TITLE: AQ-13 for Uncomplicated *Plasmodium falciparum* Malaria: Proof of Concept Study

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13th February 2017

Statement of Compliance

The study will be performed in accordance with Good Clinical Practice (GCP) as required by the following:

- U.S. Code of Federal Regulations applicable to clinical studies (45 CFR 46)
- ICH GCP E6
- Completion of Human Subjects Protection Training
- Terms of the FDA Office of Orphan Product Development Notice of Grant Award

Refer to: http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.htm#46.
http://www.fda.gov/cder/guidance/959fnl.pdf
http://grants.nih.gov/grants/guide/notice-files/NOT-OD-01-061.html

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Site Investigator (Principal Investigator):*

Signed: _____ Date: _____

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** The protocol should be signed by the local investigator who is responsible for the study implementation at his/her specific site; i.e., if Investigational New Drug study, the individual who signs the Form FDA 1572.*

Site Investigator (Mali PI):*

Signed: _____ Date: _____

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List of Abbreviations

AE	Adverse Event
AQ	aminoquinoline
AQ-13	Investigational aminoquinoline active against CQ- and multi-resistant *Plasmodium falciparum* parasites (*n*-propyl side chain)
AQ-72	mono–*N*-dealkylated metabolite of AQ-13
AQ-73	di-*N*-dealkylated metabolite of AQ-13
AUC	Integrated area under the curve of drug blood levels over time, which is used as a measure of oral bioavailability
β-hCG	Beta Human Chorionic Gonadotropin
BP	Blood Pressure (mm Hg)
C	Comparator (Coartem in these studies)
CBC	Complete Blood Count
CDC	Centers for Disease Control (and Prevention)
CFA	West African Franc ($1.00 US=500 CFA)
CFR	Code of Federal Regulations
CK	Creatine Kinase
Cl	Clearance of drug from the blood (by excretion or conversion to metabolites inactive against CQ-resistant parasites)
CPD	Citrate Phosphate Dextrose (anticoagulant)
CQ	chloroquine
CRF	Case Report Form
*CYP*450	Cytochrome P-450
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH
DSMB	Data and Safety Monitoring Board
ECG	ElectroCardioGram
EDTA	Ethylene Diamine Tetra-acetic Acid (anticoagulant)
F	Constant to adjust pharmacokinetic data for oral dosing
FDA	Food and Drug Administration
FWA	Federal-Wide Assurance (number)
GCP	Good Clinical Practice
G6PD	Glucose-6-Phosphate Dehydrogenase
H_0	Null hypothesis
H_1	Alternative hypothesis
Hb	Hemoglobin
Hct	Hematocrit
HPLC	High Performance Liquid Chromatography
ICH	International Conference on Harmonisation
ID	Identification (study) number for study participants
ICMJE	International Committee of Medical Journal Editors
IEC	Independent or Institutional Ethics Committee

List of Abbreviations - *continued*

IND	Investigational New Drug (Application)
IRB	Institutional Review Board
ITT	Intent-to treat (analysis)
K76T	Lysine to Threonine point mutation at position (amino acid) 76
M	Margin of non-inferiority
MOP	Manual of Procedures
MRT	Mean Residence Time
N	Number (typically refers to subjects)
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
OOPD	Office of Orphan Product Development (FDA)
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
P	Placebo
PCR	Polymerase Chain Reaction
PE	Physical Examination
pfcrt	*Plasmodium falciparum* Chloroquine Resistance Transporter
PI	Principal Investigator
QT	Interval from the beginning of ventricular depolarization (start of QRS) to the conclusion of repolarization (end of T wave)
QTc	QT interval adjusted (corrected) for heart rate
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
t ½	Half-Time (for concentration to decrease by 50%)
V_d	Volume of distribution (Liters)
WBC	White Blood Cell
WHO	World Health Organization
X	the percentage of the efficacy of the active control vs. placebo (C – P) that one desires to retain in the investigational drug (I) (see the Statistical Section on pages 37-40)

Title: AQ-13 for Uncomplicated *P. falciparum* Malaria: Proof of Concept Study

Population: Adult Malian males with uncomplicated *Plasmodium falciparum* Malaria
This study will include adult Malian males ≥18 years of age in good general health (who have no known medical conditions other than malaria). Subjects who agree to participate and provide their informed consent will be randomized to one of two study arms (33 per arm x 2 arms = 66 subjects) to receive either AQ-13 (1596 mg orally over 3 days) or Coartem (4 tabs twice daily x 3 days).

Number of Sites: There are several initial screening sites at: 1] the Missira/Sirakoroba Community Health Center in the Kolokani District and 2] referral outpatient clinics in and near Bamako. Although there is only one inpatient study site at the Clinical Research Center in Bamako, there will be two study follow-up sites for the 5 weeks of outpatient follow-up: the Community Health Center for Missira and Sirakoroba and the Clinical Research Center in Bamako.

Study Duration: The duration of this study will be up to 15 months (including follow-up), beginning on June 15th, 2013 and concluding on or before January 15, 2016.

Subject Duration: The duration of each subject's participation will be 6 weeks, based on a 1 week inpatient stay for treatment/observation, plus 5 weeks of twice-weekly follow-up.

Primary Objective and Primary Endpoints:

1] The primary objective is to determine and compare the cure rates for AQ-13 and Coartem. Cure is a lack of recrudescence within 42 days (PCR-corrected) – no evidence of recrudescence with the same parasite genotype(s) after reduction of the asexual parasitemia to less than 25% of the admission value by day 3 and clearance of asexual parasites and fever by day 7. Failure is defined as lack of cure.

Secondary Objectives and associated Endpoints for comparisons of AQ-13 with Coartem:

1] Adverse Events (AEs) – within 4 weeks of treatment as judged by **blinded** physician reviewers to be possibly related to the study drug(s).

2] Parasite clearance time – time from the initiation of treatment to the first of 2 successive negative thick smears.

3] Time to recrudescence or reinfection – time from the initiation of treatment to the reappearance of asexual *P. falciparum* parasites on the thick smear.

4] Effects on the QT (QTc) interval – mean changes from the baseline (pre-treatment) QTc interval to the post-treatment QTc interval after treatment with AQ-13 or Coartem.

5] Pharmacokinetic parameters for AQ-13 – will include measurements of AQ-13 blood levels over time (peak levels, AUC, MRT), drug distribution (Vd/F) and drug elimination (t½, Cl/F).

6] Fever clearance time: time in hours from the initiation of therapy until the disappearance of fever for at least 24 hours.

Tertiary Objectives and associated Endpoints for comparisons of AQ-13 with Coartem:

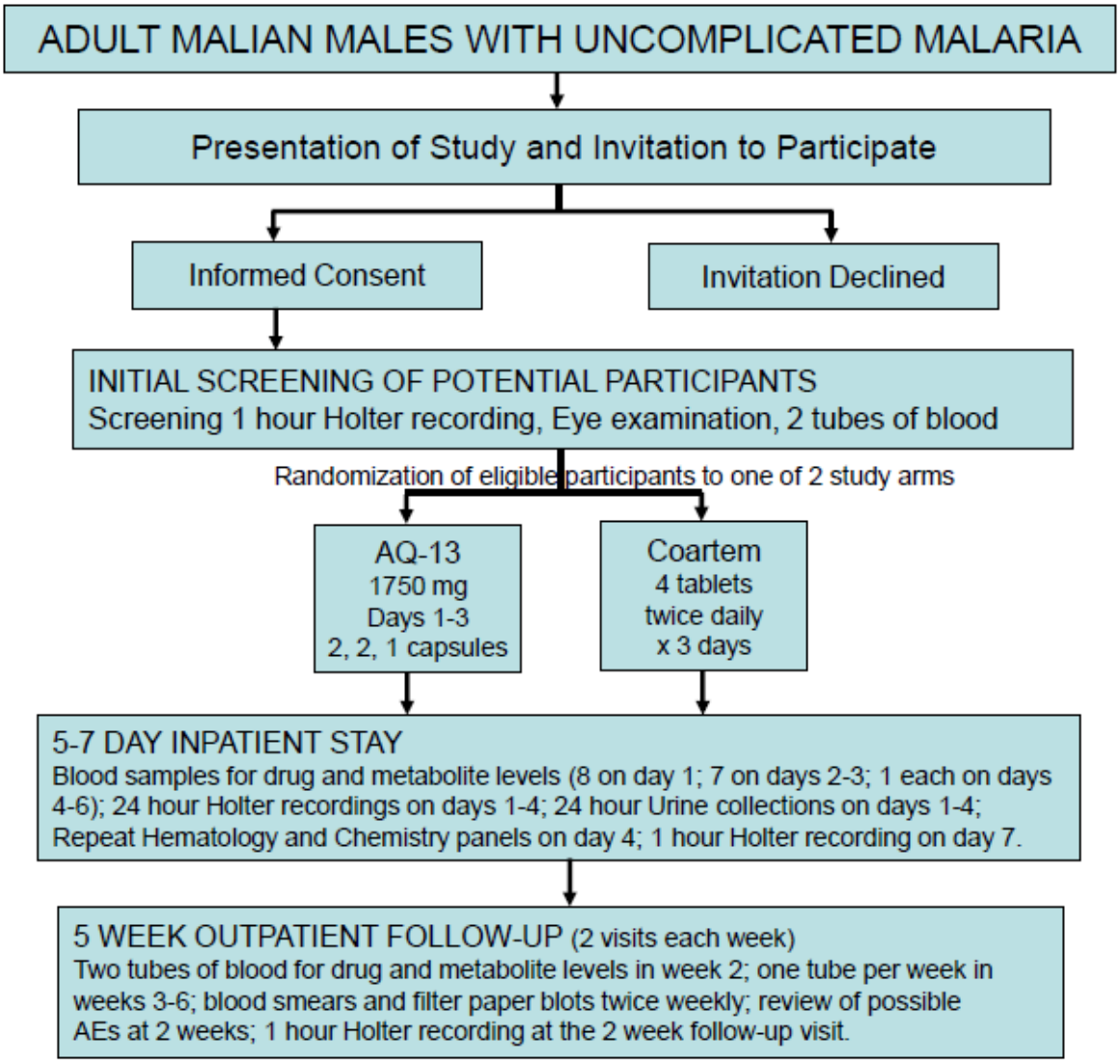
1] Pharmacokinetics of AQ-13 metabolites – estimates of pharmacokinetic parameters for the mono- and di-*N*-dealkylated metabolites of AQ-13 (AQ-72, AQ-73).

2] Aminoquinoline (CQ) pruritus – pruritus in persons receiving AQ-13 or Coartem, especially in persons with a history of pruritus after treatment with CQ.

Schematic of Study Design (Flow Chart 1, below):
This is a **randomized study in which subjects** who have provided their informed consent to participate **will be randomized to one of two study arms**: 1] oral administration of AQ-13 (1596 mg AQ-13 base over 3 days) or 2] the control arm, in which subjects will receive the standard treatment dose of Coartem (artemether + lumefantrine; 4 tablets twice daily x 3 days) which is currently recommended as the first-line treatment for uncomplicated *P. falciparum* malaria in Mali, including infections likely to be resistant to chloroquine (CQ) or pyrimethamine + sulfadoxine (Fansidar®). This is a single-blind study because the subjects (**but not the investigators)** will know which subjects are receiving AQ-13 vs. Coartem (by the number and types of capsules [AQ-13] or tablets [Coartem])**.**

Flow Chart 1

PROOF OF CONCEPT EFFICACY STUDY FOR AQ-13



1 KEY ROLES

Individuals: **FDA Representative**: Soumya Patel, PharmD

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2 BACKGROUND, RATIONALE, RISKS/BENEFITS

2.1 Background

Antimalarial resistance was the single most important factor in the recent global resurgence of malaria [**1**] and is now the greatest obstacle to the control and potential elimination of malaria [**2**]. Specifically, the increasing prevalence of chloroquine (CQ) resistance [**3**] and the recent appearance of artemisinin resistance [**4-5**] have had a profoundly negative impact on malaria morbidity and mortality [**6-7**]. Therefore, the development of new antimalarials active against otherwise drug-resistant parasites that are also safe and orally bioavailable is an urgent global health priority. In particular, it would be desirable to have alternative antimalarials that were as safe and orally bioavailable as CQ, and were also active against CQ-, mefloquine- and multi-resistant strains of *Plasmodium falciparum.*

Our recent studies have shown that aminoquinolines (AQs) with side chains shorter or longer (≤ 3 or ≥ 10 carbons) than the 5 carbon isopentyl side chain of chloroquine (CQ) are active *in vitro* against CQ-, mefloquine- and multi-resistant *P. falciparum* [**8-10**]. They have also shown that short chain AQs administered orally are effective *in vivo* in two monkey models of human malaria (the *P. cynomologi/*rhesus monkey model of human *P. vivax* infection, and the *P. falciparum/*squirrel monkey model of human infection with the CQ-resistant Indochina I strain after adaptation to the squirrel monkey).

In addition, recent Phase 1 (safety and pharmacokinetic) studies have shown that the side effects (adverse events [AEs] associated with) of the lead compound AQ-13 (which has a linear *n*-propyl side chain) in human subjects are similar to those of CQ and that the pharmacokinetics of AQ-13 and CQ are similar [**11**]. In terms of potential cost, the current estimate of the cost of goods for AQ-13 is $0.03 per dose (for an AQ-13 oral dose equivalent to 700 mg CQ base in terms of bioavailability), which is approximately $0.08 per treatment course if the treatment dose chosen is 1596 mg over 3 days. In addition, the close structural similarity between AQ-13 and CQ and the similar Adverse Events (AEs) observed with AQ-13 and CQ in the Phase 1 studies [**11**] suggest that AQ-13 may be as safe as CQ for the treatment of human subjects with malaria, and raise the possibility that AQ-13 may share the safety of CQ during pregnancy. Although these arguments are part of the rationale for studying AQ-13, please note that **adult women (including women who are pregnant or breast-feeding) are excluded from participation in these studies.** Finally, please note that AQ-13 is only one of 66 AQs which we have synthesized and shown to be active against CQ- and multi-resistant *P. falciparum* [**9-10**]. Therefore, if these studies are successful, they have the potential to create a new class of AQ antimalarials effective against otherwise drug-resistant *P. falciparum* malaria parasites.

2.2 Rationale

Therefore, the next logical step in the evaluation of AQ-13 is to determine whether it is active against CQ-resistant *P. falciparum* infection in human subjects. The hypotheses underlying this protocol are that: 1] an AQ-13 dose of 1596 mg administered orally will be efficacious for the treatment of uncomplicated *P. falciparum* malaria in human subjects, and 2] the bioavailability, pharmacokinetics and adverse events (AEs) of AQ-13 in human subjects with uncomplicated malaria will be similar to those observed previously in healthy uninfected subjects during the Phase 1 studies [**11**].

The rationales for the selection of adult men for this Proof of Efficacy Study with AQ-13 are that: 1] the involvement of only males will obviate potential concerns about inadvertent pregnancies in female volunteers who have agreed to contraception and 2] that persons who have survived to adulthood in Mali are protected against life-threatening complications and death from malaria because they have acquired the semi-immune state [**12**]. Therefore, if for reasons we have not foreseen, AQ-13 does not work (if AQ-13 is not effective for treatment of uncomplicated malaria in human subjects), the risk of severe disease or death in these subjects will still be extremely low – because they are protected by their previous acquisition of the semi-immune state.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

Immediate (Short-Term) Risks. The first potential risk of participation in this study is that the investigational drug AQ-13 may not work; it may not cure uncomplicated *P. falciparum* malaria in Malian adults. If AQ-13 does not clear malaria parasites from subjects' blood by day 7 in the AQ-13 arm, those subjects will be treated with Quinine. In addition, if Coartem does not clear malaria parasites from subjects' blood in the Coartem control arm by day 7, these subjects will also be treated with Quinine, which is recommended in Mali for rescue in persons who fail treatment for uncomplicated malaria.

The most frequent side effects of AQ-13 in the volunteers who received it during the Phase 1 studies (and therefore the most likely adverse events (AEs) in these Proof of Concept studies) are similar to those of CQ: they include headache, dizziness, and digestive tract symptoms (nausea, loss of appetite, diarrhea, vomiting and abdominal pain). One of 126 subjects developed a mild, transient rash on her lower body, and 1 had muscle damage for several days, which is thought not to have been caused by the study drug, and is often caused by vigorous exercise. Other potential risks include bleeding, bruising, discomfort, black and blue spots and infection from venipunctures to obtain blood specimens. Abnormal beating of the heart is a potential risk, and has been observed in two subjects during the previous studies. Although it is thought not to have been caused by the study drug, the study drug will be stopped and subjects will be treated with quinine if they have abnormal beating of the heart and were randomized to AQ-13.

Potential Long-Term Risks. There are no long-term risks which are likely, in part because the subjects participating in this study will receive AQ-13 for only 3 days in daily doses that are 6 to 10-fold lower than the doses of CQ used previously to treat rheumatoid arthritis and other non-infectious diseases [**13-14**]. However, there have been reports of ocular complications when other 4-AQs (CQ or hydroxy-CQ; retinopathy) or an 8-AQ (tafenoquine keratopathy) [**15**] have been given for periods as short as 3 months (tafenoquine) or as long as 4-10 years (CQ or hydroxy-CQ doses of >3 or >6.5 mg base/kg/day) [**16**]. Therefore, although the likelihood of such complications is extremely low, we will perform eye examinations before and after treatment with AQ-13 to ensure that there is no evidence for ocular AEs with AQ-13. Note that the eye examinations performed before and after AQ-13 dosing during the Phase 1 studies revealed no evidence of ocular AEs.

Potential for Unknown Risks. Because AQ-13 is an investigational drug, there may be other risks that we do not know about. Those risks have been substantially reduced – although not eliminated – by the absence of such AEs during the Phase 1 studies in healthy human volunteers.
Potential Loss of Confidentiality. Although there is a risk that information about subjects' participation in this study could become known, that risk is low. That risk will be minimized by keeping study information in password-protected computers and locked cabinets. When the results of the study are published or made available on the Internet, participants will be identified by study ID (identification) number and not by name. Officials of the US and Mali Governments, Tulane University and the University of Bamako will have access to the study records. Referrals to Malian physicians will be provided for problems related to this study and subjects will receive new information about AQ-13 that may affect their willingness to participate as that information becomes available.

Rationale (Justification) for Risks. Although these risks (including the similar AEs observed with AQ-13 and CQ) [**10**] are real, they are small in relation to the risks of untreated malaria for individual patients [**1-2**]. For the community, drug-resistant parasites are the most important obstacle to the control and potential elimination of malaria. In addition, after a candidate antimalarial has been found active *in vitro* and *in vivo* in monkey models of human malaria [**8-9**] and safe in healthy human subjects [**11**], the only way to determine whether that candidate compound has the potential to be used as an antimalarial to treat humans with CQ-resistant malaria is to perform a Phase 2 Proof of Concept Study and ultimately Phase 3 studies. Thus, at this point in the evaluation of AQ-13 as the lead compound, it is appropriate to perform a Proof of Concept Efficacy Study of AQ-13 in human subjects.

2.3.2 Known Potential Benefits.

The potential benefit to the subjects from participating in this study is the information obtained from the initial blood testing and heart and eye examinations. If there are abnormal results on those tests, they will be explained to the subjects in detail, and the subjects will be referred for initial evaluation and treatment by medical specialists in Mali. Alternatively, for subjects whose only abnormal test result is a positive malaria smear, treatment with either AQ-13 or Coartem may benefit them by clearing those malaria parasites from their blood. In addition, knowledge gained from this study may benefit both these subjects and others in the future. However, there are no expected benefits to the subjects (participants) from measuring the levels of AQ-13 and its metabolites in their blood or urine or from studying the genes that metabolize these drugs or control the host immune response to malaria infection.

3 OBJECTIVES (ENDPOINTS):

Statement of Purpose. To summarize the information in the Background and Significance section above, the next logical step in the evaluation of AQ-13 as the lead compound is to determine whether it is active against (efficacious for the treatment of) uncomplicated *P. falciparum* malaria in human subjects. That is the purpose of (rationale for) this Proof of Concept Study. The hypotheses underlying this protocol are that: 1] an AQ-13 dose of 1596 mg administered orally will be efficacious for the treatment of uncomplicated *P. falciparum* malaria in human subjects, and 2] the bioavailability, pharmacokinetics and adverse events (AEs) of AQ-13 in human subjects with uncomplicated malaria will be similar to those observed previously in healthy uninfected subjects (during the Phase 1 studies) [**11**].The rationale for beginning with studies of adults before proceeding to studies of children is based on safety. Our rationale is that it is safer to begin with studies of adults (who have acquired the semi-immune state) before performing studies of children (who may not yet acquired the semi-immune state and are therefore at greater risk of severe or complicated malaria if AQ-13 is not efficacious for the treatment of uncomplicated malaria). The rationale for performing the Proof of Efficacy Study with only males is to ensure there is no chance that an adult female volunteer who has agreed to practice contraception before and after exposure to AQ-13 will inadvertently become pregnant during or shortly after these studies.

Primary Objective and associated Endpoints:

1] The primary objective is to determine and compare the cure rates for AQ-13 and Coartem. Cure is a lack of recrudescence within 42 days (PCR-corrected - no evidence of recrudescence with the same parasite genotype(s)) after reduction of the asexual parasitemia to < 25% of the admission value by day 3 and clearance of asexual parasites and fever by day 7. Failure is defined as “lack of cure.”

Secondary Objectives and associated Endpoints for comparisons of AQ-13 with Coartem:

1] Adverse Events (AEs) – within 4 weeks of treatment as judged by **blinded** physician reviewers to be possibly related to the study drug(s) using the same criteria as in the Phase 1 study.

2] Parasite clearance time – from the initiation of treatment to the first of 2 successive negative thick smears.

3] Time to recrudescence or reinfection – time from the initiation of treatment to the reappearance of asexual *P. falciparum* parasites on the thick smear.

4] Effects on the QT (QTc) interval – mean changes from the baseline (pre-treatment) QT interval to the post-treatment QT interval after treatment with AQ-13 or Coartem.

5] Pharmacokinetic parameters for AQ-13 – these will include measurements of blood levels over time (peak levels, AUC, MRT), drug distribution (Vd/F) and drug elimination (t½, Cl/F).

6] Fever clearance time: Time in hours from the initiation of therapy until disappearance of fever for at least 24 hours.

Tertiary Objectives and associated Endpoints for comparisons of AQ-13 with Coartem:

1] Pharmacokinetics of AQ-13 metabolites – estimates of pharmacokinetic parameters for the mono- and di-*N*-dealkylated metabolites of AQ-13 (AQ-72, AQ-73).

2] Aminoquinoline (CQ) pruritus – pruritus in persons receiving AQ-13 or Coartem, especially in persons who have a history of pruritus after treatment with CQ.

4 STUDY DESIGN

Description of Study Design. This is a randomized single-blind study of uncomplicated *P. falciparum* malaria in adult Malian males (≥ 18 years of age) in which subjects who provide their informed consent to participate will be randomized to either the study arm (1596 mg oral dose of AQ-13 over 3 days) or the control Coartem arm (4 tablets twice daily x 3 days). The rationale for the Coartem controls is to relate the AQ-13 results to Coartem – which is the recommended first-line treatment (current state-of-the-art) for the treatment of uncomplicated *P. falciparum* malaria in sub-Saharan Africa [**19-22**]. **This is a single-blind study because the subjects will potentially know whether they are receiving AQ-13 (capsules) or Coartem (tablets).** However, the **investigators** evaluating AEs, the relatedness of AEs to the study drugs and other data will not know which subjects were randomized to which arm of the study.

Patient population (Flow Chart 2, page 16, below). The patient population and flow of potential subjects from screening to study accrual are summarized in Flow Chart 2. Recruitment of adult Malian subjects with uncomplicated *P. falciparum* malaria will be performed at the Missira Community Health Center for persons from Missira and the adjacent village of Sirakoroba (total population 6,000) and at the outpatient referral centers in Bamako. Please note that the inpatient studies for all subjects will be performed at the Clinical Research Center and that the subsequent outpatient follow-up visits (twice weekly for 5 weeks) will be at both the Community Health Center in Missira (for subjects from Missira or Sirakoroba) and the Clinical Research Center (for subjects from urban and per-urban regions of Bamako).

Times to obtain Blood Specimens and other Samples and Studies (Flow Charts 3-4, Gantt Chart, pages 17, 26 and 27, below). After providing their informed consent to participate, subjects will be asked to provide 2 tubes of blood for chemistry and hematology testing. Beginning at the time of dosing, participants will also be asked to provide serial blood samples for AQ-13 drug and metabolite blood levels 8, 7 and 7 times on days 1-3, once on days 4 and 5, twice in week 2 (at each of the twice-weekly outpatient visits) and once per week in weeks 3-6 (days 15-42). This information is provided in tabular form and graphically in a Gantt Chart below in Section 7 (page 27). To facilitate the drawing of multiple blood specimens, a heparin lock plastic catheter with a scalp vein needle will be placed in the arm of participants for use during inpatient days 1-4 or 1-5.

Methods for Collecting Blood Specimens. Blood specimens will be obtained by finger stick or through the heparin lock plastic catheter for the screening blood smear, filter paper blot, and Hb, AQ-13 and AQ-13 metabolite levels. Subsequently, they will be obtained by venipuncture for the AQ-13 drug and metabolite levels and for the repeat chemistry and hematology panels beginning on day 4 of the inpatient stay or at the start of outpatient studies in Week 2. A heparin lock (scalp vein attached to a plastic catheter containing heparin to keep the line open) will be placed on inpatient day 1 to permit drawing 24-26 blood samples on inpatient days 1-4 or 1-5 without separate venipunctures for each sample.

Collection of Urine Specimens. To monitor the urinary excretion of AQ-13 and its two major metabolites, subjects will be asked to save their 24 hour urines during inpatient days 1-4.

Expected Duration of Subject Participation from the time of screening and enrollment to the conclusion of their participation in the study is 42 days (6 weeks).

Description of Subject Participation. As noted above, these subjects will be asked to provide or have:

- 32 Tubes of blood for a total volume of 160 ml (5 ml per tube),
- 4 (24 hour) urine collections on inpatient days 1-4,
- 7 Holter recordings at the times of screening (1 hour), during inpatient days 1-4 (24 hours each) and at the 1 and 2 week outpatient follow-up visit (2 hours),
- 2 Eye examinations at the times of screening and the 1 week outpatient follow-up visit.

Safety Oversight will be assisted by a Data Safety and Monitoring Board (see section 8.7 on page 33, below).

Flow Chart 2

PROOF OF CONCEPT STUDY FOR AQ-13
ADULT MALES WITH UNCOMPLICATED *P. falciparum* MALARIA

Recruitment in the Villages of Missira and Sirakoroba in Northwestern Mali
and from Outpatient Referral Clinics in the city of Bamako;
In-Patient Studies at the Clinical Research Center, Hôpital Point G in Bamako

STUDY OF UNCOMPLICATED *P. falciparum* MALARIA IN MALIAN MEN
(n=132; 66 males per arm, expected losses of 10%

- INCLUSION CRITERIA:
  Adult males (≥ 18 years of age),
  Positive smear for asexual *P. falciparum*,
  Uncomplicated *P. falciparum* malaria.
- EXCLUSION CRITERIA (expected rates):
  Chronic medications (< 1%),
  Recent malaria treatment (≤ 2 weeks, 5-6%),
  Arrhythmia on screening Holter (<< 1%),
  Persistent or repetitive vomiting (2-4%).

↓

SCREEN *P. falciparum*-INFECTED MEN FOR ELIGIBILITY
(n=118; 59 men per group, expected 40% loss before consent and enrollment)

- Informed Consent
  (~90% of successfully screened volunteers),
- Positive Smear for *P. falciparum*
  (seasonally variable, ~40-50%),
- ADDITIONAL EXCLUSIONS: Evidence for severe or complicated malaria: cerebral malaria, pulmonary edema, renal failure, hyperparasitemia (≥ 100,000 per μl) or hyperthermia (≥ 40° C) (< 1%).

↓

TRANSPORT ELIGIBLE VOLUNTEERS TO THE CLINICAL RESEARCH CENTER AT HÔPITAL POINT G IN BAMAKO
(n=70; 35 each for the AQ-13 and Coartem groups)

- Review of the Inclusion and Exclusion Criteria to determine the potential eligibility of individual subjects,
- Scheduling of Transportation to the Clinical Research Center with family members beginning with 4 subjects at a time.

Flow Chart 3

PROOF OF CONCEPT STUDY FOR AQ-13
RANDOMIZATION, DRUG ADMINISTRATION AND FOLLOW-UP

Recruitment in the Villages of Missira and Sirakoroba in Northwestern Mali
and from Outpatient Referral Clinics in the city of Bamako;
In-Patient Studies at the Clinical Research Center, Hôpital Point G in Bamako

EYE EXAMS, HOLTER RECORDINGS, CHEMISTRY PANEL, HEMATOLOGY (CBC)
(n=70; 35 each for the AQ-13 and Coartem groups, expected 5% ineligible).

- INCLUSION CRITERIA:
  ≥ 2,000 asexual *P. falciparum* parasites per μl,
  Uncomplicated *P. falciparum* malaria,
- Baseline Screening Holter Recording x 1 hour,
- Visual Screening baseline acuity, visual fields,
- EXCLUSIONS: Hb ≤ 7 gms/dL, K+ ≤ 3.5 mEq/L, arrhythmias, heart block, BP ≥ 140/90 or severe or complicated malaria.

↓

RANDOMIZE ELIGIBLE ADULT VOLUNTEERS WITH UNCOMPLICATED *P. falciparum* MALARIA
(n=66; 33 each for the AQ-13 and Coartem groups, expected 20% loss to follow-up).

- Filter Paper Blots for molecular studies,
- Freeze Initial Parasites at -70° C,
- *pfcrt* PCR and other Tests for CQ Resistance,
- Oral Administration of 700 mg AQ-13 on days 1-2, followed by 350 mg on day 3, OR
  Oral Administration of Coartem, 80 mg artemether + 480 mg lumefantrine twice daily x 3 days,
- Repeat Daily Blood Smears (days 1 through 7), repeat Hematology, Chemistry panels on day 4,
- AQ Drug and Metabolite Levels at 0, 0.5, 1, 2, 4, 6, 12,18, 24, 24.5, 25, 26, 28, 30, 36, 48, 48.5, 49, 50, 52, 54, 60, 72, 96 and 120 hours x 5 ml,
- Holter Monitoring 24 hours/day x 4 inpatient days,
- One Week eye exam, 1 hour Holter recording,
- WITHDRAWAL: severe or complicated malaria.

↓

FOLLOW-UP for a total of 6 weeks, PRIMARY ENDPOINT is a lack of recrudescence with the same genotype(s)
(n=28 evaluable subjects/group)

- AQ Drug and Metabolite Blood Levels twice weekly x 1 week, then once weekly in weeks 3-6,
- Filter Paper Blots and Smears twice weekly x 5 weeks on outpatient follow-up,
- Freeze Parasites for Molecular Studies and Test for Resistance if parasitemia recurs,
- Repeat 1 hour Holter recording at the 2 week follow-up outpatient visit.

5 STUDY POPULATION

5.1 Selection of the Study Population

The population to be studied is the subset of adult Malian males with uncomplicated *P. falciparum* malaria. There are two rationales for this choice. The first is that uncomplicated malaria with asexual parasite counts ≥ 2,000 per μl is extremely common in Mali (affecting ≥ 40-90% of the population in the transmission season). The second is that adults in Mali have acquired the semi-immune state and will therefore have an extremely low risk of severe or complicated malaria if AQ-13 is not effective for the treatment of uncomplicated malaria (which we do not expect to be the case).

Target Sample Size, including the actual numbers to be enrolled. The target sample size for the number of evaluable subjects is 28 evaluable subjects in each of 2 study arms for a total of 56 evaluable subjects. However, the numbers of potential subjects that will be recruited are greater than 28 per arm because of expected losses from: 1] persons who have a negative blood smear for malaria parasites, 2] the need to spend one week (5-7 days) at the Clinical Research Center during the inpatient phase of the study, 3] the subsequent 5 weeks of twice-weekly outpatient follow-up visits and 4] the multiple blood samples, monitoring and urine collections required for participation.

Numbers of subjects (adult males) that will be recruited. For the reasons outlined above and in Flow Charts 2-3 (pages 16-17), we expect to recruit 132 men (adult Malian males ≥ 18 years of age) thought to have uncomplicated malaria to identify 118 eligible men (who have positive smears with ≥ 2,000 asexual parasites per μl and no other medical conditions that require diagnosis or treatment) in order to obtain 70 men willing to provide their informed consent to enroll and participate in the inpatient studies (33 per arm for the 2 study arms). Based on an additional 20% attrition during inpatient and outpatient follow-up, this should yield approximately 28 evaluable subjects per study arm (based on approximately 56 evaluable subjects from the initial 132 who were recruited and the 70 who provided their informed consent to participate in the study).

Indicate the source/site from which the study population will be drawn. The study population of Malian adult males with uncomplicated malaria will be drawn from several outpatient sites: 1] the Missira Community Health Center which serves the adjacent communities of Missira and Sirakoroba 160-170 km northwest of Bamako that have an estimated population of 6,000, and 2] referral clinics in urban and peri-urban Bamako, where >100 outpatients from the urban and peri-urban areas in and around Bamako seek treatment for uncomplicated malaria every day between August and November.

Identify strategies for subject recruitment and retention. Subjects will be recruited from persons who come to the Missira Community Health Center or to clinics that refer to the University of Bamako for the diagnosis and treatment of presumed uncomplicated *P. falciparum* malaria (fever, chills, headache, without signs or symptoms of severe or complicated malaria) [**23**]. We expect that close personal care and attention during the inpatient week of the study will minimize attrition during that time. To maximize cohort retention for the twice-weekly outpatient follow-up visits during the subsequent 5 weeks, we will provide financial compensation (CFA 5,000=$10 US) for each outpatient visit and transportation home at the conclusion of each follow-up outpatient visit.

Ability to accomplish participant enrollment and Follow-Up

As noted above and in Flow Charts 2-3, this study is based on the analysis of approximately 28 evaluable subjects in each of the 2 study arms (28 x 2 = 56), and therefore on ≥ 56 evaluable subjects completing the study protocol. To achieve that goal, we expect to screen and enroll substantially greater numbers of subjects. Based on the number of subjects seeking diagnosis and treatment for uncomplicated malaria at the two recruitment sites (≥ 200 per month) and on the inpatient capacity of the Clinical Research Center at the Hôpital Point G (8-10 inpatients), it should be possible to accomplish this study and determine whether the 1596 mg dose of AQ-13 is as effective as Coartem for treatment of uncomplicated malaria.

As described above (on page 18) in the section on subject recruitment and retention), the majority of the subjects for this study will be recruited because they came to the Missira Community Health Center or a referral outpatient clinic in Bamako, requesting studies to diagnose and medicines to treat uncomplicated malaria. For those subjects, the initial screening malaria smear and rapid Hb determination are part of their routine clinical care. However, if it is necessary to recruit additional subjects from the community, a protocol amendment will be prepared in order to obtain a separate informed consent before performing a malaria smear, Hb determination or other studies potentially related to this Phase 2 Proof of Concept Efficacy Study of AQ-13. As noted below, and in the enclosed Gantt Chart (page 27), informed consent will be obtained before subjects are considered for the initial referral and transportation from Missira or other sites to the Clinical Research Center in Bamako.

5.2 Eligibility (Inclusion/Exclusion) Criteria

Inclusion criteria for this study include:

1] Adult Malian males ≥ 18 years of age,
2] Uncomplicated malaria with ≥ 2,000 asexual *P. falciparum* parasites per μl, and
3] Informed consent obtained and signed.

After obtaining the initial consent to treat, a physical examination and medical history will be obtained and recorded on a standard form. Thick smears will be obtained to diagnose *P. falciparum* infection. Patients who fulfill the first two criteria above will be invited to participate in the study, and informed consent will be obtained from those who choose to participate.

Exclusion criteria for this study include:

1] Severe or complicated malaria (including temperatures ≥ 40° C),
2] ≥ 100,000 asexual parasites per μl of blood,
3] Anemia or other laboratory results (other than malaria) that require treatment (e.g., Hb ≤ 7 gm/dL, K+ ≤ 3.5 mM, BP ≥ 140/90),
4] Seizures or impaired consciousness,
5] Recent antimalarial treatment by history (within ≤ 2 weeks),
6] Chronic medications (including *CYP*3A4 inducers such as rifampin and nevirapine),
7] Ventricular or atrial arrhythmias including frequent ventricular premature beats or extrasystoles (> 20 per hour) or second or third degree heart block on the screening Holter recording,
8] Infection with other plasmodial species on the blood smear (*P. ovale, P. ovale, P. vivax*).

Enrollment and randomization will occur only after a final review to ensure that all of the inclusion criteria

have been met and that none of the exclusionary criteria are present.

6 STUDY PROCEDURES/EVALUATIONS

Table 1.

Study Schedule: PROOF OF CONCEPT EFFICACY STUDY OF AQ-13

Activity, Day(s)	Tests Performed and Potential Findings
DIAGNOSIS, Day 1: Malian males ≥ 18 years with Fever and Chills	Blood smear to diagnose malaria in an adult Malian male is positive (and the subject fulfills none of the exclusion criteria)
INVITATION, Day 1: Invite to Participate in Study of AQ-13	Decide whether to participate in the Phase 2 Proof of Concept Study of AQ-13 by providing informed consent
SCREENING, Day 1: Subjects with malaria who provide consent	Screening 1 hour Holter recording, 2 tubes of blood for chemistry and hematology testing, eye examination
ENROLLMENT, Day 1: Randomize eligible, consented subjects	Randomization of subjects to AQ-13 (1596 mg orally over 3 days) or to the control arm, Coartem (4 tabs x 2/day x 3 days)
INPATIENT STUDIES: Days 1-7	Blood samples for drug and metabolite levels on days 1-5; Two tubes of blood for hematology and chemistry panels on day 4; 24 Holter recordings on dosing days 1-4; Urine collections on days 1-4; Eye exam plus an additional Holter recording at the end of week 1.
OUTPATIENT FOLLOW-UP, Days 8-42: (Weeks 2-6)	Five weeks of outpatient follow-up with 2 visits each week, to obtain 1 tube of blood for drug and metabolite levels on both visits in week 2, and 1 tube per week (at 1 of the 2 outpatient visits) in weeks 3 to 6; During week 2, there is a repeat 1 hour Holter recording and a review (discussion) of potential side effects (AEs).

6.1 Study Procedures

As noted above and in Flow Chart 4 below, the initial study procedures for subjects with uncomplicated *P. falciparum* malaria are a history and physical examination. The history and physical examination will be performed as outlined in the enclosed Case Report Form. Other study procedures that will be performed at the times of screening and enrollment include:

- Specimen Collection – performed by medical and nursing staff at designated times for collection of blood samples and 24 hour urines during inpatient days 1-4 (Flow Chart 4, page 26),
- Medical History – emphasizing fever, chills, other symptoms and signs of malaria, as well as weight loss and other signs of long-term and potentially confounding illnesses,
- History of concomitant Medications – emphasizing: a] medications for malaria in the past 2 weeks, and b] medications other than contraceptives, especially medications such as rifampin and nevirapine which induce 3A4 *CYP*450 activity and are therefore grounds for exclusion,
- Physical Examination – emphasizing conjunctival pallor (anemia), signs of increased cardiac output consistent with fever or anemia, splenomegaly (Hackett Classes I-V) [**24**],

- Counseling – other issues of concern to the patient.

6.2 Initial Laboratory Evaluation

Subjects thought to have uncomplicated *P. falciparum* malaria will be evaluated by obtaining a finger stick blood sample for a thick smear, filter paper blot and a rapid Hb determination in order to screen for *P. falciparum* malaria and anemia.

6.2.1 Laboratory Evaluations/Assays

Subjects who satisfy the first two inclusion criteria (adult Malian males ≥ 18 years of age with uncomplicated *P. falciparum* malaria, and 2,000 to 99,999 asexual parasites per μl of blood) will be invited to participate in this study if they fulfill none of the exclusion criteria. Those who accept by providing their informed consent will have 2 additional tubes of blood drawn in Bamako for chemistry and hematology panels (1 each), including glucose-6-phosphate dehydrogenase (G6PD) assays and a separate sample for drug and metabolite blood levels that will also be used to freeze (cryopreserve) malaria parasites for future reference (Flow Chart 4, below on page 26). Laboratory evaluations or assays that will be performed in these studies include:

- Thick smear for asexual *P. falciparum* parasites with quantitative parasite counts (Mali; SOP)
- Filter paper blot for parasite and host genomic DNA (Mali; SOP)
- Rapid (Hemocue) Hb determination for anemia (Mali; SOP)
- Chemistry panel (Piccolo-Abaxis) (Mali; SOP)
- Hematology panel (Coulter A^{C}T 10) for Hb, Hct, WBC, platelets (Mali; SOP)
- Glucose-6-phosphate dehydrogenase (G6PD) determination (Mali; SOP)

6.2.2 Special Assays or Procedures

Prior to treatment with AQ-13 or Coartem, subjects will have a 1 hour baseline Holter recording to exclude pre-existing arrhythmias and other cardiac abnormalities and a baseline eye examination to ensure that there are no pre-existing ocular abnormalities before treatment or any new ocular abnormalities (potential AEs) after treatment. Randomization will be performed after the chemistry, hematology, eye and Holter recording results have been checked to ensure there are no criteria for exclusion and that informed consent to participate has been obtained and signed. Special assays or procedures that will be used in these studies include:

- PCR testing for parasite genotypes and for the K76T point mutation in *pfcrt* (Mali; SOP),
- Fluorescence HPLC assay for AQ-13 and its metabolites (New Orleans; SOP),
- Holter recordings for evidence of arrhythmias (Mali and New Orleans; SOP),
- Eye examinations pre- and post-treatment to test for evidence of keratopathy, retinopathy, other pre-existing abnormalities or treatment-related ocular side effects (adverse events) (Mali; SOP),
- Pharmacokinetic analysis of AQ-13 drug and metabolite blood and urine levels (Mali and New Orleans; SOPs for AQ-13 HPLC, CRFs for Out-Patient Follow-Up).

6.2.3 Specimen Collection, Preparation, Handling and Shipping

Blood Drawing for AQ-13 Drug and Metabolite Levels (Flow Chart, page 26, Section 7).

Because of the need to define the pharmacokinetics of AQ-13 and its metabolites, this blood sampling is more intense at the times of dosing (during days 1-3) when AQ-13 is being administered to the participants. (That is also the reason this table is below on a different page, because the substantial number of samples in Week One [n=25, Table 3, page 26] required that this table be presented in landscape orientation.)

After day 5, blood samples for the pharmacokinetic studies (drug and metabolite levels) will be drawn twice-weekly in the first week of outpatient follow-up (week 2 from day 8 to day 14) and once weekly during the subsequent 4 weeks (weeks 2 to 6 from days 15 to 42). The 25 samples obtained on days 1-5 plus the 10 samples obtained during outpatient follow-up are a total of 35 samples for the pharmacokinetic studies of AQ-13 and its metabolites at 5 ml apiece = 175 ml per subject.

6.2.3.1 Instructions for Specimen Preparation, Handling, and Storage

Specimens for chemistry, hematology and drug blood level testing are anticoagulated with lithium heparin, EDTA and CPD, respectively. Filter paper blots are kept in individually-labeled and dated envelopes in a dessicator at room or refrigerator temperature.

6.2.3.2 Specimen Shipments

Blood and urine specimens for the measurement of AQ-13 drug and metabolite levels will be shipped from Mali to Tulane University in New Orleans at 0-4° C because there have been no changes in those results when specimens have been stored at 0-4° C for ≥ 6 months. Specimens containing live frozen parasites shipped to New Orleans will be transported by commercial carrier using a CDC Permit for the Importation of Potentially Infectious Agents issued to the PI. Holter recordings will be sent from Bamako to New Orleans via the WEB using encryption to protect subject confidentiality, so they can be reviewed independently by cardiologists in both the U.S. and Mali using the *Box.com* WEB Site.

Permissible Concomitant Medications (Table 2, page 23, below)

As noted above, persons taking chronic medications and persons with chronic or acute diseases other than malaria will be excluded from participation in this study. However, in the course of uncomplicated malaria, supportive treatment is normally provided for: 1] fever and other discomforts, 2] nausea and vomiting, and 3] treatment of seizures (which would require withdrawal of the subject from this study for cerebral malaria). Therefore, those treatments (medications) will be allowed for participants in this study. Because the list provided below is based on discussions with Malian physician investigators, it focuses on drugs available in Mali, rather than drugs used for these indications in the U.S. For example, based on current practice in Mali, it lists: 1] paracetamol and aspirin including aspegic, rather than acetaminophen (Tylenol) for fever, headache, arthralgias and myalgias, and 2] metopimazine rather than prochlorperazine (Compazine) for nausea and vomiting.

Please note that: 1] these drugs and doses are not investigational (they are provided as examples of current practice for the supportive treatment of malaria in Mali) and 2] doses are provided only for adults because this study is restricted to adult Malian males ≥ 18 years of age with uncomplicated *P. falciparum* malaria.

Table 2.

Medications used for the supportive Care of Persons with Malaria in Mali

INDICATION	MEDICATION	DOSING
FEVER, DISCOMFORT		
Antipyretic Analgesics	Paracétamol Tablets 500 mg tablets	0.5-1.0 gm every 4-6 hours, not to exceed 4 doses per 24 hours,
	Paracétamol Injectable (Perfalgan), 10 mg/ml	Intravenous infusion of 1.0 gm (100 ml) not to exceed 4 doses per 24 hours,
	Aspirin Tablets	0.5-1.0 gm every 4-6 hrs, not to exceed 4 doses per 24 hours,
	Aspégic Injectable	1.0 gm every 6-8 hours, not to exceed 4 doses per 24 hours,
NAUSEA, VOMITING		
Antiemetics	Metopimazine (Volgalène) 5 and 10 mg oral, rectal	10 mg orally up to 3 doses per day, or 5-10 mg rectally not to exceed 3 doses/day
	Metopimazine Injectable	5-10 mg per injection, not to exceed 4 doses per day,
SEIZURES		
Anticonvulsants	Diazepam (Valium) 10 mg tablets	10-20 mg as needed, not to exceed 5 doses per 24 hours,
	Diazepam Injectable	10-20 mg as needed, not exceed 5 doses per 24 hours.

6.3 Screening

As noted above (section 6.2) and in Table 1, subjects thought to have uncomplicated *P. falciparum* malaria will be evaluated (diagnosed) by obtaining a finger stick blood sample for a thick smear, filter paper blot and a rapid Hb determination to screen for *P. falciparum* malaria and anemia. **The time between screening and enrollment must be ≤ 48 hours, although ≤ 24 hours is preferable** (intervals > 24 hours are acceptable if they result from consultation or deliberation related to informed consent). If a subject is eligible for the study and agrees to participate by providing their signed informed consent, other screening tests then performed will include a 1 hour Holter recording, 2 tubes of blood for chemistry and hematology testing and an eye examination (Table 1, Flow Chart 4).

6.4 Enrollment/Baseline, if applicable

Baseline data should be obtained within 24 hours of enrollment. This should allow for (permit) delays related to venipunctures for blood samples and the insertion of a heparin-lock to facilitate drawing multiple timed blood samples during the first 3 inpatient days, a 1 hour baseline Holter recording to exclude arrhythmias and a baseline eye examination.

6.5 Inpatient Period

Blood samples will be taken for drug and metabolite levels on days 1-5 and hematology and chemistry panels on day 4. After screening Holter recordings have been obtained and reviewed for pre-existing arrhythmias, continuous 24 hour Holter recordings will be performed on each of days 1-4 (focusing on the time between 2 and 6 hours after dosing for maximal effects on the QT interval). Urine collections will be performed on each of days 1-4. An eye exam plus an additional 1 hour Holter recording will be performed at the end of week 1.

6.6 Follow-up and Final Visits, if applicable

After completion of the 5-7 day inpatient stay (Flow Chart 4 below, and Table 1, above), there are 2 follow-up outpatient visits each week for the next 5 weeks (total of 6 weeks active involvement in the study). At each of the follow-up visits during Weeks 3-6, a blood sample is obtained for AQ-13 drug and metabolite blood levels to define the pharmacokinetics of AQ-13 and its metabolites. In addition, there is a final 1 hour Holter recording at the 2 week follow-up visit plus an in-depth interview about potential AEs.

Sequence of Events during the Follow-Up Outpatient Visits. The twice-weekly follow-up outpatient visits will include (in sequence): an interval history with discussion of potential AEs related to study drugs, review of current medications and a physical examination with recorded results for vital signs (blood pressure, heart rate, respiratory rate, temperature), conjunctival pallor, pulmonary, cardiac and abdominal examinations (including splenomegaly) (see the Out-Patient Follow-Up CRFs).

Allowable Windows (Times) for Follow-Up Outpatient Visits. The major purposes of the follow-up outpatient visits are to: 1] follow the pharmacokinetics of AQ-13 and its metabolites over time to estimate the terminal elimination t½ and other pharmacokinetic parameters for AQ-13 and its metabolites in subjects with uncomplicated malaria and 2] to test for recurrent infection by distinguishing new infections (which do not represent recurrences or treatment failures) from recrudescences of infection with the same parasite genotypes (which do potentially represent treatment failures). Although the best data (in terms of points on the time-concentration curve to estimate the pharamacokinetic parameters) are provided by twice-weekly follow-up blood samples during the first two weeks (Weeks 1 and 2), the software that will be used for this purpose (PharSight) can adjust and still provide reasonable estimates of the relevant pharmacokinetic parameters no matter what the time points are. For this reason and because recurrent infection should be detected as soon as possible, late return visits will be permitted up to the final 6 week (42 day) time point.

6.7 Early Termination Visit, if applicable

Evidence for the recurrence of *P. falciparum* infection (positive smear for asexual parasites) at a follow-up outpatient visit within the 42 day period of post-treatment observation is reason for re-treatment and for the termination of a subject's participation in the study. At that time, blood will be drawn for AQ-13 drug and metabolite levels and to freeze asexual parasites for study in the future, 2 filter paper blots will be obtained, and repeat chemistry and hematology testing will be performed. The rationale for performing these studies at that time is to determine whether:

a] **the recurring parasite was present at the time of the initial diagnosis and treatment** (in which case the recurrent infection would represent a treatment failure) and

b] the blood levels of AQ-13 and its metabolites are within the expected range (in order **to exclude treatment failure due to ultrarapid metabolism of AQ-13** to its *N*-dealkylated metabolites by the *CYP*450 system.

6.8 Discontinuation or Withdrawal of a Subject

Treatment with AQ-13 or Coartem in this study may be discontinued for any of the reasons below. However, whenever possible (i.e., except when informed consent to participate has been withdrawn) subjects will be followed through to the conclusion of the treatment of their illness and outcomes will be examined using both *intent to treat* and *per protocol* analyses.

1] Recurrence of uncomplicated malaria,
2] Progression to severe or complicated malaria,
3] Development of ventricular tachyarrhythmias or conduction defects (new 2nd or 3rd degree heart block),
4] Other Serious Adverse Events (SAEs) possibly related to AQ-13, or
5] The subject's decision to withdraw his/her informed consent to participate.

If lack of cure (recurrence of uncomplicated malaria) occurs with either AQ-13 or Coartem, the subject will be treated with Quinine. Likewise, if a subject develops severe or complicated malaria, he will be treated with Quinine as recommended by the Mali Ministry of Health and the National Malaria Control Programme. Treatment with either AQ-13 or Coartem will be stopped in subjects who develop ventricular tachyarrhythmias, conduction defects or other serious adverse events (SAEs) possibly related to AQ-13 or Coartem. In those instances, further antimalarial treatment (if necessary) will be provided using Quinine based on the recommendations of the Mali Ministry of Health and the Mali National Malaria Control Programme.

Flow Chart 4.

Work Sheet (schedule of procedures) for Phase 2 efficacy studies of AQ-13 in semi-immune Malian adult Males ≥ 18 years of age

Clinical	Scr	Pre-dose	0	30 m	1 hr	2	4	6	12	18	24	24:30	25	26	28	30	36	48	48:30	49	50	52	54	60	72	96	1/2 wks
History/PE	X																										X
Medication History	X																										X
Assess for AEs		X							X								X							X			X_1
Laboratory																											
CBC,WBC, diff, plt	X																									X	
Chem 7	X																									X	
Liver profile	X																									X	
G6PD	X																										
AQ & Metabolites			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X*		X
Freeze parasites			X																								
24 hr urine		X									X							X							X		
Diagnostics																											
Holter Recording	X		X_2								X_2							X_2							X_2		X**
Eye exam	X																									X	
Study Drug AQ-13 Σ=1750 mg			X_3								X_3							X_3									
Coartem X_4=4 tabs			X_4						X_4		X_4						X_4	X_4						X_4			

X1 – To be performed by study Co-Investigator while inpatient and at follow-up
X2 – To be done over 96 hours (4 days), beginning at zero time on day 1
X3 – AQ-13 dose; to be given in the morning x 3 days.
X4 – Coartem dose (4 tablets in morning and afternoon x 3 days).
½ wks – at the end of week 1 and the end of week 2
* - blood draws at 96 and 120 hours after this, then twice a week for 6 weeks.
** - Holter recording for 1 hour at 1 and 2 week follow up visits.

Table 3.

WEEK ONE BLOOD SAMPLING SCHEDULE FOR AQ-13 DRUG AND METABOLITE LEVELS

Days	Day 1								Day 2							Day 3							4	5	6
Hours	0	0.5	1	2	4	6	12	18	24	24.5	25	26	28	30	36	48	48.5	49	50	52	54	60	72	96	120

Gantt Chart

ORGANIZATION OF THE PHASE 2 PROOF OF CONCEPT STUDY OF AQ-13

	OUTPT TESTS	INPT TESTS	INPATIENT WEEK 1							OUTPATIENT FOLLOW-UP (WEEKS 2 TO 6)				
Recruitment	X													
Informed Consent	X													
Thick Smear	X		X	X	X	X	X	X	X	2 per Week	2 per Week	2 per Week	2 per Week	2 per Week
Thin Smear	X													
Filter Paper Blot	X		X	X	X	X	X	X	X	2 per Week	2 per Week	2 per Week	2 per Week	2 per Week
Physical Exam	X	X	X	X	X	X	X	X	X	2 per Week	2 per Week	2 per Week	2 per Week	2 per Week
Hb	X					X								
Transport Decision	X													
Hematology Panel		X				X								
Chemistry Panel		X				X								
Eye Examination		X							X					
Holter (1 hour)	X								X	Day 14				
Holter (24 hours)			X	X	X	X								
Randomisation		X												
Drug Treatment			X	X	X									
Pharmacokinetics			8	6	7	1	1	1		2 per Week	1 per Week	1 per Week	1 per Week	1 per Week
Parasite Clearance									X					
End of Inpatient Stay									X					
Completion of Study														Day 42

	OUTPT TESTS	INPT TESTS	INPATIENT WEEK ONE							OUTPATIENT FOLLOW-UP (WEEKS 2 TO 6)				
DAYS			1	2	3	4	5	6	7	8 to 14	15 to 21	22 to 28	29 to 35	36 to 42
WEEK						1				2	3	4	5	6

7 ASSESSMENT OF OUTCOME MEASURES

7.1 Specification of the Appropriate Outcome Measures

The major question being asked in these studies is whether AQ-13 is efficacious for the treatment of uncomplicated malaria due to *P. falciparum.* Therefore, the major outcome is whether treatment with AQ-13 clears *P. falciparum* parasites from the bloodstream and fever within 7 days without recrudescence within 42 days (3 times the 14-15 day terminal t½ of AQ-13 in human subjects) [**11**]. Because the transmission of malaria is often intense in Mali (100-300 bites per person per night during the rainy season), the data for recrudescence will be PCR-corrected [**16-17**]. Based on the amplification of selected polymorphic loci, recrudescences with parasites different from those in the initial infection will be categorized as new infections, which do not represent treatment failure. Conversely, recrudescences with parasites indistinguishable from those in the initial infection will be categorized as recurrent infections and do potentially represent treatment failures.

7.1.1 Primary Outcome Measures

Cure is a lack of recrudescence within 42 days (PCR-corrected) – no evidence of recrudescence with the same parasite genotype(s) after reduction of the asexual parasitemia to less than 25% of the admission value by day 3 and clearance of asexual parasites and fever by day 7. Failure is defined as "lack of cure".

7.1.2 Secondary Outcome Measures

1] Adverse Events (AEs) – within 4 weeks of treatment as judged by **blinded** physician reviewers to be possibly related to the study drug(s) using the same criteria as in the Phase 1 study.

2] Parasite clearance time – from the initiation of treatment to the first of 2 successive negative smears.

3] Time to recrudescence or reinfection – time from the initiation of treatment to the reappearance of asexual *P. falciparum* parasites on the thick smear.

4] Effects on the QT (QTc) interval – mean changes from the baseline pre-treatment QT (QTc) interval to the post-treatment QT (QTc) interval after treatment with AQ-13 or Coartem.

5] Pharmacokinetic parameters for AQ-13 – these will include measurements of AQ-13 blood levels over time (peak levels, AUC, MRT), drug distribution (Vd/F) and drug elimination (t½, Cl/F).

6] Fever clearance time: Time in hours from the initiation of therapy until disappearance of fever for at least 24 hours.

7.1.3 Tertiary Outcome Measures

1] Pharmacokinetics of AQ-13 metabolites – estimates of pharmacokinetic parameters for the mono- and di-*N*-dealkylated metabolites of AQ-13 (AQ-72, AQ-73).

2] Aminoquinoline (CQ) pruritus – pruritus in persons receiving AQ-13 or Coartem, especially in persons with a history of pruritus after treatment with CQ.

7.2 Assessment of Treatment Outcomes

7.2.1 Parasitologic Data

Asexual parasite counts (parasite densities per μl of blood) will be based on the average of two independent readings for each time point – performed by two independent microscopists (both blinded to the treatment the patient received). Blood smears will be obtained beginning at the time of enrollment and continuing twice daily (every 12 hours) until two successive smears are negative during the week-long (6-7 day) inpatient stay. After the patient has been discharged, two thick smears will be obtained at each of the twice weekly follow-up appointments during weeks 2 to 6 (Gantt Chart on page 27).

Laboratory Procedures: Parasite counts will be obtained by examining Giemsa-stained thick blood smears containing a minimum of 300 white blood cells and multiplying the number of asexual parasites observed by 25 to estimate the parasite count based on a white blood cell count of 7,500 white cells per μl of blood.

Quality Control: Slides for which the parasite densities estimated by the two microscopists vary by more than 20% or differ qualitatively (negative vs. positive, presence vs. absence of species other than *P. falciparum*) will be examined by a third senior microscopist to resolve those questions.

Parasitologic Outcomes that will be assessed and compared between the two study arms will include:

- Reduction of asexual parasitemia to < 25% of the admission parasite count by day 3,
- Clearance of asexual parasites from the blood on or before day 7 after treatment, and
- Recurrent asexual stage parasitemia on or before day 42.

Assessment and Reporting of Parasitologic Outcomes

Based on the parasitologic data, the PI and one physician colleague (both blinded to the treatment the patients received) will assess the parasitologic outcomes for each of the participants. Those data will then be summarized by the subject's study numbers and returned to the study statistician who will place the data in groups by study arm (retaining blindedness by using Arm 1 and Arm 2 to identify the study arm rather than AQ-13 and Coartem) before the data are tested for differences and presented to the DSMB.

7.2.2 Clinical Data

Clinical assessments of study participants at the times of screening, inpatient studies and outpatient follow-up will be performed by Malian physicians (likewise blinded to the treatments the participants have received) using the Case Report Forms prepared for this study.

Assessment of Clinical Outcomes

Judgments about progression to severe or complicated malaria and the recurrence of uncomplicated malaria will be made by the Mali PI and a physician colleague (both blinded to the treatment the patient received) based on the CRFs and parasitologic data. As for the parasitologic data, those results will then be summarized by the subject's study numbers and returned to the study statistician who will place the data in groups by study arm (retaining blindedness by using Arm 1 and Arm 2 to identify the study arm rather than AQ-13 and Coartem) before the data are tested for differences and presented to the DSMB.

8 SAFETY ASSESSMENT AND REPORTING

8.1 Definition of an Adverse Event (AE)

An **adverse event** (AE) is any adverse change in health or side-effect that occurs in a person participating in a clinical trial while the patient is receiving the treatment (study medication) or within a pre-specified period of time after their treatment has been completed (in this case, within 6 weeks or 42 days).

8.2 Definition of a Serious Adverse Event (SAE)

An adverse event (AE) is serious and should be reported when the patient outcome is one or more of the following:

- Death,
- Life-threatening illness,
- Requires an inpatient hospitalization or the prolongation of an existing hospitalization,
- Results in persistent or permanent disability,
- Is associated with a congenital anomaly or birth defect,
- Requires intervention to prevent permanent impairment or damage. *Serious AEs do not include treatment failure with recurrent parasitemia and uncomplicated malaria (i.e., failure to clear asexual parasites from the bloodstream within 7 days or recurrence within 42 days*).

8.3 Definition of the Relatedness of AEs to DRUG TREATMENT

Definitions to be used in assessing the relationship of the investigational product to the AE/SAE are:

- **Unknown:**
  Use this category only if the cause of the AE/SAE is not possible to determine.
- **Not Related:**
  The subject did not receive the investigational product, the temporal sequence of the AE/SAE onset relative to administration of the investigational product is not reasonable, or there is another obvious cause of the AE/SAE.
- **Unlikely (to be Related):**
  There is **no** evidence of exposure to the investigational product or there is another more likely cause of the AE/SAE.
- **Possibly Related:**
  There is evidence of exposure to the investigational product, the temporal sequence of the AE/SAE onset relative to administration of the investigational product is reasonable, but the AE/SAE could have been due to another equally likely cause.
- **Probably Related:**
  There is evidence of exposure to the investigational product, the temporal sequence of the AE/SAE onset relative to administration of the investigational product is reasonable, and the AE/SAE is more likely explained by the investigational product than by any other cause.
- **Definitely Related:**
  There is evidence of exposure to the investigational product, the temporal sequence of the AE/SAE onset relative to administration of the investigational product is reasonable, the AE/SAE is more likely

explained by the investigational product than by any other cause, and the AE/SAE shows a pattern consistent with previous knowledge of the investigational product or investigational product class.

8.4 Assessment of Adverse Events

8.4.1 Detection of Adverse Events

Detection of adverse events (AEs) will be based on twice daily interviews during the inpatient stay and on interviews during each of the outpatient visits. At these times, the physician will ask about common symptoms such as headache, dizziness, nausea and any other symptoms or signs patients may have. Please note that patient diaries (which were used during the Phase 1 studies in New Orleans) are unlikely to be useful (although they will be offered) because we expect that > 90% of the study participants will be illiterate.

8.4.2 Assessment (Grading) of Adverse Events (AEs)

Based on the information in the patient's record and the Case Report Forms, the Mali PI and a physician colleague (blinded to the treatments that the patients have received) will grade the AEs observed and report those results by study number to the Study Statistician, who will provide those data to the DSMB grouped by study arm, but not identified in terms of which treatments the different study arms received.

8.5 Reporting Procedures

Researchers participating in clinical trials must report all adverse events to the Food and Drug Administration (FDA) or the relevant regulatory authority in the country where the drug is to be registered. Serious AEs must be reported immediately; minor AEs are 'bundled' by the sponsor and submitted later. Specific reporting procedures are summarized below for:

- Deaths – will be reported to the Medical Monitor, the Chair and other members of the DSMB, the Mali and Tulane IRBs and the FDA by phone within 24 hours of becoming aware of their occurrence and confirmed by E-Mail also within 24 hours of awareness of their occurrence.
- Non-fatal serious AEs (regardless of their relationship to the study protocol) - will be reported to the Medical Monitor and the Chair of the DSMB via E-Mail within 72 hours after becoming aware of their occurrence.
- Other Adverse Events (AEs) – AEs that do not result in death or life-threatening events, and are judged not to be serious will be accumulated (collated) as they occur and reported with annual and other regular reports to the Medical Monitor, Data Safety and Monitoring Board (DSMB), the Mali and Tulane IRBs and the FDA at regular intervals.

8.5.1 Serious Adverse Event Detection and Reporting

For events meeting the previously described definition of Serious Adverse Events, the completion of a Serious Adverse Event Form is required. Information on where to send this form is included in the Manual of Procedures for this study. All serious AEs will be followed through resolution by a study physician and reviewed by a Senior Investigator. Any AE considered serious by the PI or the Senior Investigator must be reported according to the guidelines on the Serious Adverse Event Form.

8.5.2 Reporting of Pregnancy

Please note that this section does not apply to this Proof of Concept Study for AQ-13 because enrollment in this study is restricted to males.

8.5.3 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

New abnormalities in chemistry or hematology test results during or after treatment with AQ-13 or Coartem will be graded based on the recent FDA Guidance for Healthy Adult and Adolescent Volunteers in Preventive Vaccine Trials [**25**]. Because a number of subjects will have abnormal chemistry and hematology laboratory values at admission because of malaria, the presence of abnormal laboratory values at the time of admission~~)~~ is not an exclusion from participation (unless those values require treatment), and the presence of abnormal laboratory values during treatment is an indication of an AE ONLY IF the initial laboratory values for that parameter were normal.

8.5.4 Type and Duration of the Follow-up of Subjects after Adverse Events

Mild AEs known to occur with both AQ-13 and malaria (e.g., headache, nausea, vomiting, myalgias, arthralgias) and other AEs will be followed daily until they have resolved and treated symptomatically as described in Table 2 on page 23 (acetaminophen for headache and for muscle and joint pains; antiemetics for nausea or vomiting).

8.6 Study Halting Rules

Because of the limited numbers of subjects in this study (**33** per study arm), halting rules based on statistical significance are likely to be of limited value. Therefore, the guidelines proposed below are based on the limited numbers of subjects likely to be evaluable and on the number, severity and relatedness of adverse events (AEs). If those guidelines are met for subjects in the arm receiving AQ-13, the enrollment of new subjects into the trial will be halted until the DSMB has had time to review the information available and thus to recommend whether the enrollment and treatment of adult Malian males who have uncomplicated malaria should continue with AQ-13 and Coartem in this study.

Table 4.

Temporary Halting Rules based on Adverse Events

Number	Severity of Adverse Event	Relatedness to Drug	Action(s) Taken
1 or more	Serious Adverse Event (SAE)	Probably or Definitely	Temporary Halt for DSMB Review
1 or more	Grade 4 Adverse Event	Probably or Definitely	Temporary Halt for DSMB Review
2 or more	Grade 3 Adverse Event	Probably or Definitely	Temporary Halt for DSMB Review

Table 5.

Temporary Halting Rules based on Efficacy (based on 15 subjects per study arm)

Control Coartem Result (number of failures)	AQ-13 Study Arm Result (number of failures)	Action(s) Taken
0 or 1 of 15	0 or 1 of 15	No action
0 or 1 of 15	2 or 3 of 15	No action
0 or 1 of 15	4 or 5 (or more) of 15	Temporary Halt for DSMB Review

In addition, summary data on efficacy (both blinded and separated by treatment group) will be presented to the DSMB at one pre-specified Interim Analysis for futility (after 15 patients have been enrolled in each treatment group/arm and have completed their follow-ups).

8.7 Data Safety and Monitoring Board

Oversight of safety, accrual and – if necessary – efficacy will be provided by a Data Safety and Monitoring Board. We propose a data safety and monitoring plan similar to the one utilized for the Phase 1 Studies in New Orleans. This was initially a challenge because FDA, NIH and CDC decided that the DMSB should not include any Tulane investigators (because of potential conflicts of interest) and because all the investigators in New Orleans with malaria experience of whom we were aware were involved in this project. However, we were able to identify 5 individuals with relevant experience, who were willing to serve on the Phase 1 DSMB, and are willing to serve on the Phase 2 DSMB. Although we had planned to bring a number of investigators from overseas for this purpose, we were told on a previous review that FDA support would not be provided to cover travel expenses for a more international DSMB which had more experience with malaria and antimalarial drugs. For this reason, we have suggested that four members of the Phase 1 DSMB continue to serve on the Phase 2 DSMB. Please note that each member of the Phase 1 DSMB has gained substantial experience during their 6-8 years of service on the Phase I DSMB. Thus, they are today more knowledgeable about malaria and studies of antimalarials than they were more than 6-8 years ago when the Phase 1 Studies began. To accomplish the goal of effective monitoring while adding expertise, we have identified two investigators with extensive relevant experience (each indicated by an asterisk), who have agreed to serve with the members of the Phase 1 DSMB to monitor the Phase 2 Studies of AQ-13 in MALI. In addition, Professor Kéita (also marked with an asterisk) and Dr. Obinna Nnedu (who has worked on-site in Kenya) have been added because the DSMB was criticized because it had no investigators from malaria-endemic areas or with experience in malaria-endemic areas.

Table 6.

MEMBERS OF THE DSMB FOR THE PROOF OF CONCEPT STUDY FOR AQ-13

George A. PANKEY, MD, Chair of DSMB	Director of Research, Ochsner Clinic Foundation extensive background in infectious disease research
John R. DAVID, MD *	Millennium Project, Immunologic Studies of Tropical Disease, Professor and Chair Emeritus, Tropical Public Health, Harvard SPH
Mamadou M. KÉITA, MD *	Professor and Chair Emeritus, Department of Pediatrics, Hôpital Gabriel Touré and the Faculty of Medicine, University of Bamako, MALI
Donald MERCANTE, PhD	Professor and Director of Biostatistics, LSU School of Public Health
Adrienne MITCHELL	Director, Clinical Research Unit, Xavier College of Pharmacy
Obinna NNEDU, MD, MPH *	Infectious Disease Physician, Ochsner Clinic Foundation
Eve E. SLATER, MD *	Former Senior Investigator and Vice President, Merck, Sharp and Dohme, Assistant Secretary of Health in Health and Human Services (2002-03) Associate Professor of Medicine, Columbia University

Emel SONGU-MIZE, PhD Professor of Pharmacology Emerita, LSU School of Medicine

9 CLINICAL MONITORING STRUCTURE

Monitoring will be performed before, during and after these studies and according to the guidelines provided by the E6 Good Clinical Practice document [**24**] to ensure that the:

- Rights and welfare of the subjects are respected,
- Data reported from the trial are accurate, complete and verifiable from source documents,
- Conduct of the trial is consistent with the currently approved protocol amendments, with GCP and with applicable regulatory requirements.

9.1 Site Monitoring Plan

Selection and Qualifications of Monitors.

- Monitors will be appointed by the Sponsor,
- Monitors will be appropriately trained so they have the scientific and clinical knowledge necessary to monitor the trial adequately,
- Monitor's qualifications will be documented.

Extent and Nature of Monitoring.

- Sponsor will determine the appropriate extent and nature of monitoring based on the objectives, purpose, design, complexity, blinding, size and endpoints of the trial,
- Sponsor will then ensure that the trial is adequately monitored,
- Monitoring will be performed before, during and after the trial,
- Written documentation can support GCP training, meetings and investigators' qualifications,
- Statistically designed sampling will be used to select the data (specimens) to be verified.

Monitor's Responsibilities.

The monitor should ensure that the trial is conducted and documented properly by:

- Providing the main line of communication between the Sponsor and the Investigator,
- Ensuring that the Investigators have adequate resources and that those resources remain adequate throughout the trial,
- Ensure that the staff and facilities (laboratories and equipment) are adequate to conduct the trial and remain adequate throughout the trial,
- Verify for the investigational product that:
    - Storage times and conditions are acceptable, and supplies are sufficient throughout the trial,
    - Investigational product is supplied only to subjects eligible to receive it and at protocol-specified doses,
    - Subjects are provided with the necessary instructions on properly using, handling, storing and returning the investigational product,
    - Receipt, use and return of the investigational product at the trial sites is controlled and adequately documented,

- Disposition of unused investigational product at the trial sites complies with applicable regulatory requirements and the Sponsor's authorized procedures,

- Verify that the Investigators follow the currently approved protocol and amendments,
- Verify that written informed consent was obtained before each subject's participation in the trial,
- Ensure that the Investigator receives the current Investigator's Brochure and all documents and supplies to conduct the trial properly, and comply with applicable regulatory requirements,
- Ensure that the Investigators and the Investigators' trial staff are adequately informed about the trial,
- Verify that the Investigators and the Investigators' trial staff are performing the specified trial functions in accord with the protocol and other written agreements between the Sponsor and Investigator or Institution, and have not delegated those responsibilities to unauthorized individuals,
- Verify that the Investigators are enrolling only eligible subjects,
- Verify that the Investigators are reporting the recruitment rate,
- Verify that source data and documents are accurate, complete, up-to-date and maintained,
- Verify that the Investigators provide all required reports, notifications, applications and submissions, and that those documents are accurate, complete, timely, legible, dated and identify the trial,
- Check the accuracy and completeness of CRF entries, source data and documents, and other trial-related documents against each other. The monitor should specifically verify that:
    - Data required by the protocol are reported accurately on the CRFs and are consistent with the source data and documents,
    - Any dose or treatment modifications are well-documented for each of the trial subjects,
    - AEs, concomitant medications and intercurrent illnesses are reported on the CRFs in accordance with the protocol,
    - Visits that subjects do not make, and tests and examinations that are not performed are clearly marked (so indicated) on the CRFs,
    - All withdrawals and dropouts from the trial are reported and explained on the CRFs.
- Inform the Investigator about CRF entry errors, omissions or illegibility, The monitor should ensure that appropriate corrections (additions, deletions) are made, dated, explained (if necessary) and signed by the Investigator or an authorized [documented] member of their trial staff,
- Determine whether all AEs are reported within the time periods required by GCP, *ICH Guidance for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting*, the protocol, the IRB/IEC, Sponsor and applicable regulatory requirement,
- Determine whether the Investigator is maintaining the appropriate documents (see *Section 8. Essential Documents for the Conduct of a Clinical Trial*),
- Communicate deviations from the protocol, GCP, SOPs and applicable regulatory requirements to the Investigator and take appropriate actions to prevent recurrence of the detected deviations,

Monitoring Procedures.

The monitor(s) should follow the sponsor's established written SOPs as well as the procedures specified by the sponsor for monitoring a specific trial.

Monitoring Report.

- The monitor should submit a written report to the sponsor after each trial-site visit or trial-related communication.
- Reports should include the date, site, name of the monitor, and names of the Investigators or other individuals contacted.
- Reports should include a summary of what the monitor reviewed and the monitor's statements concerning the significant findings/facts, deviations and deficiencies, conclusions, actions taken or to be taken, and/or actions recommended to secure compliance.
- The review and follow-up of the monitoring report by the sponsor should be documented by the sponsor's designated representative.

10 STATISTICAL CONSIDERATIONS

10.1 Study Outcome Measures

The primary, secondary and tertiary objectives (Section 3, above) and outcome measures (Section 8, above) in this study will be assessed statistically as outlined below (Table 7).

Table 7.

Statistical Assessment of Study Endpoints

COMPONENTS OF THE PRIMARY ENDPOINT	STATISTICAL TESTS
Lack of recrudescence at 42 days	Pearson χ^2, Fisher Exact test
< 25% of baseline asexual parasitemia on day 3	Pearson χ^2, Fisher Exact test
Clearance of asexual parasitemia and fever by day 7	Pearson χ^2, Fisher Exact test
SECONDARY ENDPOINTS	
Adverse Events (AEs)	Pearson χ^2, Fisher Exact test
Effects on the QT (QTc) Interval (within individuals)	Wilcoxon, Friedman test
Effects on the QT (QTc) Interval (between groups)	ANOVA, Kruskal-Wallis test
Pharmacokinetic Parameters for Parent Compounds (AUC_t, $t_{1/2}$, Vd/F, Cl/F, MRT)	Mann-Whitney, Kruskal-Wallis
Time to parasite Clearance, Recrudescence or Reinfection (between groups)	Kaplan-Meier, Cox Hazard
Time to fever clearance (between groups)	Kaplan-Meier, Cox Hazard
TERTIARY ENDPOINTS	
Pharmacokinetics of metabolites	as above
Aminoquinoline (chloroquine) pruritus	Fisher Exact test

AUC_t = total area under the curve; $t_{1/2}$ = elimination half-life; Cl/F = oral clearance; Vd/F = oral apparent volume of distribution; MRT = mean residence time.

10.2 Sample Size Considerations

The proposed sample sizes are 33 per group for a total of 66 subjects in 2 treatment groups (one AQ-13 group receiving 1596 mg orally + one Coartem control group) for this Proof of Concept Study of AQ-13 in adult Malian males ≥ 18 years of age with uncomplicated malaria (Flow Charts 2-3 on pages 16-17 and Flow Chart 4 on page 26).

Because sample sizes vary markedly with the efficacy of the comparator, accurate estimation of the Coartem cure rate is a key issue. The sample sizes estimated were based on an expected efficacy (cure rate) of 95% for Coartem [**20-22, 25-27**] and a conservative 20% estimate of attrition after treatment . *An expected cure rate of 95% has been used for Coartem* because this estimate is at the lower end of the range of cure rates reported in the recent literature (93.9%, 95.5%, 97.1%, and 97.5%) [**20-22, 36**], and because it simultaneously recognizes recent concerns about *in vivo* selection by Coartem for the 86N allele of *pfmdr1* in *P. falciparum,* which has the potential to further reduce the cure rate [**37-38**].

Another major factor is the width of the non-inferiority margin (*M*), the range within which the investigational drug is considered non-inferior to the comparator (control). We have used a value of *M* = 15%. In the recent Guidance Document "Non Inferiority Clinical Trials" [Mar 2010], *M* equal to 40% of the efficacy of the positive control is stated to be a statistically persuasive result for phase III trials of drugs in general and elsewhere in this document it is stated that a non-inferiority margin of 50% for cardiovascular drugs is equivalent to a non-inferiority margin of 10-15% for antibiotics. From these statements, we infer that for a candidate antimalarial such as AQ-13, a non-inferiority margin of 10-15% would be statistically persuasive for a phase 3 trial, in which case this margin should also be sufficient for the present Phase 2 Proof of Concept Efficacy Trial of AQ-13.

With *M*=15%, a Coartem efficacy assumed to be 95% and a dropout rate up to 20%, a table from Machin and Campbell [Statistical tables for the design of clinical trials. Blackwell, Oxford: 1987, pp. 35-53] gives 33 patients per group as a sample size [80% power] (an excerpt from that table is provided as Table 8).

Table 8.
Effects of the Margin and the Dropout Rate on Sample Sizes necessary to test for Non-Inferiority of AQ-13 to Coartem

	Dropout Rate (percent)		
Non-Inferiority Margin	0%	10%	**20%**
5%	235	261	294
7.5%	104	116	130
9%	73	81	91
10%	59	66	74
15%	26	29	**33**
20%	15	17	19

Although the sample size calculation was based on the primary endpoint of cure for all patients, based on studies we have performed in Mali previously, we expect that approximately 65% of the 26 evaluable subjects in each group will have CQ-resistant *P. falciparum* [35]. Therefore, subgroup analysis according to chloroquine susceptibility should make it possible to generate hypotheses about the efficacy of AQ-13, compared to Coartem, for subjects with either CQ-resistant or –susceptible parasites.

10.3 Analysis Plan

This is a Proof of Concept Efficacy Study of AQ-13 in comparison to the gold standard (Coartem). Thus, this study in adult Malian males can be viewed as a non-inferiority study, comparing one dose of AQ-13 (1596 mg orally over 3 days) to Coartem [**39**].

The null and alternative hypotheses to be tested are:

$H_0 = (C - E) \geq M$ (control is superior to experimental)
$H_1 = (C - E) < M$ (experimental is not inferior to control)

C is the efficacy of the comparator (Coartem), *E* the efficacy of the investigational drug (AQ-13) and *M* the non-inferiority margin. *M* = 15% will be used (see above). AQ-13 will be considered equivalent to (not inferior to) the comparator (Coartem) if the null hypothesis is rejected with a one-sided alpha of 0.025 [**29, 40**]. Cure rates, adjusted for reinfection, will be calculated for AQ-13 and Coartem and differences between cure rates with AQ-13 and Coartem will be calculated with 95% confidence intervals. Non-inferiority of AQ-13 will be established if the upper limit of the 95% CI for the difference between AQ-13 and Coartem is less than the non-inferiority margin of 15% [**28-29**].

Analyses will be performed based on both the original randomization (intent-to-treat, ITT) and the subset of patients who complete the study protocol, for whom complete information is available (per-protocol analysis). Paradoxically, intent-to-treat (ITT) analysis poses a special problem in non-inferiority trials. ITT is in fact a non-conservative approach in non-inferiority trials because the inclusion of non-compliant subjects and dropouts dilutes the differences between treatments and therefore, favors the conclusion that the drugs are equivalent [**39**]. However, we expect the differences between ITT and per-protocol analyses will be small because drug administration under direct observation should reduce noncompliance to very low levels. Nevertheless, every effort will be made to obtain follow-up data on non-compliant subjects and subjects who drop out because of adverse events (AEs). In contrast, excluding subjects with protocol "violations" (per-protocol analysis) can bias the analysis toward concluding equivalence of the two drugs. This would be the case if drop outs from the investigational treatment were related to non-response (treatment failure). Thus, excluding those dropouts from the per-protocol analysis could inflate the efficacy estimate for the investigational drug and lead to an inappropriate conclusion of non-inferiority. Careful examination of the data from subjects who are excluded from the analysis helps to detect such bias and facilitates the correct interpretation of trial results.

Times to recrudescence and reinfection will be analyzed using life tables, and compared using the log rank test. Cox regression will be used to compare multiple (multivariate) predictors of times to recrudescence and reinfection. Pharmacokinetic parameters will be examined for AQ-13 doses and compared to data from the Phase 1 studies, as appropriate. **Using the same protocol as in the Phase 1 study [11], the relatedness of adverse events (AEs) to study drugs will be classified independently by two blinded physicians according to the definitions in section 8.3 on page 30 as *unknown, not related, unlikely to be related, or possibly, probably or definitely related* to the study drugs, based on temporal relationships and biological plausibility.** The frequencies of AEs will be compared between AQ-13 and Coartem using Pearson Chi-square. Because more than one AE may occur in an individual patient, the proportion of patients receiving AQ-13 with AEs will be compared with Coartem using Chi-square or the Fisher Exact test. The severity of AEs will be classified using the recent FDA Guidance for Healthy Adult and Adolescent Volunteers in Preventive Vaccine Trials [**23**], and the severity of AEs across the study arms will be compared using Chi-square. QT interval corrections for heart rate (QTc) will be made using both Bazett's and Fridericia's formulas [**41-42**]. Within-patient maximum changes from baseline will be assessed using the Wilcoxon test for paired observations. Between-group QT interval changes will be compared using ANOVA or Kruskal-Wallis.

HYPOTHESES BEING TESTED.

The primary hypothesis is that the efficacy of AQ-13 for uncomplicated *P. falciparum* malaria is similar to

that of Coartem - based on a non-inferiority margin of 15%. That is, that AQ-13 will produce a cure rate within 15% of the Coartem control (standard). The secondary hypothesis is that AQ-13 will have safety profiles in subjects with uncomplicated malaria comparable to those for AQ-13 in healthy volunteers [**11**].

EXPECTED OUTCOMES.

Our preliminary (previous) studies have shown that AQ-13 is:

- *active in vitro* against CQ-resistant and multi-resistant *P. falciparum* [**8-9**],
- *active in vivo* against CQ-resistant *P. falciparum* in the squirrel monkey model of human infection with CQ-resistant *P. falciparum* (enclosed Figures 1 and 2 in the Appendix), and
- *safe in human subjects* based on Phase 1 studies recently performed in New Orleans [**11**].

Therefore, we expect this Proof of Concept Study of AQ-13 in adults will demonstrate that AQ-13 is:

- *efficacious* for the treatment of human subjects with uncomplicated *P. falciparum* malaria – i.e., that the efficacy of AQ-13 will be similar to that of Coartem, and
- *safe* – i.e., that the adverse events (AEs) observed with AQ-13 will be similar to those of Coartem in these studies, as they were to CQ in the Phase 1 studies of AQ-13 and CQ [**11**].

10.4 RANDOMIZATION

Randomization of individual subjects will be performed after screening is complete, signed informed consent has been obtained, and the inclusion and exclusion criteria have been satisfied and reviewed. Randomization to the 2 treatments (study arms) will involve permuting blocks of 4 and 6 with a random number generator [**11**]. Only the pharmacist will have access to the computer generated form.

11 ACCESS TO SOURCE DATA/DOCUMENTS

Access to Source Data and Documents for Study Forms and other Record-Keeping will be provided both electronically and in hard copy. Electronic access will be provided via a password-protected WEB Site portal (Box.com) which will be used to post and retain both original and amended versions of study protocols such as Case Report Forms, Standard Operating Procedures, Equipment Maintenance and Performance Records and Investigator Training and Job Description records. Please note that the WEB Site Portal with the source data and documents for this study will be hosted in a cloud which has full back-up and is therefore protected against power failures, hurricanes, fire and other potential local catastrophes that could occur at either site.

Access to Source Data/Documents for Participant-based Information (patient records). In contrast, patient records (for screening, informed consent, the inpatient stay and outpatient follow-up) will not be posted on the WEB Portal. However, they will be available in electronic format after scanning for review by authorized individuals under controlled conditions (on designated password-protected microcomputers).

Data and Document Storage. Electronic records will be stored on a dedicated server at the University of Bamako with duplicate electronic files maintained on a separate server at Tulane in New Orleans. Hard copies of forms and patient records will be maintained and available for review at one site in Bamako: in the Clinical Research Center at the Faculty of Medicine (Hôpital Point G). Hard copies of patient records will be kept under lock and key (in locked cabinets within rooms that have doors with locks); electronic copies of patient records will be protected (in both Mali and New Orleans) by storage in password-protected files within password-protected computers. Patient files will be organized by the subject's study number; the document linking study ID numbers to patient names will be stored under lock and key with the PI, the Mali PI and the Study Monitor (3 hard copies).

Case Report Forms will be maintained in individual folders (one folder for each type [category] of Case Report Form) on the Box.com WEB Site and will therefore be accessible to all investigators participating in this study. Each revision of a Case Report Form will be identified by the date on which it was approved, and each folder will have a chronologic record of revisions, which will be entered as they are posted and implemented and beginning with the date of implementation.

Standard Operating Procedures will likewise be maintained in individual folders (one folder for each Standard Operating Procedure [SOP], e.g., for Hb determinations and PCR). As with the Case Report Forms, both the original and revised versions of each SOP will be available via the SharePoint WEB Site. Likewise, each SOP folder will have a chronologically-organized record of the revisions of that SOP, including the dates on which they were posted and implemented.

Equipment Inventory, Maintenance and Performance will likewise be recorded for each of the instruments that will be used for these studies. During these studies, those forms (records) will be updated at least once every 3 months (quarterly), and modified on Box.com WEB Site as new information becomes available. Hard copies of these forms will be available at two sites in Mali: the Faculty of

Medicine (Clinical Research Center at the Hôpital Point G) and the Faculty of Science of the University of Bamako.

Investigator Training, Job Descriptions and Responsibilities will be documented in hard copy and in electronic format for each of the investigators participating in these studies. Electronic copies of these records will be available on the Google Apps WEB Portal; the hard copies will be available at two sites in Mali: the Faculty of Medicine (Clinical Research Center at the Hôpital Point G) and at the Faculty of Science of the University of Bamako.

The Manual of Procedures for these studies will also be available in electronic form on the Box.com WEB Site and in hard copy at two sites in Mali: the Faculty of Medicine (Clinical Research Center at the Hôpital Point G) and at the Faculty of Science of the University of Bamako.

Individual Participant Records and Files will be available in hard copy under lock and key in a locked room at the Faculty of Medicine (Clinical Research Center at the Hôpital Point G) which is part of the University of Bamako. Electronic versions of these records (Adobe Acrobat pdf versions of participant records and files) will be available, but will not be posted on the Box.com WEB Site because of concerns about patient confidentiality. Identifying information in the hard copies and electronic copies of these files will be limited to participants' study ID numbers and initials because of concerns about patient confidentiality.

12 QUALITY CONTROL AND QUALITY ASSURANCE

- The sponsor is responsible for implementing and maintaining quality assurance and quality control systems with written SOPs to ensure that trials are conducted and data are generated, documented (recorded) and reported in compliance with the protocol, GCP, and the applicable regulatory requirement(s).
- The sponsor is responsible for securing agreement from all involved parties to ensure direct access (see section 1.21) to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by domestic and foreign regulatory authorities.
- Quality control should be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.
- Agreements, made by the sponsor with the investigator/institution and/or with any other parties involved with the clinical trial, should be in writing, as part of the protocol or in a separate agreement.

These goals will be accomplished using two complementary methods:

1] by the random selection of a fraction of specimens for blind retesting (without their original identification) for all procedures, and is described in the relevant SOPs. The fraction of specimens selected for blind retesting will initially be 10% and will then decrease to 5% as reproducibility increases and coefficients of variation decrease (reproducibility).

2] by participating in quality control testing programs that distribute unknown specimens for testing by participating laboratories at overseas sites (accuracy).

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 Declaration of Helsinki

The investigator will ensure that this study is conducted in full conformity with the current revision of the Declaration of Helsinki, or with the International Conference for Harmonisation Good Clinical Practice (ICH-GCP) regulations and guidelines, whichever affords the greater protection to the subject.

13.2 Institutional Review Board

Each participating institution must provide for the review and approval of this protocol and the associated informed consent documents and recruitment material by an appropriate independent ethics review committee (IEC) or Institutional Review Board (IRB) registered with OHRP. Any amendments to the protocol or consent materials must also be approved before they are placed into use. In both the United States and in other countries, only institutions holding a current U. S. Federal-Wide Assurance issued by OHRP may participate. Refer to: http://ohrp.osophs.dhhs.gov.

13.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation in this study will be provided to the subjects and their families. Consent forms describing in detail the study procedures and risks are given to the subject and written documentation of informed consent is required prior to enrolling in the study. Consent forms will be IRB-approved. Literate subjects will be asked to read and review the document. Illiterate subjects will receive oral presentations of the content of consent document by study staff in their native Bambara language. Upon reviewing the document, the investigator or their staff will explain the research study to the subject and answer any questions that may arise. The subjects will sign the informed consent document prior to being enrolled in the study. The subjects should have the opportunity to discuss the study with their surrogates and think about it prior to agreeing to participate. The subjects may withdraw consent at any time throughout the course of the study. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they choose not to participate in this study.

13.3.1 Informed Consent/Assent Process (in Case of a Minor or others unable to consent for themselves)

Not Applicable. This study will enroll only adult Malian males ≥ 18 years of age.

13.4 Exclusion of Women, Minorities, and Children (Special Populations)

Minorities will not be excluded from these studies. Because Mali is in sub-Saharan Africa, all the subjects recruited will be minorities by U.S. criteria (African).

In contrast, both adult women and children will be excluded from these studies. The rationale for excluding children from these initial Phase 2 dose-finding efficacy studies of AQ-13 is to ensure that the doses of AQ-13 proposed for use in children (10 or 15 mg base per kg daily x 3 days, based on scaling down the mg base per kg doses from the doses used in adults) do not have untoward or unexpected effects in adults before they are tested in children for the treatment of uncomplicated malaria. The rationale for excluding women from this Proof of Concept Study for AQ-13 is to ensure that adult women volunteers who volunteer to participate and commit to contraception do not inadvertently become pregnant while receiving AQ-13 or shortly thereafter.

13.5 Subject Confidentiality

Subject confidentiality is held strictly in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests, in addition to the clinical information relating to participating subjects. **As noted above and in the consent form, participants will be identified in hard copies and electronic copies of case report forms and other patient records by their initials and study ID numbers, but not by name.**

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

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

13.6 Future Use of Stored Specimens

As noted above and in the consent form, residual specimens will be held for a minimum of three years after the study is complete to permit repeat testing as necessary. As also noted in the consent, participants have the option to withdraw their consent at any time, which includes the withdrawal and removal of their specimens if they so choose. Specimens will be maintained at the University of Bamako in Mali and at Tulane University in New Orleans. As also noted in the consent, participants are asked to consent to genetic studies of the parasites in their blood and of the genes responsible for the metabolism of drugs such as AQ-13 and the host immune response to uncomplicated malaria. As with other data, that testing will be performed with specimens identified only by the participant's study ID number.

14 DATA HANDLING AND RECORD KEEPING

The steps noted below will be taken to assure that the data collected are accurate, consistent, complete, reliable and in accordance with ICH GCP guidelines and 21 CFR Part 11. Included below are references to source documentation, case report forms, data handling procedures and procedures for data monitoring. Details may be provided in a Manual of Procedures, User's Guide or other citable reference document. Refer to: http://www.fda.gov/ora/compliance_ref/part11/

14.1 Data Management Responsibilities

The responsibilities for data handling and record keeping as they relate to the sponsor, clinical site, laboratory and data coordinating center are outlined in Table 9, below. This information includes the roles of groups and individuals in data collection, review of data, study materials and reports and the retention of source documents, files and records. At the conclusion of the study, a copy of all data sets will be provided to DMID, FDA, the Mali Ministry of Health and other participating federal and international agencies. These data sets will be sent by the Information Technology director for this project, Ayouba Diarra, at the Faculty of Science of the University of Bamako.

All source documents and laboratory reports will be reviewed by the clinical team and data entry staff in order to ensure they are accurate and complete. Adverse Events will be graded, assessed for severity and causality, and reviewed by the Principal Investigator and co-investigators (who will be blinded to the treatment arms to which the subjects were assigned).

Table 9.

Overview of Data Management Responsibilities

Data Management	Clinical Information	Clinical Lab Data	Holters	Molecular Results
Data Collection (Source Documents)	Screening, Enrollment CRFs and scans	Clin- Laboratory Data Printouts	Screen, Enroll, Holter reports	Initial & Follow-Up Lab reports
Data Review	as CRFs (*StudyTRAX*)	Printouts & CRFs	Holter reports	Lab rep's & CRFs
Data Entry, Cleaning	Bamako, *StudyTRAX*	Bamako, Tulane	Bamako	Bamako
Data Storage (source docs, electronic)	Bamako, Tulane	Bamako, Tulane	Bamako, Tulane	Bamako, Tulane
Initial Summary	Epidemiol, Mali PI	Epidemio, Mali PI	Cardiol Point G	Faculty of Science
Initial Data Review	Biostat, Clin Monitor	Clinical Monitor	Cardiol Mali	Mali PI
Additional Review	Tulane	Tulane	Cardiol Tulane	Sequence data
Evaluation, Grading of Adverse Events	PI, colleagues blind to treatment arm	PI, others blind to treatment arm	PI and Tulane Cardiology	Interpretation of recurrent infections
Reports	PI, Sponsor	PI, Sponsor	PI, Sponsor	PI, Sponsor
Long-term Storage	Hard copy, electronic	Mali and U.S.	Mali and U.S.	Mali and U.S.

14.2 Data Capture Methods

Data capture methods will begin with hard copy source documents such as Case Report Forms (CRFs) which will be entered into the data base (*StudyTRAX* forms for the AQ-13 Proof of Concept Study) after

they have been reviewed for accuracy by the Data Manager and Clinical Monitor. This approach will also be used for the clinical forms (Screening, Enrollment and Follow-Up) and for the clinical laboratory forms (Screening, Enrollment and Follow-Up). For other types of data (Holter recordings, molecular results - see section 14.3, below), the original records will be retained and used as source documents at the time of data entry, rather than case report forms (CRFs). The expectation is that data from CRFs and other source documents will be entered within 2 weeks after they have been obtained and reviewed.

14.3 Types of Data

The types of data that will be collected in this Phase 2 Proof of Concept Efficacy Study include:

Clinical and Historical Data during the processes of screening, enrollment and follow-up (CRFs; *StudyTRAX*),

Physical Examination information from the times of screening, enrollment and follow-up (CRFs, *StudyTRAX*),

Clinical Laboratory information from the times of screening, enrollment and follow-up (CRFs; *StudyTRAX*),

Electrocardiograms and Holter recordings from the times of screening, enrollment, dosing and follow-up (CRFs, Rozinn Holter Monitoring System),

Molecular results in terms of *Apo*I endonuclease cleavage and sequence data for the chloroquine resistance locus (positions 72-76 of *pfcrt* – CRFs and reports of sequence data).

14.4 Timing of Reports

For this abbreviated Phase 2 Proof of Concept Efficacy Study, there will be two reports. The first report will be provided after 15 subjects have been enrolled in each of the two arms of the protocol (AQ-13 and Coartem) and have completed their follow-ups (Interim Analysis for Futility – section 8.6 of this protocol on page 32). The second report (Final Analysis for Efficacy, Adverse Events and Pharmacology) will be provided after 18 additional subjects have been enrolled in each arm (total of 66 subjects) and have completed their follow-ups.

14.5 Study Records Retention

Records and specimens will be maintained (stored under protected conditions) for the duration of the study and a minimum of three years thereafter to permit repeat testing and re-examination as necessary. At that time, both records and specimens will be destroyed if there is no further need for them.

14.6 Protocol Deviations

Information about potential deviations from the study protocol will be sought by the Clinical Monitor (who will visit the sites of inpatient and outpatient follow-up weekly during the course of this study), the director for Information Technology, the Clinical Coordinator, the Mali PI and his colleagues (again based on weekly follow-up visits) and the PI. Information about protocol deviations will be summarized monthly during the course of these studies and reported each month (within 5 days) to the Mali PI and the study PI and sponsor.

15 PUBLICATION POLICY

Following completion of the study, the investigator may publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. It is the responsibility of DMID to register this trial in an acceptable registry. Any clinical trial starting enrollment after 01 July 2005 must be registered either on or before the onset of patient enrollment. For trials that began enrollment prior to this date, the ICMJE member journals will require registration by 13 September 2005 before considering the results of the trial for publication.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., Phase 1 trials), would be exempt from this policy.

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SUPPLEMENTS/APPENDICES

PREVIOUSLY CITED MANUSCRIPTS THAT ARE PROVIDED:

De D, Krogstad FM, Cogswell FB, Krogstad DJ: Aminoquinolines that circumvent resistance in *Plasmodium falciparum in vitro. Am J Trop Med Hyg* 1996; 55(6): 479-583.

De D, Krogstad FM, Byers LD, Krogstad DJ: Structure-activity relationships for antiplasmodial activity among 7-substituted 4-aminoquinolines. *J Med Chem* 1998; 41(25): 4918-4926.

Lakshmanan V, Bray PG, Verdier-Pinard D, Johnson DJ, Horrocks P, Muhle RA, Alakpa GE, Hughese RH, Ward SA, Krogstad DJ, Sidhu ABS, Fidock DA: A critical role for *PfCRT* K76T in *Plasmodium falciparum* verapamil-reversible chloroquine resistance. *EMBO J* 2005; 24(13): 2294-2305.

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FIGURES PROVIDED FROM THE INITIAL IND SUBMISSION:

Figures 1 and 2 illustrating the effect of AQ-13 on *P. cynomolgi* in the rhesus monkey model of human *P. vivax* infection and on the CQ-resistant Indochina I strain of *P. falciparum* from page 15 of the Initial IND Submission in April 1998.

STANDARD OPERATING PROCEDURES (submitted January 2010):

AQ-13 HPLC ASSAY: CALCULATING AQ-13 AND METABOLITE CONCENTRATIONS. Deng H, Krogstad DJ: Drafted Dec 8, 2009; Last revision Jan 23, 2010.

AQ-13 HPLC ASSAY: EXTRACTING (PREPARING) WHOLE BLOOD AND URINE SAMPLES. Deng H, Krogstad DJ: Drafted Dec 5, 2009; Last revision Jan 23, 2010.

AQ-13 HPLC ASSAY: FLUORESCENCE HPLC ASSAY FOR AQ-13 AND ITS METABOLITES. Deng H, Krogstad DJ: Drafted Dec 4, 2009; Last revision Jan 5, 2010.

CENTRIFUGATION OF BLOOD AND RED CELL SUSPENSIONS. Sangaré L, Koita OA: Drafted Oct 30, 2009; Last revision Jan 23, 2010.

CLEANING (PREPARING) SLIDES FOR MALARIA BLOOD SMEARS. Sangaré L, Koita OA: Drafted Oct 30, 2009; Last revision Jan 23, 2010.

COULTER A^{C}T 10 HEMATOLOGY ANALYZER. Sangaré L, Koita OA: Drafted Oct 30, 2009; Last revision Jan 23, 2010.

CRYOPRESERVATION (FREEZING) OF MALARIA PARASITES. Sangaré L, Koita OA: Drafted Oct 30, 2009; Last revision Nov 5, 2009.

DATA ACQUISITION, TRANSFER AND STORAGE. Diarra A, Koita OA: Drafted Dec 29, 2009; Last revision Jan 23, 2010.

DNA ELECTROPHORESIS IN AGAROSE. Sangaré L, Koita OA: Drafted Oct 30, 2009; Last revision Jan 23, 2010.

DNA EXTRACTION FROM FILTER PAPER BLOTS. Sangaré L, Koita OA: Drafted Oct 30, 2009; Last revision Jan 23, 2010.

DNA QUANTITATION BY SPECTROPHOTOMETRY. Sangaré L, Koita OA: Drafted Nov 2, 2009; Last revision Jan 23, 2010.

G6PD (GLUCOSE-6-PHOSPHATE DEHYDROGENASE) TESTING. Sangaré L, Koita OA: Drafted Jan 6, 2010; Last revision Jan 7, 2010.

HEMOCUE RAPID HB DETERMINATION. Sangaré L, Koita OA: Drafted Oct 30, 2009; Last revision Jan 5, 2010.

PCR (POLYMERASE CHAIN REACTION) AMPLIFICATION OF DNA. Sangaré L, Koita OA: Drafted Nov 2, 2009; Last revision Jan 23, 2010.

PICCOLO CHEMISTRY ANALYZER. Sangaré L, Koita OA: Drafted Nov 2, 2009; Last revision Jan 23, 2010.

PREGNANCY TESTING. Sangaré L, Koita OA: Drafted Nov 2, 2009; Last revision Jan 5, 2010.

RNA EXTRACTION. Sangaré L, Koita OA: Drafted Oct 30, 2006; Last revision Jan 23, 2010.

STORAGE OF BIOLOGICAL SPECIMENS. Sangaré L, Koita OA: Drafted Oct 30, 2009; Last revision Jan 23, 2010.

THICK SMEARS, THIN SMEARS AND FILTER PAPER BLOTS. Sangaré L, Koita OA: Drafted Oct 30, 2009; Last revision Jan 5, 2010.

Appendix A: Study Schedule

This information is provided in the Study Proposal, particularly in Section 6 (Screening and Study Procedures), which has a flow chart on screening and first week of inpatient studies (Flow Chart 4, page 20) and a color-coded Gantt Chart on page 21, which provides an overview of the entire 42 day study period. In addition, an initial overview is provided in Flow Chart 1 (page viii), and flow charts describing the screening (Flow Chart 2) and inpatient drug administration and outpatient follow-up (Flow Chart 3) are provided on pages 11 and 12.

Material provided in tabular form includes information about the estimation of sample size (Table 1 on page 6), the persons on the Data Safety and Monitoring Board (Table 2, page 9), medications used in Mali for supportive care of persons with malaria in Mali – Permissible Concomitant Medications (Table 2, page 16), an overview of the Study Schedule (Table 3, page 17), a list of blood samples to be drawn during week one for the pharmacokinetic studies of AQ-13 and its metabolites – Week One Blood Sampling Schedule for AQ-13 Drug and Metabolite Levels (Table 5, page 20) and a table on the Statistical Assessment of Study Endpoints (Table 6, page 28).