National Institute of Allergy and Infectious Diseases (NIAID)
National Institutes of Health (NIH)

PROTOCOL FACE SHEET

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Protocol Number:	13-I-N209
Version Description:	Version 1.0
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Protocol Title:	Effect of Oral Activated Charcoal on Parasite Clearance Rates in Response to Intravenous Artesunate in Malian Children with Uncomplicated *Plasmodium falciparum* Malaria
Abbreviated Title:	Oral Activated Charcoal in Uncomplicated Malaria
IRB Name:	NIAID
Institute Name:	NIAID
Proposed Dates:	August-December, 2013
Total Subjects to be Accrued:	200 maximum (to enroll 70 children from whom complete sample *and* data sets are collected)
Ionizing Radiation Use:	None
Is Tissue Being Collected for Research Purposes?	Yes (whole blood only)
Location of the Study	Kenieroba, Mali
Research Contact: Address: Phone: Email:	Prof. Mahamadou Diakite BP 1805, Point G, Bamako, Mali (223) 76 23 11 91 mdiakite@icermali.org
Medically Advisory Investigator:	None (Dr. Fairhurst is a member of the NIH Clinical Center Junior Credentialed Medical Staff; Dr. Walther is a member of the NIH Clinical Center Senior Credentialed Medical Staff)

STUDY STAFF ROSTER

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Multi-site Collaboration: Foreign Sites Only

Organization Name	Country	City/State	Assurance Type/Number
Faculte de Pharmacie, Universite des Sciences, Techniques et Technologies de Bamako (USTTB), Mali	Mali	Bamako	FWA #1769, IRB #1983 Expires 12/14/2013

TABLE OF CONTENTS

ABBREVIATIONS

AE	adverse event
AQ	Amodiaquine
AS	Artesunate
AUC	area under the curve
BSI	Biological Sample Inventory
CBC	complete blood count
CM	cerebral malaria
C_{max}	maximum drug concentration
CRF	case report form
DCA	Dichloroacetate
DHA	Dihydroartemisinin
ECM	experimental cerebral malaria
FWA	Federal-wide Assurance
GCP	Good Clinical Practice
h	Hour
ICF	informed consent form
IRB	Institutional Review Board
IV	Intravenously
LMIV	Laboratory of Malaria Immunology and Vaccinology
LMVR	Laboratory of Malaria and Vector Research
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
oAC	oral activated charcoal
OHRP	Office for Human Research Protections
PbA	Plasmodium berghei ANKA
Ph	parasite half-life
pi	post infection
PI	principal investigator
PO	Orally
RBC	red blood cell
RCHSPB	Regulatory Compliance and Human Subjects Protection Branch
SAE	serious adverse event
sec	Second
SM	severe malaria
$t_{1/2}$	drug clearance half-life
t_{max}	time to maximum drug concentration
UM	uncomplicated malaria
UPnonAE	unanticipated problem that is not an adverse event
USTTB	Universite des Sciences, Techniques, et Technologies de Bamako

PRECIS

While the incidence of *Plasmodium falciparum* malaria declines (1) the proportion of cases with severe malaria (SM) may increase (2). The mortality associated with SM in endemic countries remains high despite the use of artesunate (AS) (3). Safe, cheap, and effective adjunct therapies preventing the development of, or reducing the mortality from, SM could have considerable and rapid public health impact. We discovered that oral administration of activated charcoal (oAC), a safe treatment for acute poisoning (4), protects mice from experimental cerebral malaria and demonstrated in a randomized controlled trial (RCT) in African adults that oAC is safe and does not interfere with the pharmacokinetics of AS (5). Here, we propose the next step to evaluate the efficacy of adjunct treatment with oAC in Malian children and to explore its mode of action. Before testing adjunct treatment with oAC in children with SM, we will perform an open-label RCT in children with uncomplicated malaria (UM) to demonstrate non-inferiority of intravenous (IV) AS plus adjunct oAC *vs.* IV AS alone with regards to parasite clearance rate. This study will be conducted in African children, because they are the primary target population for such an intervention. Although the adequate standard-of-care treatment for UM is oral (PO) administration of an artemisinin-based combination therapy (ACT), we will treat participants with IV AS. Like ACT treatment of UM, AS is the WHO-recommended first-line treatment for SM (1). In order for the data obtained from UM cases to be meaningful for our future studies in children with SM, we will administer AS to the UM cases in this trial via the same IV route that is used to administer AS to SM cases. Exploratory objectives include: (i) to compare the kinetics of plasma cytokines in both groups, and (ii) to preserve RNA for gene transcription analysis for future studies into the mode of action of oAC. The study will be nested within an NIAID-funded study (Principal Investigators Drs. Fairhurst, Diakite) that assesses parasite clearance rates in response to AS treatment in Kenieroba (6). Children aged 2-10 years with UM and initial parasite densities 10,000–70,000 parasites per µL will be enrolled. Parasite clearance rates will be expressed as the parasite half-life (Ph), estimated from parasite clearance curves using a formula that has been validated in this cohort (7). Children will be randomized 1:1 to receive IV AS+oAC or IV AS only, respectively, until complete specimen and data sets for 35 children per group are obtained. oAC will be administered as Actidose Aqua® at 0, 6, 12, and 18 hours. AS will be administered IV following WHO recommendations for use of AS in SM (8), followed by 3 daily doses of amodiaquine (AQ). Subsequently and in a separate study, we plan a proof-of-concept RCT to determine whether adjunct oAC reduces disease severity and morbidity (assessed by scoring systems (9)) in hospitalized children with SM and to define the mode of action of oAC. Since oAC is a licensed, inexpensive drug without sophisticated storage requirements, which has an extremely long shelf life at room temperature and can be given orally or via nasogastric tube at high doses without major side effects (4, 10), this drug has an ideal profile for use at the primary health-care level to reduce mortality from SM, or even prevent the development of SM.

1 INTRODUCTION

1.1 Background

The fight against malaria has received considerable attention recently, with major funding organizations even proposing eradication (2, 3). However, despite documented declines in clinical malaria in sub-Saharan Africa over the last decade (4-6), coinciding with substantial reductions in overall mortality in children <5 years old, malaria-attributable mortality remained stable in West Africa, and even increased in East and South Africa throughout this period (7, 8). Estimates of case-fatality rates from severe malaria (SM) still range from 10% to 50% (9, 10) and have recently been reported as 15% and 8.5% in Southeast Asia (11) and Africa (12), even when using parenteral artesunate (AS) under optimized study conditions. New tools to prevent the development of, or reduce mortality from, SM are thus urgently required.

Over several decades, dexamethasone, low-molecular-weight dextrans, osmotic agents, heparin, adrenaline, cyclosporine A, prostacyclin, pentoxifylline, hyperimmune globulin, desferrioxamine, prophylactic anticonvulsants, and monoclonal anti-TNF antibodies have all been investigated as adjunct treatments for SM without success (13). While dichloroacetate (DCA) was evaluated (14-17) and shown to reduce hyper-lactatemia in patients with SM, no published studies have assessed mortality. Volume expansion with saline or albumin suggested some benefit (18), but a recent multi-center study in African children showed that any fluid bolus is associated with a significantly increased risk of dying (19). Levamisole was found to impair the sequestration of infected red blood cells (RBCs) in patients with uncomplicated malaria (UM) (20), while exploitation of N-acetylcysteine's anti-oxidant properties failed (21). Rosiglitazone, an oral anti-diabetic drug, enhanced parasite clearance and reduced inflammatory responses in UM (22), and improved outcome in mice with experimental cerebral malaria (ECM) (23). Case reports suggest benefits from drotrecogin alfa in patients with cerebral malaria (CM) and disseminated intravascular coagulation (24). The compatibility of levamisole, DCA, and rosiglitazone with AS is not yet established. Due to parenteral administration, the use of DCA remains limited to the clinic setting. The efficacy of levamisole and rosiglitazone in SM is not yet established, and the use of the latter in resource-poor settings is limited by current pricing and the need to monitor for hepatotoxicity. Penet et al. recently proposed pantethine as adjunct therapy, though multiple parenteral doses from 1 day post-infection (pi) was required for efficacy in mice (25). Human recombinant erythropoietin prevents death in mice with ECM (26, 27) and will soon enter clinical trials (28, 29), but its price will limit its use to developed countries.

Oral activated charcoal (oAC) is a licensed, orally-administered drug that is widely used to treat acute poisoning (30), and more recently to treat some inflammatory diseases (31). oAC provides excellent levels of protection against ECM, following infection of C57BL/6 mice with 10^4 *Plasmodium berghei* ANKA (PbA)-infected RBCs (32), a model acknowledged to share many characteristics with human CM (33). As shown in Figure 1, only 21.7% (5/23) of untreated PbA-infected mice survived past Day 7 and 0% (0/23) survived past Day 9. In contrast, mice administered oAC (Actidose-Aqua®) by oral gavage on Days 3 and 5 pi were highly resistant to developing ECM, with 87.1% (27/31) surviving past Day 7 and 54.8% (17/31) surviving past Day 9. As no antimalarial drugs were administered, oAC-treated mice eventually developed hyperparasitemia and died. Nevertheless, oAC significantly prolonged overall survival time (χ^2=37.8, p<0.0001; hazard ratio 16.4, 95%CI 6.73-40.1).

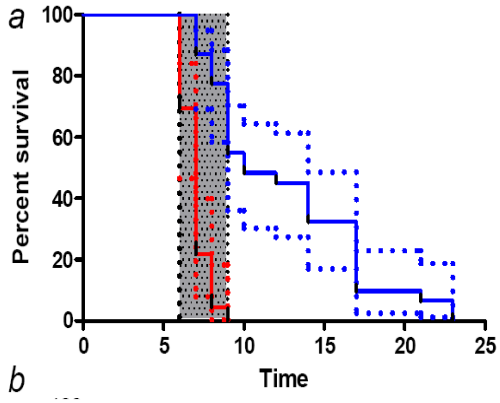


Figure 1:. oAC treatment prevents ECM. C57BL/6 mice were infected with PbA. On day 3 and day 5 pi, mice were left untreated (red) or treated with oAC (blue). Survival was monitored over 25 days. The window for CM deaths (days 6-9) is indicated by the hatched bar. Data pooled from 5 independent experiments (n= 23 untreated, n= 31 oAC-treated mice) are shown with 95%CIs (dotted lines). Overall survival was significantly improved by oAC treatment ($p<0.0001$).

A separately-sourced stock of oAC (Aktivkohle®) was also highly effective in preventing ECM (40% protection, $p<0.002$) and also had no impact on parasitemia (not shown). There was no difference in ECM protection between mice receiving oAC on Days 4, 5, and 6 pi and those receiving oAC on Days 3 and 5 pi ($p=0.54$). Most importantly, mice receiving only one dose of oAC on Day 6 pi (at the onset of clinical symptoms) were also protected against ECM (100% *vs.* 40% survival at Day 7 for oAC-treated and untreated PbA-infected mice), but ultimately died from the disease.

We conclude that oAC given shortly prior to the onset of severe illness is highly efficient in preventing ECM, and that repeated administration of oAC maximizes benefit. Even when oAC was given at the onset of ECM symptoms, it clearly postponed death from ECM, suggesting that repeated dosing even at this stage – which is readily feasible in humans, but technically challenging in mice – has the potential to reduce mortality from SM.

While the precise mode of action of oAC in preventing ECM is unknown, its efficiency in reducing the progression of chronic kidney disease (31) – an essentially chronic inflammatory process – suggests oAC has immune-modulatory properties. In line with this possibility, we observed a lower pro-inflammatory response by $CD4^+$ and $CD8^+$ T cells in the spleen of oAC-treated compared to untreated PbA-infected mice (Appendix 1). Whole-blood gene expression profiling (Appendix 2) identified transcriptional biomarkers potentially associated with oAC-mediated ECM protection, with many differentially-regulated genes controlling the acute phase of inflammation, and suggested that oAC treatment failures can be detected (32). Preliminary bioinformatics analysis identified butyric acid to be one of the differentially-regulated metabolites in oAC-treated mice (Aziz and Kaye, unpublished), which is known for its anti-inflammatory properties (34). Alternatively, other studies suggest that oAC adsorbs cytokines secreted in bile, thereby preventing their enterohepatic recirculation (35). In the murine ECM model, however, oAC did not influence the TNF clearance rate in serum (Kaye et al., unpublished).

To assess whether oAC affects the pharmacokinetics of intravenous (IV) AS, a controlled, randomized, open-label phase 1 pharmacokinetics study (ISRCTN #64793756) was conducted by the UK Medical Research Council in The Gambia with healthy (i.e., not infected with *P. falciparum*) adults being randomized to one of three study arms to receive: (1) water and AS simultaneously, (2) 50g oAC and AS simultaneously, or (3) 50g oAC 1 h after AS (32). Drugs were administered on admission and 12 h later. In addition to standard clinical and biochemical safety assessments, plasma levels of AS and its active metabolite dihydroartemisinin (DHA) were measured by liquid chromatography – mass spectrometry at 5, 10, 15, 30, 60, and 90 min, and at 3 and 6 h after the second dose of AS was given (at 12 h) to determine C_{max}, t_{max}, $t_{½}$, and AUC parameters. Study drugs were safe and well-tolerated, and no serious adverse events

(SAEs) occurred. No clinically-relevant deviations from normal ranges were observed for complete blood count (CBC) and biochemistry tests. For AS and DHA, the geometric mean AUC was similar between the three study arms (32), indicating that oAC does not affect the pharmacokinetics of IV-administered AS and its DHA metabolite.

Based on intriguing data we obtained in murine ECM and human pharmacokinetics studies (32), we propose to clinically evaluate adjunct oAC treatment in Malian children with *P. falciparum* malaria. Unlike other candidate adjunct therapies, oAC perfectly fits the ideal profile of a drug to be successfully used at the community level in developing countries: oAC is inexpensive, has undemanding storage requirements, has an extremely long shelf-life at room temperature, and can be given orally or via nasogastric tube at high doses without major side effects (30, 31, 36). In addition to an adjunct treatment for children with SM, oAC may also complement existing prevention strategies (37). For example, when administered to children with UM in a primary health-care setting, oAC may prevent the development of SM and thus reduce mortality.

The ultimate aim of this protocol and future clinical studies is to determine whether adjunct oAC treatment reduces SM mortality and/or prevents the progression of UM to SM episodes. We envision a three-step process in which we have already completed the 1st step: demonstrating that IV AS and oAC can be co-administered without oAC interfering with the pharmacokinetics of AS or DHA (32). Here we describe an open-label, randomized controlled trial (2nd step) to determine whether adjunct oAC treatment of children with UM affects parasite clearance rates in response to IV AS. If this study concludes that oAC does not slow parasite clearance rates, we will conduct a randomized controlled trial (3rd step) to (i) test whether adjunct oAC treatment reduces SM morbidity and mortality, and (ii) exploit gene profiling to explore the mode of action of oAC and define transcriptional biomarkers for treatment response.

On a previous NIAID protocol (#08-I-N120), we treated UM in 215 Malian children aged 0.5-15 years with directly-observed, weight-based doses of AS (0, 24, 48 h) and amodiaquine (AQ) (72, 96, 120 h), both given orally. We estimated the parasite half-life (a measure of parasite clearance rate) using parasite density counts in blood every 6 h until parasitemia was undetectable, and evaluated the effects of age, sex, ethnicity, and RBC polymorphisms on half-life. The half-life, the time it takes for parasite density to decrease by 50%, was calculated from the linear portion of the parasite clearance curve. The geometric mean half-life was 1.9 h (95%CI 1.8-2.0) (38). In a linear model accounting for host factors, half-life decreased by 4.1 min for every 1-year increase in age. We thus established the parasite clearance kinetics in response to AS in Malian children with UM, and have used the relevant data to design the present study. Here we propose to investigate whether adjunct oAC treatment affects the parasite half-life in response to AS in Malian children with UM. To mimic the treatment scenario for SM patients, we intend to treat UM patients with IV AS.

2 STUDY OBJECTIVES

2.1 Primary Objective

- Demonstrate that co-administration of IV AS and oAC is not inferior to administration of IV AS alone, with regard to the parasite half-life.

2.2 Exploratory Objectives

- Assess the effects of oAC on the kinetics of selected cytokine levels in plasma.
- Assess the effects of oAC on whole-blood transcriptional profiles over time.

3 STUDY DESIGN AND METHODS

3.1 Study Sites

We will conduct this protocol in Kenieroba, Mali, where malaria transmission is highly endemic and seasonal. In this village, we recently completed a longitudinal cohort study of malaria incidence in which we followed 1350 children aged 0.5-17 years and diagnosed 4200 malaria episodes over 4 transmission seasons in 2008-2011. In a subset of 160 children aged 2-10 years, the geometric mean parasite half-life in response to oral AS was 1.9 h (38). It could be argued this study should initially be performed in adult patients. However, this is not feasible logistically, since adults living in malaria-endemic areas are immune to clinical malaria, and thus are very unlikely to present to clinic when they are infected with *P. falciparum*. Also, parasite densities in such adults are very low, which precludes proper calculation of parasite clearance rates.

3.2 Study Procedures

In an open-label, randomized controlled study we will compare parasite half-lives between children receiving AS or AS+oAC, and assess the safety of AS+oAC, in the 2013 transmission season.

While it would be desirable to perform a double-blinded study, this is not possible because, to our knowledge, there is no appropriate placebo that would match Actidose®-Aqua in taste, appearance, and viscosity. We are aware that the lack of double blinding is a potential source of bias. However, we feel that in this study risk is minimal because the primary outcome measure - parasite clearance rate - is an objective parameter that cannot be influenced willingly or unconsciously by either the participant or the investigator administering the study drug. The slide reader producing the raw data for parasite clearance rates will be blinded to the participant's group allocation.

Children will be randomized (1:1) to either the "AS" or "AS+oAC" study arms using block randomization, with block sizes ranging from 6 to 12 children. After obtaining informed consent from a parent or legal guardian, we will enroll children aged 2-10 years with UM and parasite density 10,000–70,000/µL. We will record axillary temperature, blood pressure, heart and respiratory rates, weight, and findings from history and physical examination. Recruitment will end when complete sets of data and specimens are obtained for 35 children in each group.

All children will have an IV catheter placed in the arm. All children will receive AS 2.4 mg/kg IV at 0 and 12 h, 24 h, and 48 h. Children in the AS+oAC group will be given weight-based doses of oAC (Table 1) at 0, 6, 12, and 18 h. Children in the AS group will receive a weight-based volume of clean water to drink. After the last dose of AS is given, the IV catheter will be removed. All children will receive AQ 10 mg/kg PO at 72 h, 96 h, and 120 h. This drug regimen mimics the current WHO-recommended treatment for SM: AS 2.4 mg/kg IV at 0, 12, 24, and 48 h, followed by an appropriate partner drug on Days 4, 5, and 6 (1). This drug regimen also avoids the potential adsorption of AQ by oAC, since 54 h will elapse between the last oAC dose and the first AQ dose.

We will hospitalize children for 48 h to monitor their clinical status and IV catheter, and ensure the accurate timing of blood sampling. Vital signs, state of consciousness, and selected symptoms (nausea, vomiting, diarrhea, constipation, abdominal pain, headache, and dizziness) will be monitored at 0, 2, 4, 6, 8, and 12 h, and then every 6 h until 48 hours or until parasitemia is undetectable (one negative thick blood film), whichever is later. Finger-prick blood samples will be obtained at 0, 2, 4, 6, 8, and 12 h, and then every 6 h until parasitemia is undetectable. In

Kenieroba, we previously found that 7%, 13%, 34%, 28%, 16%, and 2% of children aged 2-10 years (n=160) cleared their parasitemia by 12, 18, 24, 30, 36, and 42 h, respectively (38). We therefore expect that finger-prick blood sampling will be completed within 48 h for all children.

We will obtain 3 mL venous blood at 0, 24, and 48 h to perform a complete blood count (CBC) with differential and platelet counts, preserve RNA, and store plasma for research purposes. Transcriptional profiles and plasma cytokine levels in children with UM children will later be compared to those with SM.

We previously found that parasite density did not drop in 61% (98/160), 10% (16/160), and 1.9% (3/160) of Malian children (aged 2-10 years) by 6, 12, and 18 h, respectively. These "lag" phases reflect the fact that, while AS-treated parasites are being cleared, the simultaneous release of merozoites from sequestered rupturing schizonts and their development into new ring forms yield an *apparent* steady-state parasite density. In the 16 children with a 12-h lag phase, the mean ratio of the peak parasite density (measured at 0 or 6 h) compared to the 12-h parasite density was 1.53 (95%CI 1.31-1.88). Based on these data, children receiving AS+oAC who show less than a 2.0-fold reduction in parasite density at 18 h, as calculated by dividing the peak parasite density (i.e., the highest value measured at either 0, 2, 4, 6, 8, or 12 h) by the 18-h parasite density will *not* receive oAC at 18 h. These children will complete the study per protocol, *except* they will *not* have blood drawn for research purposes at 24 and 48 h. Children receiving AS only who show less than a 2.0-fold reduction in parasite density at 18 h will also complete the study per protocol, *except* they will *not* have blood drawn for research purposes at 24 and 48 h.

At the discretion of the study clinician, the clinical monitoring for children who 'failed' to begin clearing parasites at 18 h may be intensified and an additional dose of 2.4 mg/kg artesunate IV may be considered.

If the IV line becomes compromised and cannot be replaced, treatment will be continued with intramuscular artemether at the recommended maintenance dose of 1.6 mg/kg every 24 h. A change to oral amodiaquine will be made after at least 56 h have passed since the last dose of oAC was given.

4 STUDY POPULATION

4.1 Rationale for Subject Selection

Our recent study (#08-I-N120) established baseline parasite half-lives in response to oral AS in children aged 2-10 years in Kenieroba, Mali. We are thus in a unique position to test whether adjunct oAC treatment affects this parameter in the same study population. The age range 2-10 years was selected to represent that group of children most likely to experience SM in Mali. While new drug regimens are usually tested initially in adult patients, this is not feasible logistically in this case, since adults living in malaria-endemic areas are immune to clinical malaria, and thus very unlikely to present to clinic when they are infected with *P. falciparum*. Also, parasite densities in such adults are very low, which precludes proper calculation of parasite clearance rates.

4.2 Recruitment Plans and Procedures

At Kenieroba Health Center, we will recruit patients from children aged 2-10 years who present with malaria symptoms and are not enrolled on our concurrent cohort study (#13-I-N107).

4.3 Description of the Consent Process

The study will initially be discussed with Kenieroba leaders, seeking their permission to perform this trial. If community leaders agree that the trial can take place, study physicians will obtain informed consent from an adult parent or guardian (usually uncles/aunts) of eligible children. The Malian Ethics Committee has established that the age of consent in Mali is 18 years and the age of assent is 12 years; therefore, we will not obtain assent from children in this study. We will present written informed consent forms (ICFs) in French. Most adults will not be able to read, and so oral consent will typically be obtained in the local language, documented by the study physician, and witnessed (signed) by a third party.

4.4 Subject Inclusion/Exclusion Criteria

4.4.1 Inclusion Criteria

1. Age 2 to 10 years, inclusive
2. Resident of Kenieroba
3. Uncomplicated malaria*
4. *P. falciparum* density 10,000–70,000/µL, inclusive
5. Willingness to participate in the study as evidenced by informed consent of the child's parent or guardian
6. Ability to swallow oral medication

*Uncomplicated malaria: axillary temperature >37.5°C *or* history of fever in the past few days *and* no criteria of SM (see next paragraph) *and* no other etiologies of febrile illness (e.g., respiratory tract infection) on clinical examination.

Severe *P. falciparum* malaria: parasitemia of any density *and* any one of the following: coma (Blantyre coma score ≤2), convulsions (witnessed by investigator), severe prostration, severe anemia (hemoglobin ≤6 g/dl), respiratory distress, hypoglycemia (serum glucose ≤40 mg/dl), jaundice/icterus, shock (systolic blood pressure ≤70 mmHg, rapid pulse, cool extremities), cessation of eating and drinking, repetitive vomiting.

4.4.2 Exclusion Criteria

1. Severe malaria
2. Any medical condition or history, including allergy to AS, AQ, artemether or lumefantrine, that poses a risk to the prospective participant
3. Any condition that in the opinion of the investigator would render the participant unable to comply with the protocol (e.g., psychiatric disease)
4. Any health condition that in the opinion of the investigator would confound data analysis or pose unnecessary risks to study participants (e.g., severe malnutrition, acquired or inherited immunodeficiency)
5. Requirement for any medication for any concurrent illness or condition
6. Participation on cohort study #13-I-N107
7. Repetitive vomiting

4.5 Criteria for Withdrawal of Subjects from the Study

A child will be considered to *not* have completed the study for any of these reasons:

1. *Research terminated by Sponsor or Investigator* – applies to termination of the entire study by the Sponsor or Investigator, or other regulatory authority for any reason
2. *Withdrawal of consent* – applies to a child whose parent or guardian withdraws consent to participate in the study for any reason

3. *Noncompliant with protocol* – applies to a child who does not comply with protocol-specific visits or evaluations on a consistent basis, such that adequate follow-up is not possible and the child's safety may be compromised by continuing the study
4. Developed an AE which requires discontinuation of the study involvement or results in inability to continue to comply with study procedures
5. *Other* – is used when previous categories do not apply (written explanation required).

If a child is withdrawn prior to completion of the study, the reason for this decision will be recorded in the case report form (CRF). If a child is withdrawn because of an adverse event (AE) or SAE, research interventions will be discontinued; however, the child will be followed for clinical care purposes until resolution of the event. Antimalarial treatment will be continued with artesunate and amodiaquine.

4.6 Plan for Maintaining Privacy and Confidentiality of Subject Records

Standard CRFs containing clinical and laboratory data will be completed, signed by study investigators, and stored in folders in a locked room while in use. Relevant patient data will be entered onto a hybrid system (Datafax©) capable of collecting, processing, and storing both images of paper documents and data through intelligent character recognition software which reads and stores handwritten numbers, dates, and check boxes. Electronically-captured data will be checked by study personnel and verified by a data manager. Data will be exported to a password-protected relational database program (Access©). All electronic data will be stored on a secure server. Access to patient charts and databases for data analysis will be restricted to the investigators listed on this protocol and those working under their direct supervision. Access to ICFs, which link identifying numbers to names, will be restricted to the Principal Investigators (PIs) and the Associate Investigators authorized to obtain consent. The Regulatory Compliance and Human Subjects Protection Branch (RCHSPB) and their authorized representatives may also have access to the subjects' study records, including ICFs. CRFs and ICFs will eventually be transported to our offices in Bamako, where they will be retained under lock and key by the Malian PI and made available for clinical monitoring.

Upon enrollment into the protocol, all patients will be assigned a unique identifying number. The ICF will serve to link this identifying number with patient name and signature/thumbprint. CRFs, databases, and samples will be coded with unique identifying numbers and will not contain subject names. Study personnel involved in data acquisition, entry, and analysis will therefore not have access to names, assuring the privacy and confidentiality of subjects.

5 STUDY AGENT

5.1 Study Agent, Storage, and Stability

oAC will be obtained as Actidose Aqua® (Paddock Laboratories), which is sold "over the counter" in the United States in bottles containing 25 g/120 mL (NDC # 0574-0121-04) or 50 g/240 mL (NDC # 0574-0121-08). oAC is stable at room temperature. For further information, see Package Insert (Appendix 3) and Drug Safety Sheet (Appendix 4).

AS will be obtained from Guilin Pharma (Shanghai), the only pharmaceutical company GMP pre-qualified by the WHO. The product 'artesunate for injection + 5% sodium carbonate inj + 0.9% sodium chloride inj' will be delivered in 60-mg vials and will be dosed at 2.4 mg/kg as recommended for SM treatment by the WHO (1).

Amodiaquine will be obtained from Pfizer (Dakar), is provided as 200-mg tablets or syrup (10 mg/mL), and will be provided as age-based doses per the manufacturer's directions.

5.2 Preparation, Administration, and Dosage

Considering that oAC is an inert substance that is not absorbed from the gut, we estimated a human dose equivalent to the 2.6 mg dose we used in a 20 g mouse (32), based on the ratio of calculated gut volumes. Chivers et al. established linear relationships (y= mx + c) between log gut volume (x) and log bodyweight (y) for faunivore, frugivore, and folivore primates (39), and we used the mean values for constants m (slope) and c (intercept) reported in these three equations to develop an equation for gut volume for omnivore primates. Gut volumes calculated for different human body weights were divided by the calculated mouse gut volume to derive a factor by which the mouse 2.6 mg dose was to be multiplied. Chivers et al. give error bounds for the constant m based on 95%CIs, and since intestinal volumes may vary depending on food intake (40), we have introduced an error term of ± 2SE for constant m. We propose to test the dose calculated from the equation, m + 2SE (Table 1). These oAC doses are well within the range of doses recommended for the treatment of acute poisoning: 1 g/kg in children aged <1 year and 25-50 g in children aged 1-12 years (30). Since intestinal transit time in African pupils from a semi-urban area is about 18 h (41), and both the efficacy of oAC to prevent ECM and treat poisoning is enhanced by repeat dosing (32, 42, 43), we will administer oAC on admission and 6, 12, and 18 h later. Actidose Aqua® (in the oAC plus AS group) or an equivalent amount of clean water for the control group will be given at the following weight-based doses:

Body weight (kg)	Dose (g)	Actidose- (mL)
6	4.3	20.7
8	6.3	30.1
10	8.4	40.2
12	10.6	50.9
14	13.0	62.2
16	15.4	74.0
18	18.0	86.2
20	20.6	98.9
22	23.3	111.9
24	26.1	125.4
26	29.0	139.1
28	31.9	153.2
30	34.9	167.5
32	38.0	182.2
34	41.1	197.1
36	44.2	212.3
38	47.5	227.8
40	50.7	240.0

Table 1. oAC dose by body weight, derived using formulae in Chivers et al.(39).

Actidose Aqua® will be administered PO at 0, 6, 12, and 18 h. Actidose Aqua® is licensed for use in infants and children at single or multiple doses of 25-50 g (120-240 mL) in children 1-12 years old. In multiple-dose regimens, doses are administered 4 to 6 h apart. The doses and dosing schedule proposed here are well within the licensed ranges. Per manufacturer's directions, the bottle will be shaken for 30 sec prior to opening and the appropriate volume

measured in a beaker. There are no absolute contraindications to the use of Actidose Aqua®. Side effects may include mild transient constipation and stools will be black for several days. If a child vomits within 60 min of taking any dose of oAC or water, the child will be withdrawn from the protocol.

6 ANALYSIS OF THE STUDY

6.1 Sample Size and Power Calculations

The sample size was calculated to demonstrate non-inferiority for 'AS+oAC' compared to 'AS' with regard to parasite half-life. The inferiority margin was defined based on the following consideration: the variation in parasite half-life we previously observed in patients treated with AS is considered acceptable. For children 2-10 years of age with initial parasite densities 10,000–70,000/µL, the parasite half-life is approximately normal-distributed around a mean of 1.946 h, with a standard deviation of 0.636 h. Such a distribution is shown in Figure 2A. The 99.5 percentile is at 3.59 h, indicating that under this distribution 99.5% will have a parasite half-life ≤ 3.59 h. We postulate that allowing 3% of children in the AS+oAC group to have a parasite half-life slightly above the 99.5 percentile of the normal distribution of the children in the AS group can be considered as non-inferior. Assuming that a similarly-shaped normal distribution for parasite half-life is obtained for children in the AS+oAC group, the corresponding shift in mean parasite half-life can be derived from the graph (Figure 2B) as 25.57 min.

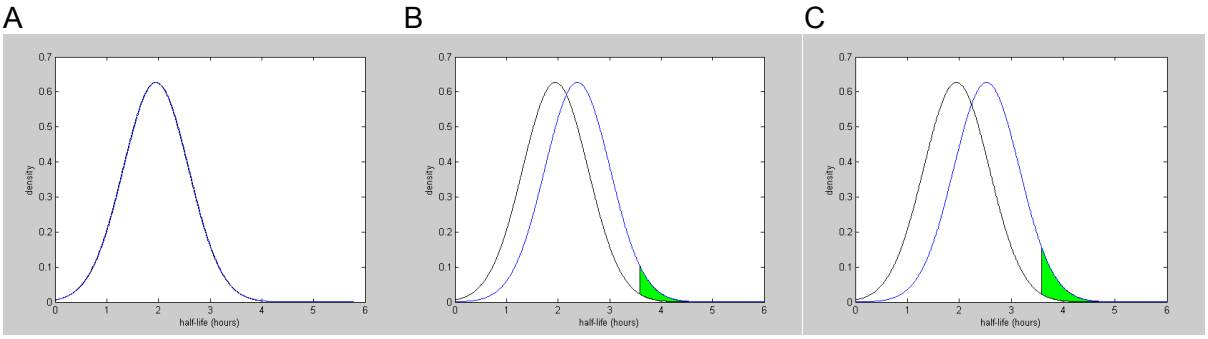


Figure 2. Distribution of parasite half-life in children treated with AS (A). Assumed shift of the parasite half-life distribution if 3% (B) or 5% (C) of children have a parasite half-life longer than that defined by the 99.5 percentile of the distribution depicted in (A).

If, for instance, 5% of children in the AS+oAC group would be allowed a parasite half-life above the 99.5 percentile, the mean parasite half-life of this distribution would be 35.05 min longer (Figure 2C). Further examples can be derived from Figure 3 below.

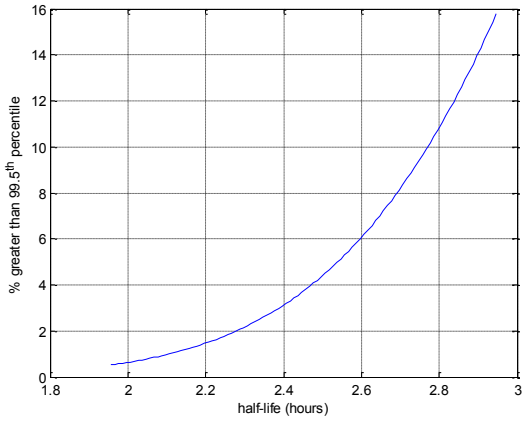


Figure 3. This graph depicts how the mean parasite half-life distribution shown in Fig. 2A changes, depending on what proportion of patients with a parasite half-life greater than the 99.5 percentile of the original distribution is measured.

Considering a mean parasite half-life of 2.37 h (i.e., a 25.57-min shift corresponding to 3% having parasite half-lives above the 99.5 percentile of the initial distribution) as non-inferior, 35 children in both groups is required to detect a difference as small as this with 80% power at the 2.5% significance level. Table 2 provides further examples.

Inferiority margin defined as X % > 99.5% percentile	corresponding to a shift of mean T_H hrs	(same in min)	n for 1:1 per group
2.0%	0.33	20.00	58
3.0%	0.43	25.57	35
4.0%	0.52	30.94	24
5.0%	0.58	35.05	19

Table 2. Sample size scenarios for different inferiority margins

6.2 Analysis plan

Analysis of safety data:

The local reactogenicity at the site where the IV catheter is placed will be assessed using the grading scale provided in Appendix 5, which is based on the FDA toxicity grading scale for healthy adults and adolescents.

Descriptive summaries of the frequency and (where applicable) duration of systemic signs and symptoms such as nausea, vomiting, diarrhea, abdominal pain, headache, and dizziness will be provided for each group.

Clinical and laboratory assessments resulting in numerical values (pulse, blood pressure, respiratory rate, state of consciousness assessed by the Blantyre coma score, axillary temperature, white blood cell counts, hemoglobin, and platelet counts) will be compared at each time-point between groups in light of age-specific normal values available for African pediatric populations (44-46).

It is expected that all the above listed parameters will be influenced to various degrees by the malaria parasite infection itself, and most of the systemic signs or symptoms are also listed as

common side effects of the antimalarial drugs used to treat the infection. Therefore, it won't be possible to establish the relatedness of any such findings to either the parasite infection, or any of the study-related interventions. For that reason, we will limit our analysis to a comparison of the frequency and duration (or, for numeric parameters the level) of such findings between both groups. This will enable us to determine whether adjunct treatment with oAC significantly alters the clinical course of disease, or whether the safety of IV AS is compromised by adding oAC.

Analysis of parasite clearance data:

Parasite clearance will be calculated for each participant as previously described (38), and compared between groups using a one sided test with a 2.5% significance level. The hypotheses are: H_0: T_H for AS + oAC – AS >25.57 min and H_1: T_H for AS + oAC – AS <25.57 min. The Welch's t-test will be performed and its associated 95% CI will be calculated for the mean difference between AS+oAC – AS. If the upper limit of this interval is <25.57 min, then the null hypothesis will be rejected and non-inferiority will be demonstrated.

The number/proportion of children who did not receive their last dose of oAC because their peak parasite density / 18-h parasite density was <2.0 will be reported in both groups, and their parasite clearance rates will be compared with the children who received the full course of oAC.

6.3 Primary and Secondary Outcomes

6.3.1 Primary Outcome

1. To assess the safety of adjunct treatment with oAC
2. To compare parasite clearance half-life in patients treated with oAC + IV AS or IV AS

6.3.2 Secondary Outcomes

1. Serum cytokine levels at 0, 24, and 48 h
2. Whole-blood transcriptional profiles at 0, 24, and 48 h

7 STUDY PROCEDURES AND EVALUATION

7.1 Detailed Study Procedure Schedule

0 hours

1. Verify that informed consent was obtained.
2. Ensure that all inclusion/exclusion criteria are met.
3. Measure child's weight and vital signs: axillary temperature, blood pressure, heart and breathing rates, and state of consciousness.
4. Perform abbreviated history and physical examination and review for specific symptoms (i.e., nausea, vomiting, diarrhea, constipation, abdominal pain, headache, dizziness).
5. Place IV catheter in the arm.
6. Obtain 3 mL blood via IV catheter for research purposes: CBC with differential and platelet count, RNA preservation, and plasma preparation.
7. Prepare thick/thin blood films from finger-prick blood sample, count parasite density. Slide readers will be blinded to study participant's group allocation.
8. Administer AS 2.4 mg/kg IV to all children.
9. Administer a weight-based dose of Actidose Aqua® to children in the AS+oAC group, and a weight-based volume of clean water to children in the AS group.

2 hours (±10 min)

1. Prepare thick and thin blood films and count parasite density. Slide readers will be blinded to study participant's group allocation.
2. Measure vital signs, assess consciousness, and review specific symptoms (i.e., nausea, vomiting, diarrhea, constipation, abdominal pain, headache, dizziness).

4 hours (±10 min)

1. Prepare thick and thin blood films and count parasite density. Slide readers will be blinded to study participant's group allocation.
2. Measure vital signs, assess consciousness, and review specific symptoms. (i.e., nausea, vomiting, diarrhea, constipation, abdominal pain, headache, dizziness)

6 hours (±10 min)

1. Prepare thick and thin blood films and count parasite density. Slide readers will be blinded to study participant's group allocation.
2. Measure vital signs, assess consciousness, and review specific symptoms. (i.e., nausea, vomiting, diarrhea, constipation, abdominal pain, headache, dizziness)
3. Administer a weight-based dose of Actidose Aqua® to children in the AS+oAC group, and a weight-based volume of clean water to children in the AS group.

8 hours (±10 min)

1. Prepare thick and thin blood films and count parasite density. Slide readers will be blinded to study participant's group allocation.
2. Measure vital signs, assess consciousness, and review specific symptoms. (i.e., nausea, vomiting, diarrhea, constipation, abdominal pain, headache, dizziness)

12 hours (±10 min)

1. Prepare thick and thin blood films and count parasite density. Slide readers will be blinded to study participant's group allocation.
2. Inspect IV catheter.
3. Measure vital signs, assess consciousness, and review specific symptoms. (i.e., nausea, vomiting, diarrhea, constipation, abdominal pain, headache, dizziness).
4. Administer AS 2.4mg/kg IV to all children.
5. Administer a weight-based dose of Actidose Aqua® to children in the AS+oAC group, and a weight-based volume of clean water to children in the AS group.

18 hours (±45 min)

1. Prepare thick and thin blood films and count parasite density. Slide readers will be blinded to study participant's group allocation.
2. Measure vital signs, assess consciousness, and review specific symptoms. (i.e., nausea, vomiting, diarrhea, constipation, abdominal pain, headache, dizziness)
3. Identify the highest parasite density measured between 0 h and 12 h and divide it by the parasite density measured at 18 h. If this ratio is <2.0, complete the protocol as outlined below but do not administer the last dose of Actidose®-Aqua (for children in the AS+oAC group) or water (for control group), and do not draw venous blood at 24 h and 48 h (if the child is either in the AS or the AS+oAC group). Consider intensified clinical monitoring and an additional dose of 2.4 mg/kg AS IV for children with a ratio <2.0, if warranted by clinical signs and symptoms.
4. Administer a weight-based dose of Actidose Aqua® to children in the AS+oAC group and a weight-based volume of clean water to children in the AS group whose ratio of peak parasite density/18-h parasite density is ≥2.0 or if the 18-h parasitemia is zero.

24 hours (±10 min)
1. Prepare thick and thin blood films and count parasite density. Slide readers will be blinded to study participant's group allocation.
2. Inspect IV catheter.
3. Measure vital signs, assess consciousness, and review specific symptoms. (i.e., nausea, vomiting, diarrhea, constipation, abdominal pain, headache, dizziness)
4. Obtain 3 mL blood via IV catheter for research purposes.
5. Administer AS 2.4 mg/kg IV to all children.

30 hours (±10 min)
1. Prepare thick and thin blood films and count parasite density. Slide readers will be blinded to study participant's group allocation.
2. Measure vital signs, assess consciousness, and review specific symptoms. (i.e., nausea, vomiting, diarrhea, constipation, abdominal pain, headache, dizziness)

36 hours (±10 min)
1. Prepare thick and thin blood films and count parasite density. Slide readers will be blinded to study participant's group allocation.
2. Inspect IV catheter.
3. Measure vital signs, assess consciousness, and review specific symptoms. (i.e., nausea, vomiting, diarrhea, constipation, abdominal pain, headache, dizziness)

42 hours (±10 min)
1. Prepare thick and thin blood films and count parasite density. Slide readers will be blinded to study participant's group allocation.
2. Measure vital signs, assess consciousness, and review specific symptoms. (i.e., nausea, vomiting, diarrhea, constipation, abdominal pain, headache, dizziness)

48 hours (±10 min)
1. Prepare thick and thin blood films and count parasite density. Slide readers will be blinded to study participant's group allocation.
2. Inspect IV catheter.
3. Measure vital signs, assess consciousness, and review specific symptoms. (i.e., nausea, vomiting, diarrhea, constipation, abdominal pain, headache, dizziness)
4. Obtain 3 mL blood via IV catheter for research purposes.
5. Administer AS 2.4 mg/kg IV to all children.
6. Remove IV catheter.
7. If the parasitemia is undetectable, discharge participant from inpatient ward.

If parasitemia is detected at 48 h, we will retain the child as an inpatient, and continue preparing thick and thin blood films and counting parasite density until parasitemia is undetectable on thick smear in 6-hourly intervals.

Three age-based doses of AQ will be given orally on Days 4 (approx. 72 h), 5 (approx. 96 h), and 6 (approx. 120 h). On each day, we will record whether the child has passed stool.

On day 7 (approx. 168 h), we will follow up the participant to ensure (s)he is in good health. The site where the IV catheter was placed will be inspected, vital signs will be monitored, specific symptoms will be reviewed, and the outcome of any AE will be documented. At the discretion of the investigator, a focused clinical exam may be performed. A finger-prick blood sample will be

obtained from all children. If the child is healthy-appearing, the slide will be read at a later time. If the axillary temperature is >37.5^{o}C, the slide will be examined immediately for malaria parasitemia. If the child is parasitemic, we will provide a full 3-day course of Coartem as directly-observed therapy. One day after the last dose of Coartem, we will take a finger-prick blood sample to document undetectable parasitemia.

A summary of study procedures is shown in Table 3.

Intervention / Procedure	0 hrs	2 hrs	4 hrs	6 hrs	8 hrs	12 hrs	18 hrs	24 hrs	30 hrs	36 hrs	42 hrs	48 hrs	72 hrs	96 hrs	120 hrs	168 hrs
admission for 48 hrs	x															
History and physical exam + weight	x															
focussed physical exam																x*
IV catheter remains in place for 48 hrs	x															
inspection of IV catheter						x		x		x		x				x
Artesunate IV dosage	x					x		x				x				
Charcoal dosage per os (1)	x			x		x	x									
Amodiaquine per mouth													x	x	x	
microscopic slide (2)	x	x	x	x	x	x	x	x	x	x	x	x				x
Ratio peak parasitemia/current parasitemia							x									
clinical assessment (2, 3)	x	x	x	x	x	x	x	x	x	x	x	x				x
blood for RNA preservation (4)	1.5							1.5				1.5				
Plasma for cytokine kinetics (4)	1							1				1				
blood for CBC (4)	0.5							0.5				0.5				

(1) for participants randomized to the oral activated charcoal arm only

(2) will be repeated every 6 hrs until parasitemia is undetectable

(3) comprises of axillary temperature, pulse, blood pressure, respiratory rate, state of consciousness, and review of specific clinical symptoms

(4) numbers in fields refer to mL of whole blood collected. In total, 3 mls of blood will be collected at 0, 24 and 48hrs

at the discretion of the clinical investigator are in brackets

x* will be performed at the discretion of the investigator

Table 3: Summary of study procedures

7.2 Clinical Evaluation

The clinical laboratory at Universite des Sciences, Techniques, et Technologies de Bamako (USTTB) will perform routine CBCs with differential and platelet counts.

7.3 Laboratory Evaluation

In an exploratory analysis, the levels of multiple cytokines will be measured in plasma using a LUMINEX assay platform. Cytokines will include (but may not be limited to) those we previously found to be up-regulated in response to malaria (47): IL-4, IL-6, IL-10, IL-12, IFN-γ, and TNF.

8 POTENTIAL RISKS AND BENEFITS

8.1 Potential Risks

Potential risks to participants are associated with the following procedures:

Venipuncture and finger sticks. Risks occasionally associated with venipuncture include pain, bruising, bleeding, and infection at the site of venipuncture, lightheadedness, and rarely, syncope.

Indwelling venous catheterization. Risks rarely associated with the placement of indwelling venous catheters include line infection and venous thrombosis.

If the IV line becomes compromised and cannot be replaced, treatment will be continued with intramuscular artemether. In rare circumstances, intramuscular injection may cause infection of superficial and deep tissues, including muscle abscess. Inappropriate injection techniques may result in nerve damage that may be permanent.

Drug administration. Expected AEs of drugs used in this study are generally mild in nature and not treatment-limiting.

Repeat dosing of oAC may cause constipation, and stools may be blackened. Aspiration of oAC in patients who vomit has been reported to produce airway obstruction (which is one reason why children presenting with repeated vomiting will not be eligible). oAC can reduce the absorption of other oral medications for as long as it is present in the intestines. The effect is greatest when oAC is given simultaneously (i.e., within 1 h) with other oral medication, additional doses of which may be required to obtain the same clinical effect.

Gastrointestinal effects (nausea, vomiting, diarrhea, abdominal discomfort) have been noted with AQ treatment, but have been extremely uncommon in our study population in the past 5 years. Rarely, itching and skin rash may occur.

No AEs are expected from AS or artemether treatment. The drugs are well tolerated, and there are no known common side effects. Rarely, allergic reactions and skin rash have been described.

Study participants will be made fully aware that this is the first time that patients with malaria will receive adjunct treatment with oAC. While there is currently no evidence that oAC might do any harm, we cannot exclude the theoretical possibility that oAC may produce unexpected, less desirable outcomes.

Coartem is now widely used for the treatment of acute uncomplicated malaria in many African countries. In 10% of patients, Coartem may cause headache, dizziness, abdominal pain and loss of appetite. In 1-10% of patients, Coartem may cause insomnia, palpitations, nausea, vomiting, diarrhea, itchiness, skin rash, cough, myalgias, arthralgias and malaise.

8.2 Potential Benefits

Children that participate in this study will directly benefit from malaria diagnosis and treatment. Study participants will be made aware that IV administration of AS is not the standard of care for UM and that the malaria episode can be treated equally well with oral ACTs that will be provided to the child in case the caregiver chooses for the child not to participate in this study.
Information gained from this study might contribute to the development of a safe and effective adjunct treatment to prevent the development of SM or lessen the morbidity and mortality of SM. The alternative to participating in this study is to not participate.

9 ASSESSMENT OF SAFETY AND ADVERSE EVENT REPORTING PLAN

9.1 Definitions

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

SAE: Any AE that

- results in death;
- is life-threatening (places subject at immediate risk of death from event as it occurs);
- results in inpatient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- results in a congenital anomaly/birth defect; or
- based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

Unanticipated Problem that is not an Adverse Event (UPnonAE): Any unanticipated problem that does not fit the definition of an AE but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data, for example, occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug will be reported.

Protocol Violation: Any change, divergence, or departure from the study procedures in an Institutional Review Board (IRB)-approved research protocol that has a major impact on the subject's rights, safety, or well-being, and/or the completeness, accuracy, or reliability of the study data.

Protocol Deviation: Any change, divergence, or departure from the IRB-approved study procedures in a research protocol that does not have a major impact on the subject's rights, safety or well-being, or the completeness, accuracy, and reliability of study data.

Unanticipated Problem: Any incident, experience, or outcome that is

1. unexpected in terms of nature, severity, or frequency in relation to
   a. the research risks described in the IRB-approved protocol and ICF, or other study documents; and
   b. the characteristics of the subject population being studied; and
2. related or possibly related to participation in the research; and
3. places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

9.2 Recording/Documentation

AEs discovered by appropriate questioning and examination will be recorded in the AE section of the CRF. Mild or moderate events that are reasonably expected in this study (symptoms of malaria such as fever, myalgias, arthralgias, anorexia, nausea, and malaise) will be recorded in the Review of Symptoms section of the CRF. Source documents will include progress notes and laboratory reports.

9.3 Reporting Procedures

9.3.1 Expedited Reporting

The only research-related procedures in this protocol are finger-prick and venous blood sampling, indwelling intravenous catheterization, IV administration of AS, and oAC administration. The risk of these procedures is minimal and is generally confined to the time at which they are performed. It is expected that all patients will complete these procedures within 48 h. All unanticipated problems, related SAEs, and any protocol violations that occur between 0 and 168 h, will be reported to the NIAID IRB and USTTB EC within 7 days of PI awareness.

9.3.2 Annual Reporting

The following items will be reported to the NIAID IRB at the time of Continuing Review:

- All unanticipated problems (including AEs and non-AEs)
- Expected SAEs that are possibly, probably, or definitely related to the research
- SAEs that are not related to the research
- All protocol deviations that the investigator believes should be reported
- Any trends or events that the investigator believes should be reported

10 DATA AND SAFETY MONITORING PLAN

The NIH and USTTB PIs will monitor protocol data that could affect patient safety or confidentiality, including occurrence of AEs, documentation of patient evaluation, treatment, and disposition, and coding of patient data and samples.

11 DATA MANAGEMENT PLAN

The PI is responsible for assuring that the data collected is complete, accurate, and recorded in a timely manner. CRFs may be used as source documents for some information. CRFs will be completed and signed by study personnel, stored in folders while in use, and maintained in locked file cabinets on site during the transmission season. CRF data will be entered

electronically into Datafax© within 2 weeks of a study visit. Free-form data (e.g., physician comments) will be entered manually. Electronically-captured and manually-entered data will be checked by an on-site study manager and verified by an off-site data manager. After two-tiered validation, data will be exported to a password-protected relational-database program (Access©) and stored on a secure server. Data from this study will be prepared for presentation at scientific meetings and publication in peer-reviewed scientific journals. Under no circumstances will a patient's identity or identifying characteristics be revealed.

12 PROTOCOL MONITORING PLAN

The study will be conducted in accordance with the design and specific provisions of this IRB-approved protocol, in accordance with the ethical principles originating in the Declaration of Helsinki, and that are consistent with the ICH-GCP and any applicable regulatory requirements, as well as in accordance with NIAID policies. The PIs will assure that no deviation from, or changes to, the protocol will take place without prior agreement and documented approval from the NIAID IRB and USTTB EC, except where necessary to eliminate an immediate hazard to study participants. The PI will promptly report to the NIAID IRB and USTTB EC any changes in research activity and all unanticipated problems involving risk to human subjects or others.

As per ICH-GCP 5.18, clinical protocols are required to be adequately monitored by the study sponsor. This monitoring will be conducted according to the "NIAID Intramural Clinical Monitoring Guidelines." Monitors under contract to the NIAID/RCHSPB will visit the study site to monitor aspects of the study in accordance with appropriate regulations and the approved protocol. The objectives of a monitoring visit will be to (1) verify the prompt reporting of data points, including SAEs, (2) verify the existence of signed ICFs, (3) examine individual subject CRFs and source documents, and (4) help ensure protection of study subjects, compliance with the protocol, and accuracy and completeness of records. The monitors will also inspect the clinic site regulatory files to ensure that regulatory requirements [Office of Human Research Protections (OHRP)/ICH-GCP] are being followed. At monitoring visits, the PIs [and/or designee(s)] and other study personnel will be available to discuss the study progress and monitoring visit. The PIs [and/or designee(s)] will make study ICFs, CRFs, source documents, and data readily available for inspection by the site monitors and NIAID staff for confirmation of study data. A specific protocol-monitoring plan will be discussed with the PIs and study staff prior to enrollment. The plan will outline the anticipated frequency of monitoring visits based on factors such as study enrollment, data collection status, and regulatory obligations.

The investigators conducting this clinical trial have reported potential conflicts of interest according to NIAID guidelines. Specifically, assets, income, liabilities, outside positions, agreements, arrangements, gifts, and travel reimbursements of the investigators, their spouses, and their minor children have been reported according to the guidelines. No reportable conflicts of interest have been identified by any of the investigators conducting this trial.

13 RESEARCH USE OF STORED HUMAN SAMPLES, SPECIMENS, OR DATA

All bio-specimens will be stored at the Laboratory of Malaria and Vector Research (LMVR), Laboratory of Malaria Immunology and Vaccinology (LMIV), or USTTB, and may be used to study malaria and related conditions. Access to research samples will be limited using a locked

room. Samples will be tracked using the Biological Sample Inventory (BSI) software system for bio-specimen management. Samples and data will be stored using codes assigned by the investigators [or their designee(s)]. Data will be kept in password-protected computers. Only PIs [or their designee(s)] will have access to samples and data. In the future, other investigators (both at NIAID and elsewhere) may wish to study these samples and/or data. In that case, approval from the NIAID IRB will be sought prior to any sharing of samples, as well as any clinical information shared about the sample with or without patient identifiers. Institutions and individuals approved to receive samples from this study will be listed in an appendix as needed. Recipients will sign an agreement stating that they will receive no identifying information with the shared samples and that they will not request such information in the future. At the completion of the protocol (termination), samples and data will either be destroyed or, after IRB approval, transferred to another existing protocol or a repository. Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) will be reported to the NIAID IRB. Additionally, subjects may decide at any time not to have their samples stored. In this case, the PIs will destroy all known remaining samples and report what was done to both the subject and to the NIAID IRB. This decision may not affect the subject's participation in this protocol or any other NIH-sponsored protocols.

14 RECORD RETENTION

The investigator is responsible for retaining all essential documents listed in the ICH Good Clinical Practice Guideline. All essential documentation for all study subjects is to be maintained by the investigators in a secure storage facility for a minimum of 3 years per NIAID policies. These records are also to be maintained in compliance with IRB/EC, state, and federal medical records retention requirements, whichever is longest. All stored records are to be kept confidential to the extent required by federal, state, and local law.

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

15 REMUNERATION PLAN FOR SUBJECTS

At the completion of core protocol activities (anticipated to be at the 48-h time point for all patients), we will provide a selection of local staple foods (i.e., milk, millet, rice, sugar, etc.) equivalent to 32 USD to the parent/guardian of each child to compensate for the time, inconvenience, and discomfort of the enrollment process, intravenous catheterization, finger-prick and venous blood sampling, and hospitalization. The total of 32 USD is based on the USD amounts we have previously used in Kenieroba to compensate for particular types of protocol activities: enrollment (1 USD), venipuncture (5 USD), finger-prick blood sampling (0.5 USD), and IV catheterization (10 USD).

SCIENTIFIC REFERENCES

1. WHO. 2010. Guidelines for the treatment of malaria. www.who.int/malaria/publications/atoz/9789241547925/en/index.html.
2. Grabowsky M. 2008. The billion-dollar malaria moment. Nature 451: 1051-1052.
3. Roberts L, Enserink M. 2007. Malaria. Did they really say ... eradication? Science 318: 1544-1545.
4. Ceesay SJ, Casals-Pascual C, Erskine J, Anya SE, Duah NO, et al. 2008. Changes in malaria indices between 1999 and 2007 in The Gambia: a retrospective analysis. Lancet 372: 1545-1554.
5. O'Meara WP, Bejon P, Mwangi TW, Okiro EA, Peshu N, et al. 2008. Effect of a fall in malaria transmission on morbidity and mortality in Kilifi, Kenya. Lancet 372: 1555-1562.
6. O'Meara WP, Mwangi TW, Williams TN, McKenzie FE, Snow RW, et al. 2008. Relationship between exposure, clinical malaria, and age in an area of changing transmission intensity. The American journal of tropical medicine and hygiene 79: 185-191.
7. Korenromp EL, Williams BG, Gouws E, Dye C, Snow RW. 2003. Measurement of trends in childhood malaria mortality in Africa: an assessment of progress toward targets based on verbal autopsy. The Lancet infectious diseases 3: 349-358.
8. Ndugwa RP, Ramroth H, Muller O, Jasseh M, Sie A, et al. 2008. Comparison of all-cause and malaria-specific mortality from two West African countries with different malaria transmission patterns. Malaria journal 7: 15.
9. Sachs J, Malaney P. 2002. The economic and social burden of malaria. Nature 415: 680-685.
10. Snow RW, Craig MH, Deichmann U, le Sueur D. 1999. A preliminary continental risk map for malaria mortality among African children. Parasitol Today 15: 99-104.
11. Dondorp A, Nosten F, Stepniewska K, Day N, White N. 2005. Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. Lancet 366: 717-725.
12. Dondorp AM, Fanello CI, Hendriksen IC, Gomes E, Seni A, et al. 2010. Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial. Lancet 376: 1647-1657.
13. Warrell DA, Gilles HM. 2002. Essential Malariology. Book Power Fourth Edition: 296.
14. Agbenyega T, Planche T, Bedu-Addo G, Ansong D, Owusu-Ofori A, et al. 2003. Population kinetics, efficacy, and safety of dichloroacetate for lactic acidosis due to severe malaria in children. J Clin Pharmacol 43: 386-396.
15. Krishna S, Agbenyega T, Angus BJ, Bedu-Addo G, Ofori-Amanfo G, et al. 1995. Pharmacokinetics and pharmacodynamics of dichloroacetate in children with lactic acidosis due to severe malaria. Qjm 88: 341-349.
16. Krishna S, Supanaranond W, Pukrittayakamee S, Karter D, Supputamongkol Y, et al. 1994. Dichloroacetate for lactic acidosis in severe malaria: a pharmacokinetic and pharmacodynamic assessment. Metabolism 43: 974-981.

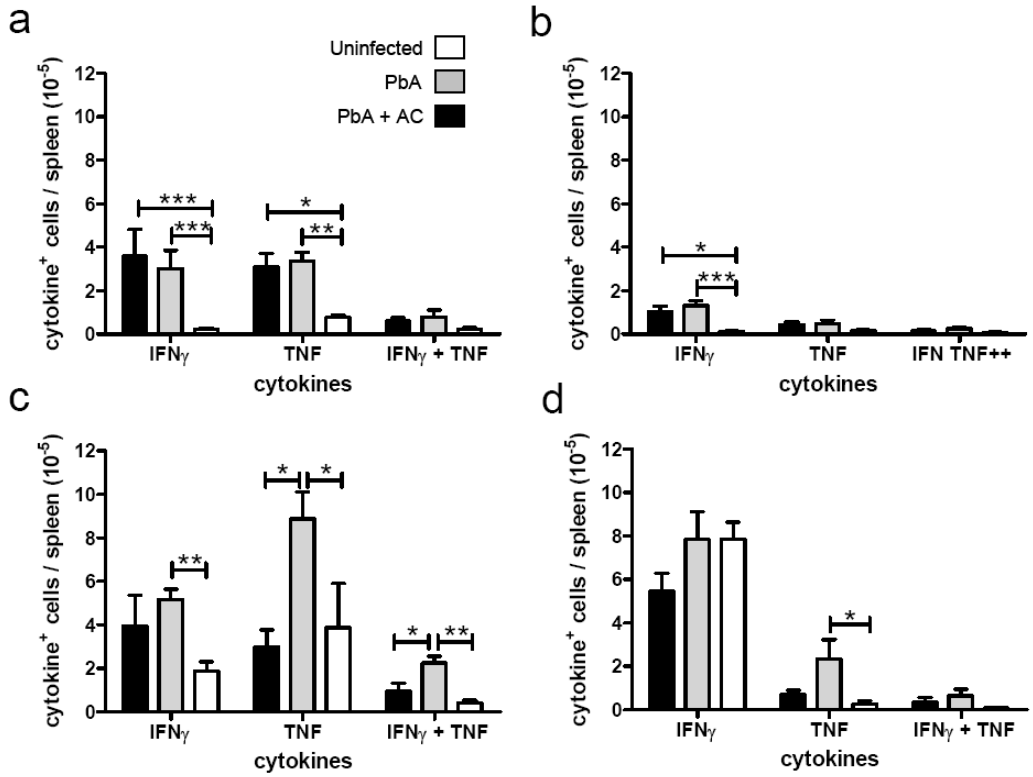
17. Krishna S, Supanaranond W, Pukrittayakamee S, Kuile FT, Ruprah M, et al. 1996. The disposition and effects of two doses of dichloroacetate in adults with severe falciparum malaria. Br J Clin Pharmacol 41: 29-34.
18. Maitland K, Pamba A, English M, Peshu N, Marsh K, et al. 2005. Randomized trial of volume expansion with albumin or saline in children with severe malaria: preliminary evidence of albumin benefit. Clin Infect Dis 40: 538-545.
19. Maitland K, Kiguli S, Opoka RO, Engoru C, Olupot-Olupot P, et al. 2011. Mortality after fluid bolus in African children with severe infection. The New England journal of medicine 364: 2483-2495.
20. Dondorp AM, Silamut K, Charunwatthana P, Chuasuwanchai S, Ruangveerayut R, et al. 2007. Levamisole inhibits sequestration of infected red blood cells in patients with falciparum malaria. The Journal of infectious diseases 196: 460-466.
21. Charunwatthana P, Abul Faiz M, Ruangveerayut R, Maude RJ, Rahman MR, et al. 2009. N-acetylcysteine as adjunctive treatment in severe malaria: a randomized, double-blinded placebo-controlled clinical trial. Crit Care Med 37: 516-522.
22. Boggild AK, Krudsood S, Patel SN, Serghides L, Tangpukdee N, et al. 2009. Use of peroxisome proliferator-activated receptor gamma agonists as adjunctive treatment for Plasmodium falciparum malaria: a randomized, double-blind, placebo-controlled trial. Clin Infect Dis 49: 841-849.
23. Serghides L, Patel SN, Ayi K, Lu Z, Gowda DC, et al. 2009. Rosiglitazone modulates the innate immune response to Plasmodium falciparum infection and improves outcome in experimental cerebral malaria. The Journal of infectious diseases 199: 1536-1545.
24. Robak O, Bojic A, Locker GJ, Laczika K, Ramharter M, et al. 2010. The use of drotrecogin alfa in severe falciparum malaria. Anaesth Intensive Care 38: 751-754.
25. Penet MF, Abou-Hamdan M, Coltel N, Cornille E, Grau GE, et al. 2008. Protection against cerebral malaria by the low-molecular-weight thiol pantethine. Proceedings of the National Academy of Sciences of the United States of America 105: 1321-1326.
26. Kaiser K, Texier A, Ferrandiz J, Buguet A, Meiller A, et al. 2006. Recombinant human erythropoietin prevents the death of mice during cerebral malaria. The Journal of infectious diseases 193: 987-995.
27. Wiese L, Hempel C, Penkowa M, Kirkby N, Kurtzhals JA. 2008. Recombinant human erythropoietin increases survival and reduces neuronal apoptosis in a murine model of cerebral malaria. Malaria journal 7: 3.
28. Casals-Pascual C, Idro R, Picot S, Roberts DJ, Newton CR. 2009. Can erythropoietin be used to prevent brain damage in cerebral malaria? Trends Parasitol 25: 30-36.
29. Mishra SK, Wiese L. 2009. Advances in the management of cerebral malaria in adults. Curr Opin Neurol 22: 302-307.
30. 1999. Position statement and practice guidelines on the use of multi-dose activated charcoal in the treatment of acute poisoning. American Academy of

Clinical Toxicology; European Association of Poisons Centres and Clinical Toxicologists. J Toxicol Clin Toxicol 37: 731-751.
31. Schulman G. 2012. A nexus of progression of chronic kidney disease: tryptophan, profibrotic cytokines and charcoal. J Ren Nutr 22: 107-113.
32. de Souza JB, Okomo U, Alexander ND, Aziz N, Owens BM, et al. 2010. Oral activated charcoal prevents experimental cerebral malaria in mice and in a randomized controlled clinical trial in man did not interfere with the pharmacokinetics of parenteral artesunate. PLoS One 5: e9867.
33. de Souza JB, Riley EM. 2002. Cerebral malaria: the contribution of studies in animal models to our understanding of immunopathogenesis. Microbes Infect 4: 291-300.
34. Saemann MD, Bohmig GA, Osterreicher CH, Burtscher H, Parolini O, et al. 2000. Anti-inflammatory effects of sodium butyrate on human monocytes: potent inhibition of IL-12 and up-regulation of IL-10 production. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 14: 2380-2382.
35. Jackson GD, Dai Y, Sewell WA. 2000. Bile mediates intestinal pathology in endotoxemia in rats. Infection and immunity 68: 4714-4719.
36. Bradberry SM, Vale JA. 1995. Multiple-dose activated charcoal: a review of relevant clinical studies. J Toxicol Clin Toxicol 33: 407-416.
37. Walther B, Walther M. 2007. What does it take to control malaria? Annals of tropical medicine and parasitology 101: 657-672.
38. Lopera-Mesa TM, Doumbia S, Chiang S, Zeituni AE, Konate DS, et al. 2013. Plasmodium falciparum Clearance Rates in Response to Artesunate in Malian Children With Malaria: Effect of Acquired Immunity. J Infect Dis 207: 1655-1663.
39. Chivers DJ, Hladik CM. 1980. Morphology of the gastrointestinal tract in primates: comparisons with other mammals in relation to diet. J Morphol 166: 337-386.
40. Schiller C, Frohlich CP, Giessmann T, Siegmund W, Monnikes H, et al. 2005. Intestinal fluid volumes and transit of dosage forms as assessed by magnetic resonance imaging. Aliment Pharmacol Ther 22: 971-979.
41. Walker AR, Richardson BD, Walker BF, Woolford A. 1973. Appendicitis, fibre intake and bowel behaviour in ethnic groups in South Africa. Postgrad Med J 49: 243-249.
42. Ilkhanipour K, Yealy DM, Krenzelok EP. 1992. The comparative efficacy of various multiple-dose activated charcoal regimens. Am J Emerg Med 10: 298-300.
43. Park GD, Radomski L, Goldberg MJ, Spector R, Johnson GF, et al. 1983. Effects of size and frequency of oral doses of charcoal on theophylline clearance. Clinical pharmacology and therapeutics 34: 663-666.
44. Adetifa IM, Hill PC, Jeffries DJ, Jackson-Sillah D, Ibanga HB, et al. 2009. Haematological values from a Gambian cohort--possible reference range for a West African population. International journal of laboratory hematology 31: 615-622.
45. Robins EB, Blum S. 2007. Hematologic reference values for African American children and adolescents. American journal of hematology 82: 611-614.

46. Gordon Cook AZ. 2009. Manson's Tropical Diseases. Saunders Elsevier.
47. Walther M, Woodruff J, Edele F, Jeffries D, Tongren JE, et al. 2006. Innate immune responses to human malaria: heterogeneous cytokine responses to blood-stage Plasmodium falciparum correlate with parasitological and clinical outcomes. Journal of immunology 177: 5736-5745.

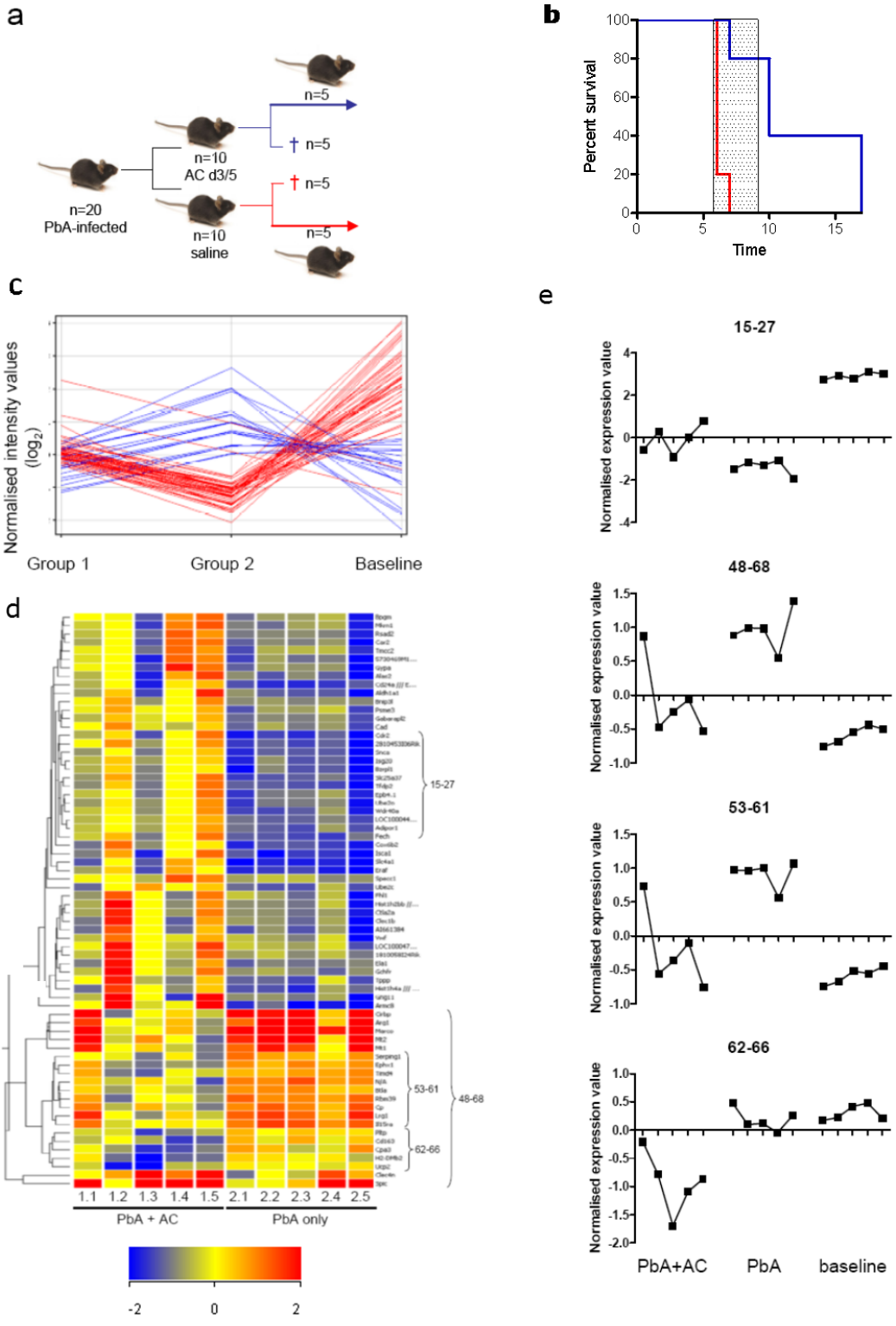
Appendix 1: **oAC treatment of mice with *P. berghei* ANKA (PbA) infection affects T cell cytokine production.**

a-d) PbA infected (grey bars) and PbA-infected oAC-treated (black bars) mice were killed on day 6 and cytokine measured either directly (a, b) or after PMA stimulation (c, d). The absolute number of splenic IFNγ$^+$, TNF$^+$ and IFNγ$^+$TNF$^+$ CD4$^+$ (a, c) and CD8$^+$ (b, d) are shown. Uninfected mice (open bars) are shown as baseline. Data represent mean ± 1SE (n=10 individual mice from 2 independent experiments; *: p<0.05; **: p<0.01; ***: p<0.001).



Appendix 2: **Gene expression profiling of AC-treated PbA-infected mice**

a) 20 C57BL/6 mice were infected with PbA, and at d3 and d5, 10 were treated with oAC. At day 6, 5 mice per group were killed for gene expression analysis and the remainder monitored for survival. b) Survival of PbA-infected oAC-treated mice (blue line) was significantly greater than PbA-infected and untreated mice (red line). p<0.0021. c) 68 genes identified as differentially expressed (red, up-regulated; blue, down-regulated) when comparing oAC-treated PbA-infected mice to untreated PbA-infected mice are shown, with normalized $\log_2$ expression values. Expression of these genes in uninfected mice (baseline) is also shown for comparison, though this data was not used to identify this gene set. d) Heat maps for individual gene expression in individual mice (n=5 group) clustered by gene expression pattern. Genes were numbered sequentially from top to bottom to provide cluster designations indicated. (red = upregulated, blue = downregulated). e) Transcriptional vectors for each cluster were calculated as the arithmetic mean of the normalized gene expression level for each transcript in the cluster. Data for uninfected mice are also shown for comparison (baseline).



See attached pdf documents for appendices 3 and 4.

Appendix 3: Actidose Aqua ® package insert (see separate pdf)

Appendix 4: Actidose Aqua ® safety data sheet (see separate pdf)

Appendix 5: Grading of local reactogenicity at IV catheter site

Local Reaction to Injectable Product	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hrs or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Severity requiring hospital based management
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	Severity requiring hospital based management
Erythema/Redness	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis

(Based on FDA recommended Toxicity Grading Scale for Healthy Adults and Adolescents)