance with IRB guidance and expectations and will comply with other IRB expectations:

- Submitting the required continuing review reports and associated documents to the applicable HSPB, allowing sufficient time for review and continuation determination prior to the established continuing review date.
- Responding to all requests for information from the IRB of record, and complying with any determinations made by the IRB regarding the continuing review.
- Ensuring that no human subject research, including data analysis, is conducted on the protocol until IRB continuation is granted if the IRB approval lapses without continuing review approval.
- Tracking IRB approvals from the collaborative institutions if the research is collaborative in nature to ensure that they are all submitted in a timely manner.

#### 12.7.2.2. Closeout Report

The PI is responsible for submitting a closeout report and associated documents to the IRB upon the completion of the study.

## 13. PUBLICATION POLICY

All data collected during this study will be used to support this IND. All data may be published in the open medical or military literature with the identity of the subjects protected. Anyone desiring to publish or present data obtained during the conduct of the study will conform to local site policies and then forward the publication to USAMRMC ORA prior to submission.

# 14. LIST OF REFERENCES

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# 15. APPENDICES

# APPENDIX A. RECRUITMENT SCRIPT

**Study Title:**

"Bridging Trial to Evaluate the Infectivity Equivalence of the Current and New Lots of Plasmodium falciparum strain NF54 (clone 3D7) within the WRAIR Controlled Human Malaria Infection (CHMI) Model"

**Study Objective:**

This study is being conducted to evaluate the safety and effectiveness of a new lot of malaria parasites in causing human infection.

**Study Duration:**

Including the screening period, people who participate in this trial will be in the study for approximately 3 months.

**Number of People in the Study:**

12 volunteers12volunteers will be enrolled to undergo the malaria challenge procedure. A few others may be recruited to serve as alternates in case one of the original 12original12 cannot be challenged.

**Study Background:**

Malaria is a parasitic disease transmitted by mosquitoes. It is a significant cause of death and disability in tropical areas of Africa, Asia, Oceania (Pacific islands) and Latin America. The U.S. military and other groups around the world are working to develop treatments and vaccines against this disease. One of the best research tools these groups have to evaluate the effectiveness of their potential treatments is called the Controlled Human Malaria Infection (CHMI) model. In this model, subjects who have been treated with a drug or vaccine are exposed to malaria infection via the bite of laboratory-raised mosquitoes containing malaria parasites. If the drug or vaccine works, subjects remain healthy; if not, the subjects become ill with malaria and are treated. This model has worked safely and well for over 30 years, with over 1,500 people challenged in studies performed by the Department of Defense. Unfortunately, the current supply of primary type of parasites used for this challenge model is running out and needs to be replaced. A new supply of the parasites has been created, but needs to be tested in a small group of subjects to ensure it can cause infection, before it can be used in future drug and vaccine studies. That is the purpose of this trial.

**Brief Review of Inclusion / Exclusion Criteria (Full Criteria in Protocol):**

Inclusion:

- Men and non-pregnant, non-breast-feeding women, civilian or military
- Ages 18-50 (inclusive)
- In good health
- Able to make all study appointments
- No plans to travel to a country with malaria throughout the study

Exclusion:

- History of malaria
- History of living for more than 5 years total in an area with malaria
- Travel to a country with malaria in the last 6 months, or planned travel to a country with malaria during the study
- History of receiving of an investigational malaria vaccine at any time
- Use of malaria medications or certain antibiotics within the past 28 days
- History of allergy or other severe reaction to either mosquito bites or the malaria medications to be used in the trial [atovaquone/proguanil (Malarone), artemether/lumefantrine (Coartem), chloroquine]
- Heart, lung, liver, or kidney disease (high blood pressure, diabetes)
- Neurologic disease or chronic migraine headaches
- Splenectomy
- History of sickle cell trait, sickle cell disease, or other blood diseases
- Positive for HIV or viral hepatitis
- Use of any investigational drug or non-registered vaccine within 30 days before challenge
- Pregnancy or planned pregnancy during the study time period
- Active duty military volunteers will require approval from their supervisory chain
- Alcohol or drug abuse
- Any other significant finding that in the opinion of the clinical investigators would make participation in the study unsafe

**Study Plan:**

Screening

A person who wants to enroll in the study will be screened to ensure they are eligible to participate.

Screening includes:

- A detailed explanation of the study
- Completion of the informed consent documents
- Brief medical history and physical exam
- Urine and blood tests
- EKG (electrocardiogram which measures the "electrical activity" of the heart)

Challenge

- Volunteers will consent to participate in a malaria challenge to find out if the new lot of parasites is effective in causing malaria infection. The challenge involves being bitten by mosquitoes infected with the malaria parasite. The mosquitoes are contained in a small cup with a screen on top. This procedure doesn't hurt, but the volunteer's arm may itch later, as with any mosquito bite. Volunteers are expected to develop malaria from the mosquito bite.
- A urine pregnancy test will be performed for all women before the challenge

**Post-challenge and Hotel Phase:**

- Volunteers will need to return to the Clinical Trials Center daily from Days 1-9 after the challenge to provide blood for testing.
- Starting the night of the 9$^{th}$ (ninth) day after the initiation of malaria challenges, volunteers will need to check in to a local hotel for a maximum of 10 nights to allow for rapid assessment and treatment by study staff. The reason is that this is the period of time volunteers are most likely to develop malaria.
- Each morning, volunteers will be seen by a study physician and have a small amount of blood drawn to test for malaria.
- Each afternoon, volunteers will speak with a staff member to get test results and report how they feel.
- Volunteers will only have to sleep at the hotel, but can come and go for work and outside activities as long as they return for clinical visits.
- The symptoms of malaria include fever, chills, headache, fatigue, vomiting, diarrhea, muscle aches, and stomachache.
- If malaria does develop, volunteers will be treated with a medication taken by mouth. All drugs that might be used (chloroquine, Malarone, Coartem) are FDA approved and have been used safely for many years. These medications are expected to completely cure the malaria.
- All volunteers who do not develop malaria by Day 19 after challenge will be treated for malaria, regardless of whether they have any evidence of infection.

**Post-Hotel Phase:**

- Volunteers who do not complete their malaria treatment in the hotel will be required to come to the clinic for one or more days to complete their treatment and to have blood testing to confirm they are cured.
- All volunteers will have a final visit approximately 1 month after challenge.
- Blood will be drawn at most of the visits for this trial.

**Risks to the Volunteers may include:** (Note: study staff have methods to decrease or limit most, if not all, side effects)

- From challenge:

  - Local reaction at the site where mosquitoes bite
  - Side effects from the FDA approved anti-malaria medications (such as upset stomach, nausea, diarrhea, tiredness, ringing in ears)
- From malaria:
  - Fever
  - Chills
  - Headache
  - Fatigue
  - Vomiting
  - Diarrhea
  - Muscle aches
  - Stomach ache
- From blood draws: bruising at the site
- Loss of confidentiality: For each volunteer that participates in this study, there is a chance that limited volunteer information may be disclosed to persons outside of this study to include: the USAMRMC, the Food and Drug Administration (FDA), and the WRAIR Institutional Review Board (IRB). These representatives may have access to review research records as part of their responsibility to protect humans in research but they must also maintain confidentiality of volunteer records within the limits of the law.

**Compensation:**

Volunteers will be financially compensated for their time and effort.

**Study Setting:**

Outpatient

**Closing Remarks:**

If you are interested in learning more about this study or the Clinical Trials Center and what we do, please call us using the contact information below and provide the following information to the staff:

- Name
- Date of Birth
- Phone number
- Email address

Contact Information:WRAIR Clinical Trials Center

503 Robert Grant Ave, Silver Spring, MD 20910

**Hours:** Mon-Fri, 6:00am – 2:30pm

**Call:** 1-866- 428-2788

**Email:** usarmy.detrick.medcom-wrair.mbx.clinical-trials@mail.mil

**Text:** 301-215-0388

**Email:** usarmy.detrick.medcom-wrair.mbx.clinical-trials@mail.mil. We can store your information in a database if you wish to be contacted for future studies.

# APPENDIX B. INFORMED CONSENT COMPREHENSION ASSESSMENT

Name: _____

1. Medical screening for this study will include which of the following?
   A. Laboratory tests (including an HIV test)
   B. Physical examination
   C. Review of medical history
   D. All of the above

2. By participating in this study, you are expected to develop malaria.
   A. True
   B. False

3. The challenge phase of the study involves subjects being bitten by which of the following?
   A. Spiders infected with malaria
   B. Mosquitoes infected with malaria
   C. Ants infected with malaria

4. What are common findings associated with malaria infection?
   A. Fever
   B. Chills
   C. Headache
   D. All of the above
   E. None of the above

5. If you participate in this study, for how long will you be unable to donate blood?
   A. 3 years
   B. 25 years
   C. Never able to donate blood again

6. If you participate in this study, you may:
   A. Withdraw voluntarily at any time
   B. Never withdraw from the study

7. If our specific medical instructions are not followed in this study, malaria infection can cause

A. Death

B. Serious illness

C. Multi-organ failure

D. All of the above

8. Which of the following are true regarding pregnancy and participation in this study?

A. Pregnant women may participate in this study

B. Women should not get pregnant for 12 months after getting malaria

C. Women in the study must agree to use a form of birth control while participating in this study up until the last study visit (3 months after the challenge)

9. Which of the following is a component of this study?

A. Staying overnight in a hotel each night for up to 10 nights during the challenge phase

B. Staying overnight in a hospital for up to 10 nights during the challenge phase

C. Coming and going as I please throughout the study

10. Which one of the following is true regarding medical care for problems resulting from your participation in this study?

A. We will provide all necessary medical care for injury or disease resulting from your participation in this study as long as you are seen in an Army or DoD, Hospital or Clinic.

B. You or your personal insurance will be charged for any medical care rendered for problems associated with your participation in this study if you are not seen in an Army or DoD, Hospital or Clinic.

C. (A) and (B) are both correct.

Score: _____ Pass: _____ Not Pass: _____

_____ _____

Signature of Subject Date

_____ _____

Signature of Reviewer Date

**Answer Key:**

**Informed Consent Comprehension Assessment: Infectivity Control Subjects**

1) D

2) A

3) B

4) D

5) A

6) A

7) D

8) C

9) A

10) C

# APPENDIX C. NHANES I CARDIOVASCULAR RISK CRITERIA

**Figure 1: NHANES I Cardiovascular Risk Criteria for Males**

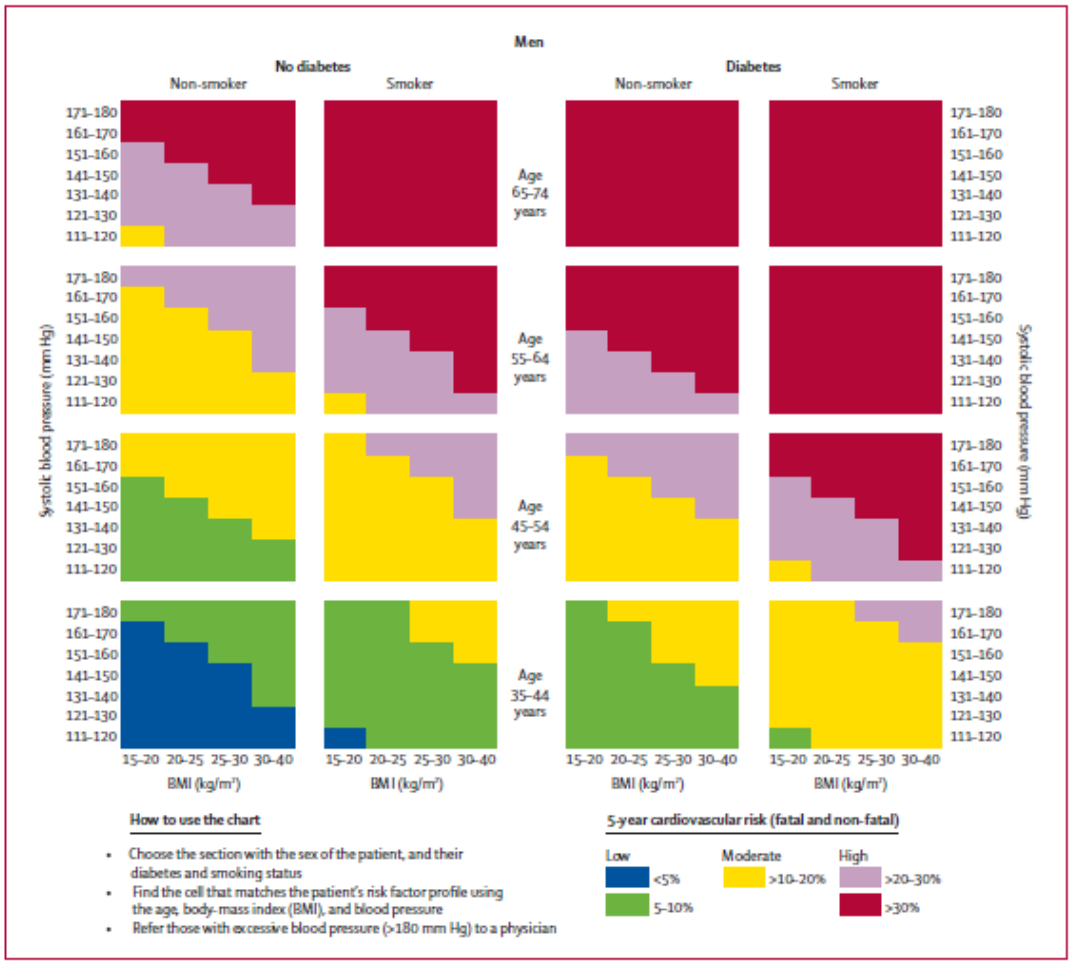
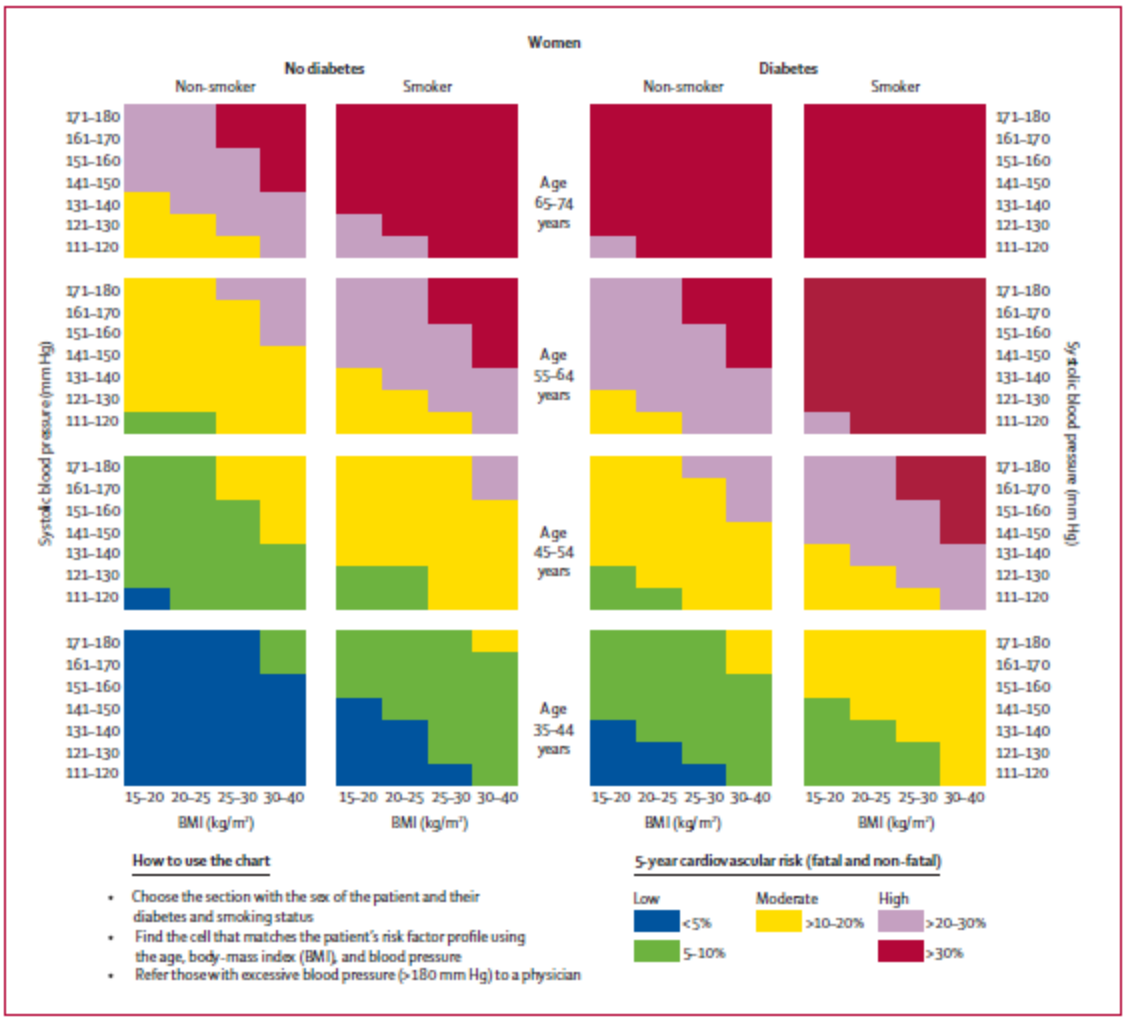


**Figure 2: NHANES I Cardiovascular Risk Criteria for Females**



# APPENDIX D. TOXICITY GRADING SCALE FOR ADVERSE EVENTS FOLLOWING MALARIA CHALLENGE

**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<br>> 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 |

| **Systemic AE** | **Mild (Grade 1)** | **Moderate (Grade 2)** | **Severe (Grade 3)** | **Potentially Life Threatening (Grade 4)** |
|---|---|---|---|---|
| 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 |
| 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 |

**Grading Scale for Abnormal Laboratory Values**

| 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 |
| 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 |

| Adverse event | Intensity grade | Intensity [a] |
|---|---|---|
| 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 |
| | 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 |

ULN: upper limit of normal range

[a] Grading scale adapted from "Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials", FDA, September 2007. Laboratory values provided in the table serve as guidelines.