Phase IIa Randomized, Single- blinded, Placebo-controlled Clinical Trial of the Reprofiled Drug Auranofin for GI Protozoa

DMID Protocol Number: 15-0015

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Principal Investigator: Sharon Reed, MD

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DMID Medical Monitor: Mo Elsafy, MD

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Statement of Compliance

This trial will be conducted in accordance with Good Clinical Practice (GCP) as required by the following:

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

ICH E6; 62 Federal Register 25691 (1997)

- NIH Clinical Terms of Award

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

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 U.S. federal regulations and ICH guidelines.

Site Investigator:*

Signed: _____ Date: _____

Sharon Reed, MD

**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.*

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List of Abbreviations

AE	Adverse Event/Adverse Experience
CAR	Clinical Agents Repository
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CRO	Contract Research Organization
DHHS	Department of Health and Human Services
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
FWA	Federalwide Assurance
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
icddr,b	International Centre for Diarrhoeal Disease Research-Bangladesh
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
JAMA	Journal of the American Medical Association
MedDRA®	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NCI	National Cancer Institute, NIH, DHHS
NDA	New Drug Application
NEJM	New England Journal of Medicine
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS

NIH	National Institutes of Health
OCRA	Office of Clinical Research Affairs, DMID, NIAID, NIH, DHHS
OHRP	Office for Human Research Protections
OHSR	Office for Human Subjects Research
ORA	Office of Regulatory Affairs, DMID, NIAID, NIH, DHHS
PHI	Protected Health Information
PI	Principal Investigator
PK	Pharmacokinetics
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SDCC	Statistical and Data Coordinating Center
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
US	United States
WHO	World Health Organization

Protocol Summary

Title:	**Phase IIa Randomized, Single-blinded, Placebo-controlled Clinical Trial of the Reprofiled Drug, Auranofin for GI Protozoa**
Short Title:	Auranofin for GI Protozoa
Phase:	IIa
Population:	136 symptomatic adults ≥18 to 65 years old from Bangladesh with amebiasis or giardiasis

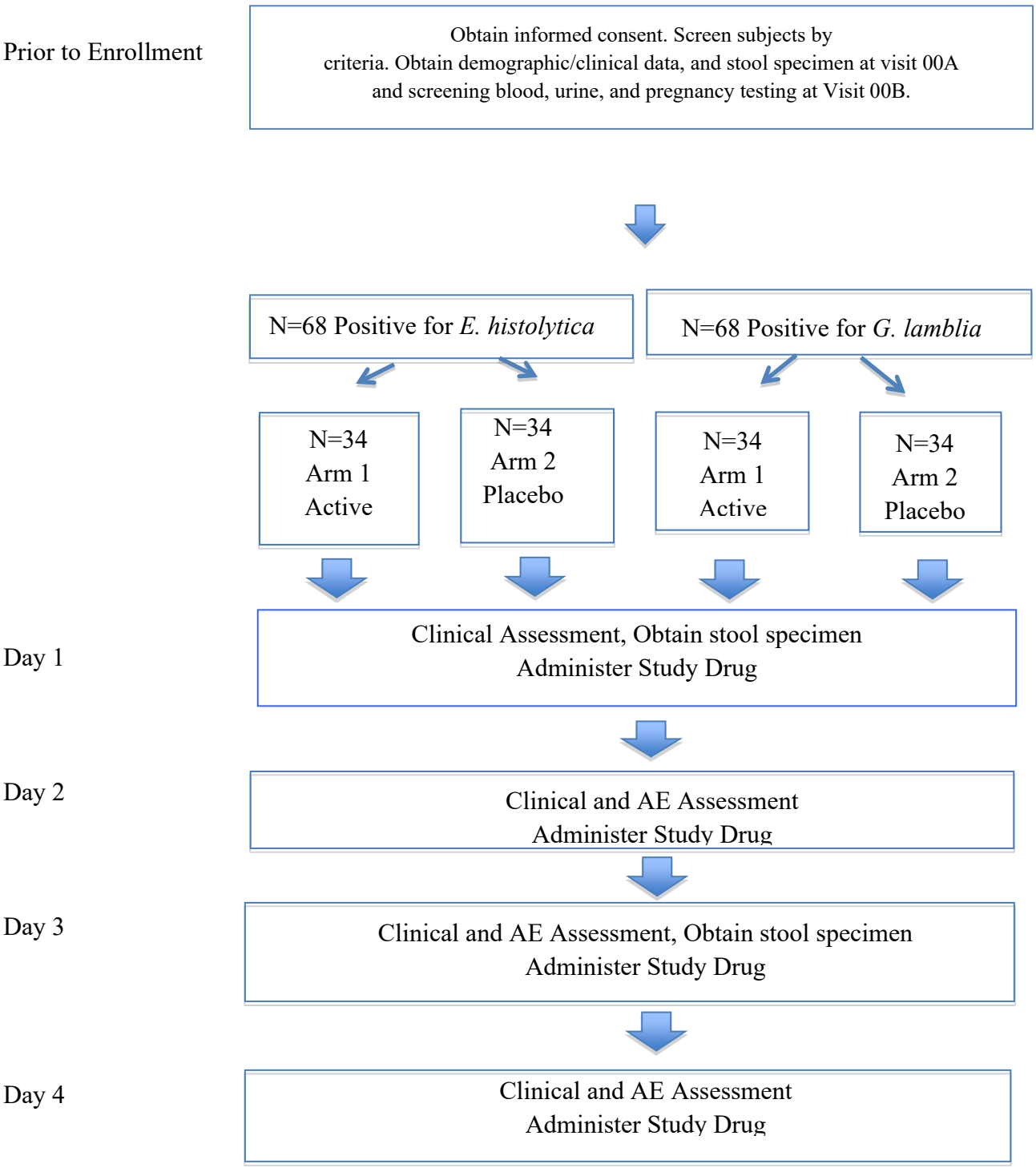
Number of Sites: 1

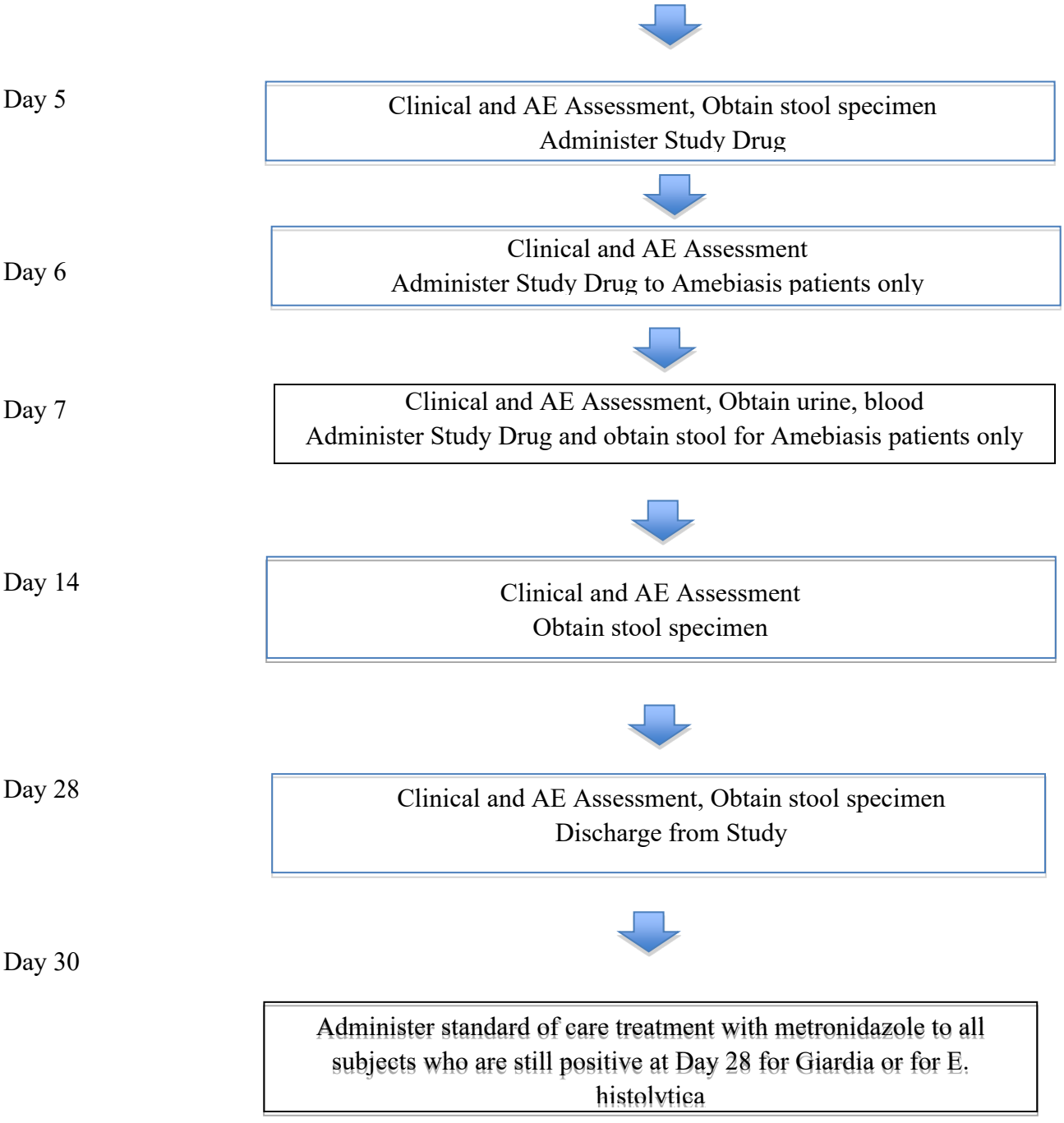
Study Duration:	3.5 years
Subject Participation Duration:	7 days treatment for amebiasis and 5 days for giardiasis with follow-up through Day 28
Description of Agent of Intervention:	Auranofin (Ridaura) 6 mg, or similar placebo
Objectives:	Primary *E. histolytica:* • To compare the proportion of subjects with stools positive by rapid EIA and positive antigen detection EIA for *E. histolytica* at enrollment with resolution of diarrhea (less than 3 loose stools/24 hrs) by Day 7 Secondary *E. histolytica:* • To compare the proportion of subjects with stools positive by rapid EIA and positive antigen detection EIA for *E.* histolytica and trophozoites on smear at enrollment with parasitological response (no detection of trophozoites of *E. histolytica* on microscopic exam by Days 7 • To compare the proportion of subjects with stools positive rapid EIA and positive antigen detection EIA for *E.* histolytica and trophozoites on smear at enrollment with parasitological response

	(no detection of trophozoites on microscopic exam or negative antigen detection) by Day 3 and 5 • To compare the rate of decrease of trophozoites/cyst load by qPCR in stools by Days 3, 5, and 7 • To compare the proportion of subjects with negative stool antigen tests by days 3, 5, 7, and 14 • To compare the proportion of subjects with sustained cure (no detection of trophozoites by microscopic exam) at 14 and 28 days • To compare the proportion of subjects with relapse (same strain) or re-infection (new strain) with positive stools at 14 and 28 days by genotyping the initial vs. subsequent strain • To compare the time to resolution of diarrhea (less than 3 loose stools/24 hours) Primary *Giardia:* • To compare the proportion of subjects with stools positive by rapid EIA and positive antigen detection EIA for Giardia at enrollment with resolution of diarrhea (less than 3 loose stools/24 hours) by Day 5 Secondary *Giardia:* • To compare the proportion of subjects with parasitological response (no detection of trophozoites on microscopic exam) on Days 3 and 5 • To compare the rate of decrease of trophozoites/cyst load by qPCR in stools by Days 3 and 5 • To compare the proportion of subjects with negative stool antigens by days 3 and 5. • To compare the proportion of subjects with sustained cure (no detection of r trophozoites by microscopic exam) at 14 and 28 days

	• To compare the proportion of subjects with relapse (same strain) or re-infection (new strain) with positive stools at 14 and 28 days by genotyping the initial vs. subsequent strain • To compare the time to resolution of diarrhea (less than 3 loose stools/24 hours)
Description of Study Design:	Randomized, placebo-controlled, single-blinded superiority treatment study to compare placebo to once daily doses of auranofin for adults with amebiasis or giardiasis. A sample size of 68 subjects enrolled with amebiasis (34 per arm) and 68 with giardiasis (34 per arm); Power based on 60 subjects with amebiasis and 60 with giardiasis completing the study
Estimated Time to Complete Enrollment:	3.5 years

Schematic of Study Design:




Day 5
Clinical and AE Assessment, Obtain stool specimen
Administer Study Drug
Day 6
Clinical and AE Assessment
Administer Study Drug to Amebiasis patients only
Day 7
Clinical and AE Assessment, Obtain urine, blood
Administer Study Drug and obtain stool for Amebiasis patients only
Day 14
Clinical and AE Assessment
Obtain stool specimen
Day 28
Clinical and AE Assessment, Obtain stool specimen
Discharge from Study
Day 30
Administer standard of care treatment with metronidazole to all subjects who are still positive at Day 28 for Giardia or for E. histolytica

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

2.1.1 Summary of Previous Pre-clinical Studies

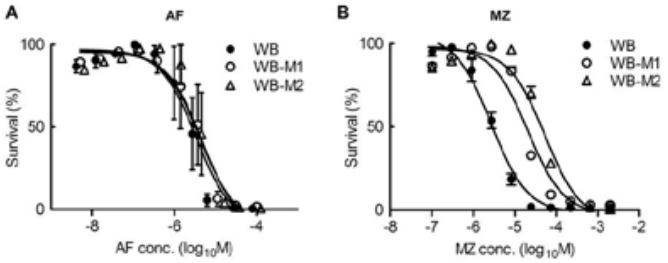
This proposed clinical trial follows from key findings in studies supported by UO1 AI0778822 entitled, "Novel Therapeutics for Class B Protozoa," to design and develop highly effective antiparasitic agents for Class B Protozoa. The development of the first high-throughput whole cell screens for *E. histolytica* (Debnath, 2012) and *G. lamblia* (Gut et al., 2011) against FDA-approved drugs and bioactive compounds (a "repurposing" screen), found that auranofin had a ten-fold lower IC50 (0.5□M) compared with metronidazole (5.2 □M) for *E. histolytica* and an equivalent IC50 for *Giardia* (4.0 □M). Auranofin is an orally available gold-containing compound that has been in clinical use in the treatment of rheumatoid arthritis for 25 years. The ability to reprofile an existing drug will significantly shorten the time and expense required to develop a drug against these important parasitic infections (Ashburn and Thor, 2004).

In most organisms there are two largely independent systems to detoxify reactive oxygen species, one based on glutathione and the other based on thioredoxin, but *E. histolytica* and *Giardia* lack both glutathione reductase activity and glutathione synthetic enzymes (Fahey et al, 1984). So the thioredoxin reductase is a major defense in both parasites in the prevention, intervention and repair of damage caused by oxidative stress (Arias et al., 2007). Auranofin inhibited recombinant EhTrxR with an IC50 of 400 nM (Debnath, 2012) and recombinant GiardiaTrxR with an IC50 of 150 nM (Tejman-Yarden, 2013). In addition, inhibition of EhTrxRled to increased susceptibility of the trophozoites to oxidants as well as the accumulation of intracellular reactive oxide species. We also showed that auranofin caused the accumulation of oxidized thioredoxin in amebic trophozoites *in vitro* and *in vivo.* Therefore, auranofin is the first drug active against *Entamoeba* and *Giardia* with a clearly defined target. Based on these studies, auranofin was given Orphan Drug status by the FDA.

Activity of auranofin against metronidazole-sensitive and resistant strains of *Giardia.*
The drug susceptibility of a metronidazole sensitive, Assemblage A strains (WB (ATCC 50803) compared to their isogenic metronidazole resistant strains (WB-M1, WB-M2). The viability of 2 X 10^3 trophozoites in 96 well plates with auranofin concentrations of 5 nM -100□□M and metronidazole concentrations of 0.1 □M-2 mM was determined at 48 hours in a luminescence-based ATP assay (Tejman-Yarden, 2013). Auranofin had equivalent efficacy with Mtz-sensitive

and resistant strains, whereas the EC50 for Mtz-resistant strains was increased 8-40 fold (Figure 1).

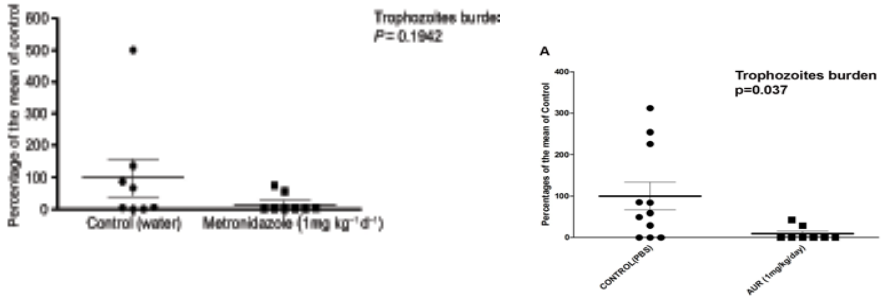
Figure 1: Killing of metronidazole-sensitive and resistant strains by metronidazole (Mz) and auranofin (AF)



The % viability of a sensitive parental strain (WB) and two resistant strains (WB-M1 and M2) to serial dilutions of metronidazole and auranofin are shown.

Efficacy of auranofin in animal models of amebiasis and giardiasis. Auranofin was effective in an *in vivo* model of murine amebic colitis, in which trophozoites invade the mouse cecum following surgical inoculation (Debnath, 2012). Auranofin was delivered by gavage at a concentration of 1 mg/kg for 7 days. The parasite burden of auranofin-treated mice by quantitative PCR significantly decreased compared to the control (Figure 2A) in contrast to metronidazole (Figure 2B), which required ten times the dose for a significant effect.

Figure 2: Effect of auranofin on amebic colitis in mice.

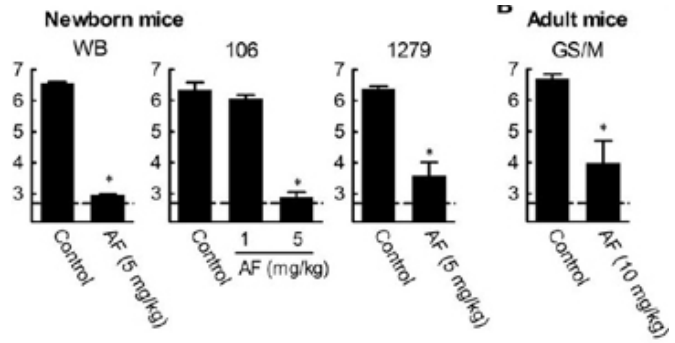


The treatment of mice with (A) auranofin at 1 mg/kg daily dose or (B) metronidazole is presented as the percentage of trophozoites/ gm of tissue.

The efficacy of auranofin was also tested in the suckling mouse models of giardiasis. Newborn C57BL/6 mice (5-7 days old) were gavaged with 10^7 *G. lamblia* trophozoites (Assemblage A

strain WB or Assemblage B strain 1279, infection established over two days, and then treated daily with 5 mg/kg auranofin for 5 days. The number of trophozoites remaining after drug treatment was determined by direct counts of the small intestinal contents. Auranofin treatment significantly decreased the trophozoite load (Figure 3).

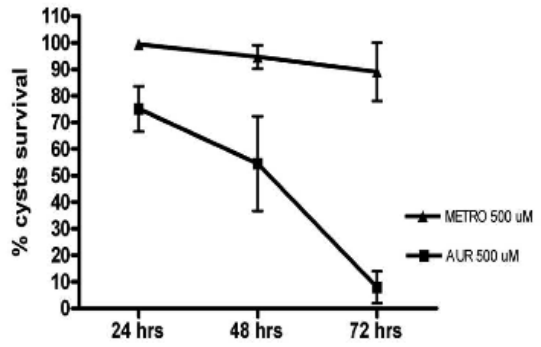
Figure 3: Efficacy of auranofin in rodent models of giardiasis



Newborn (5-7 days) or adult mice were infected with trophozoites of strains 106, 1279, or GS/M and auranofin started 2 days afterwards for 5 days. Values represent the log number of trophozoites present in the small intestine.

Efficacy of auranofin against cysts. We also tested the efficacy of auranofin against *Entamoeba* cysts as *E. histolytica* cyst are resistant to metronidazole (Upcroft, 2001). Because *E.histolytica* cannot be induced to encyst *in vitro,* we used *E. invadens,* which do. Viability was tested with a fluorescent ATP assay (GoLive). *E. invadens* cysts were resistant to metronidazole, in contrast to auranofin (Figure 4). We will test the *in vitro* efficacy of auranofin against clinical *E. histolytica* cysts both *in* vitro and in treated patients in this study.

Figure 4: Efficacy of auranofin and metronidazole against Entamoeba cysts



Viability was determined as the % survival compared to controls in ethanol alone.

2.1.2 Summary of Relevant Clinical Studies

The hypothesis is that auranofin/Ridaura will be an important drug for use against metronidazole resistant parasites, particularly *Entamoeba histolytica, Giardia,* and *Trichomonas.* We have both *in vitro* and *in vivo* data supporting this therapeutic hypothesis. The FDA approved auranofin in 1985 with limited PK/PD data because current sensitive techniques such as mass spectrometry were not available. To fill this gap, DMID performed a Phase I, single center, open label, multiple dose study to evaluate the PK of gold, administered as auranofin (Clinical Study Report 12-0101, finalized May 13, 2015). Fifteen healthy subjects were enrolled to receive the same treatment dose (6 mg daily for 7 days) as proposed for amebiasis in this study. The pharmacokinetics of gold was evaluated by Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) in plasma (up to Day 126) and in feces (up to Day 42). Treatment-emergent adverse events (TEAEs) were monitored from Day 1 until Day 14 and serious adverse events (SAEs) until Day 126. All subjects (15) were included in the summary analyses for fecal gold, but two subjects were excluded (13 final) from summary analyses of plasma gold concentration because of quantifiable Day 1 pre-dose plasma gold concentrations higher than 5% of their Cmax.

All subjects had quantifiable plasma gold levels throughout the 24 hour dosing period with a T_{max} at 1.5 post dose. The plasma $AUC_{(0-24)}$ increased approximately six-fold from 0.971 $\pm$ 0.51 to 5.05 $\pm$1.07 □g-h/mL and the C_{max} three-fold from 0.102 $\pm$ 0.04 to 0.312 $\pm$0.08 □g/mL. On Day 7, 11 of 15 subjects had fecal gold concentrations of 8.80 $\pm$ 7.83 mg/kg and undetectable levels at Day 14. The study concluded that auranofin at 6 mg per day dose for 7 days was generally safe

and well tolerated with no deaths or SAEs. There were no clinically notable TEAEs or subject withdrawn from the study (see 2.3.1 for more details).

2.1.3 Summary of Epidemiological Data

Up to 10% of the world's population is infected with *Entamoeba,* resulting in morbidity second only to malaria and schistosomiasis among parasitic infections (Amoebiasis, 1997). Infection is initiated by the ingestion of environmentally resistant cysts in contaminated water or food, which release motile trophozoites in the intestine. *E. histolytica* trophozoites can then invade either the mucosa to cause symptomatic colitis, or the bloodstream to cause distant abscesses of the liver, lungs, or brain (Haque et al., 2003). In endemic areas such as our clinical site in Bangladesh, 40% of children develop amebic colitis a year (Haque, 2006).

Giardiasis is the most common gastrointestinal parasitic infection in the U.S. (Yoder, 2010). In developing countries, giardiasis is a major public health problem with infection rates in many regions as high as 15-20% in adults and close to 100% in children (Gilman et al., 1985), leading to dehydration and malnutrition. Because of its worldwide prevalence, *Giardia* was added to the WHO Neglected Disease Initiative (Savioli et al., 2006).

2.2 Rationale

Entamoeba histolytica and *Giardia lamblia* are Class B protozoa, which cause major water- and foodborne outbreaks worldwide. Infection is caused by the ingestion of environmentally resistant cysts in water or contaminated food. Everyone in the U.S. is susceptible to these parasites, and at least six waterborne outbreaks of amebiasis have been documented in the U.S. and 20,000 cases of giardiasis reported a year. Drug resistance is also an increasing concern with the majority of patients worldwide treated with imidazoles, particularly metronidazole, which causes cross-resistance to the newest approved drugs, tinidazole and nitazoxanide. Metronidazole has frequent side effects, is carcinogenic and mutagenic in animals, and does not kill cysts. Therefore, shorter, more tolerable, and cyst killing therapy is needed.

This proposed clinical trial will test the hypothesis that oral auranofin is effective for the treatment of amebiasis and giardiasis and follows from studies supported by UO1 AI0778822 entitled, “Novel Therapeutics for Class B Protozoa.” The overall aim of the project was to design and develop highly effective antiparasitic agents for Class B Protozoa. As part of whole-cell screening of amebic trophozoites against FDA-approved drugs, one drug, auranofin, was found to have an IC50 of 0.5□M vs. the standard drug, metronidazole (IC50= 5.2 □M). We found that auranofin targets the *E. histolytica* thioredoxin reductase (EhTrxR) *in vitro* and is efficacious against amebic colitis and liver abscess in rodent models. Studies with Giardia demonstrated that auranofin was very effective against both metronidazole-sensitive and resistant strains of *Giardia in vitro* and in rodent models. Auranofin is an orally available gold-

containing compound, FDA-approved in 1985 for rheumatoid arthritis, which has received Orphan Drug status for the treatment of amebiasis. During the planning stage of this trial, funded by R34 Grant AI098633-01, we have identified the International Centre for Diarrheal Diseases in Bangladesh (icddr,b) as the ideal clinical site under the direction of Dr. Rashidul Haque with Dr. William Petri as Co-PI. Potential patients are routinely screened for both *E. histolytica* and *G. lamblia,* so we can efficiently enroll patients infected with either parasite, supporting potential approval for two indications for auranofin in a single trial. This is an NIH Priority as the use of auranofin for amebiasis and giardiasis has been awarded both an R34 Planning Grant as well as a Phase I Clinical Trial Grant.

Because this trial will re-profile an FDA-approved drug that is still in use, we propose a Phase IIa, randomized, single-blinded, placebo-controlled superiority clinical trial comparing auranofin (standard dose of 6 mg daily) for 7 days for treatment of amebiasis or 5 days for giardiasis to placebo in adults**.** The two primary outcomes will be the proportion of patients with resolution of amebiasis by day 7 of therapy and/or resolution of giardiasis by day 5. Any patients who remains asymptomatic but stool positive at 28 days will receive standard of care therapy with metronidazole.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

Auranofin is contraindicated in patients with a gold allergy, and not recommended during pregnancy or severe hepatic or renal insufficiency. The only reported drug interaction is a single patient with elevated phenytoin blood levels. The complications listed in the package insert for long term (> 1 yr) auranofin therapy include dermatologic: rash in 26%; gastrointestinal: loose stools (42%), abdominal pain (14%), nausea (10%); hematologic: anemia, leucopenia, thrombocytopenia in up to 3%; hepatic: elevated liver enzymes: 2%; mucous membranes: stomatitis (13%); renal: proteinuria (1%).

In the DMID-sponsored Phase I Clinical Trial 12-0101 (Section 2.1.2), the safety of auranofin was assessed in 15 healthy volunteers based on adverse events collected from the first dose on Day 1 through Day 14 and through Day 126 for SAEs. Four subjects (26.7%) reported Treatment-related TEAEs, one with headache, one with headache and nausea, one with abdominal discomfort and flatulence, and one with diarrhea. All were assessed as mild by the investigator. There were no clinically meaningful trends or changes in clinical laboratory results, vital signs, or physical examination data during the study.

Auranofin is a Category C drug whose use is not recommended in pregnant women because of studies in pregnant rabbits and rats with decreased maternal and fetal weight and litter size with

4-50X the human daily dose. The risk to pregnant women in our trial will be mitigated by careful screening, pregnancy testing and providing contraception (hormonal oral or injectable contraceptives for women in addition to providing condoms for their partners) for the length of the trial (5-7 days) and for three half-lives of the drug (total 4 months). Birth control method and compliance will be assessed during screening and throughout the subject's participation.

2.3.2 Known Potential Benefits

Treatment worldwide for giardiasis relies primarily on imidazoles, particularly metronidazole, which was first introduced in 1959. Metronidazole has not been approved for use against *Giardia* in the U.S. and has been associated with treatment failures up to 20% (Upcroft 2001). It also causes frequent side effects, including a disulfiram-like effect (severe nausea and vomiting with alcohol ingestion), central and peripheral nervous system abnormalities (seizures, encephalopathy and peripheral neuropathy), and interactions with drugs metabolized by the CYP2C9 enzyme of the P450 system (Freeman et al., 1997). Metronidazole is also carcinogenic and teratogenic in animals (Bendesky et al., 2002). Nitazoxanide, a thiazolide drug, is the first drug with efficacy against *Cryptosporidium* as well as *Giardia* compared to placebo (Rossignol et al., 2001). Albendazole therapy of giardiasis has been evaluated in several clinical trials (reviewed in Solaymani-Mohammadi et al. 2010.) Quinacrine and Furazolidone also have activity, but are no longer available in the U.S. (Gardner and Hill, 2001). Metronidazole resistance is now a major concern as clinical failures have been linked to metronidazole-resistant *Giardia* (Upcroft et al., 2001; Lemee et al., 2000), and highly resistant strains are readily produced in the laboratory (Dunn et al., 2010). Unfortunately, metronidazole-resistant *Giardia* are cross- resistant to nitazoxanide (Wright et al., 2003) and all metronidazole derivatives.

In addition, auranofin may prove to be a future broad spectrum antiparasitic drug as *in vitro* and/or *in vivo* efficacy has already been demonstrated against amebiasis (Debnath, 2012), trichomoniasis (Dr. Kirk Land, University of the Pacific, personal communication), *Cryptosporidium* (Dr. Momar Ndao, personal communication), *Toxoplasma gondii* (Andrade, 2014), *T. brucei* (Lobanov, 2006), *Leishmania infantum (*Ilari, 2011), filaria (Dr. James McKerrow, UCSF, personal communication), and schistosomiasis (Kuntz, 2007; Angelucci, 2009).

3 OBJECTIVES

3.1 Study Objectives

Auranofin is a "repurposed" drug with established pharmacology and is approved for human use by the FDA. We will assess clinical efficacy for two new indications, amebiasis and giardiasis, with a randomized Phase IIa, placebo-controlled, single-blinded, superiority trial comparing auranofin for treatment of amebiasis and giardiasis to placebo in adults. Resolution of diarrhea and clearance of parasites will be the endpoints, and all subjects who remain positive at Day 28 for giardiasis or amebiasis will receive standard of care treatment with metronidazole. Ultimately, the goal will be to expand the trial to include symptomatic children after proving efficacy and safety in adults.

All subjects will have the same screening protocol, but the trial will evaluate subjects with amebiasis or giardiasis with separate primary and secondary endpoints.

Asymptomatic subjects enrolled under prior versions of the protocol will be analyzed as per the corresponding objectives and study SAP.

Primary *E. histolytica:*

To compare the proportion of subjects with stools positive by rapid EIA and positive antigen detection EIA for *E. histolytica* at enrollment with resolution of diarrhea (less than 3 loose stools/ 24 hours) by Day 7.

Secondary *E. histolytica:*

- To compare the proportion of subjects with positive rapid EIA and positive antigen detection EIA for *E. histolytica* and trophozoites on smear (wet mount or concentrated trichrome) at enrollment with parasitological response (no detection of trophozoites on microscopic exam by Day 7.
- To compare the proportion of subjects with positive rapid EIA and positive antigen detection EIA for *E.* histolytica and trophozoites on smear at enrollment with parasitological response (no detection of trophozoites on microscopic exam) by Day 3 and 5.
- To compare the rate of decrease in trophozoite/cyst load by qPCR in stools by Days 3, 5, and 7.
- To compare the proportion of subjects with negative stool antigen test by Days 3, 5, 7, and 14.

- To compare the proportion of subjects with sustained cure (no detection of trophozoites by microscopic exam) at 14 and 28 days.
- To compare the proportion of subjects with relapse (same strain) or re-infection (new strain) with positive stools at 14 and/or 28 days by genotyping the initial vs. subsequent strain.
- To compare the time to resolution of diarrhea (less than 3 loose stools/24 hours)

Primary *Giardia:*

To compare the proportion of subjects with stool positive rapid EIA and positive antigen detection EIA for *Giardia* at enrollment with resolution of diarrhea (less than 3 loose stools/24 hours) by Day 5.

Secondary *Giardia:*

- To compare the proportion of subjects with parasitological response (no detection of trophozoites on microscopic exam) by Day 3 and 5.
- To compare the rate of decrease in trophozoite/cyst load by qPCR in stools by Days 3 and 5.
- To compare the proportion of subjects with negative stool antigens by days 3 and 5.
- To compare the proportion of subjects with sustained cure (no detection of trophozoites by microscopic exam) at 14 and 28 days.
- To compare the proportion of subjects with relapse (same strain) or re-infection (new strain) with positive stools at 14 and/or 28 days by genotyping the initial vs. subsequent strain.
- To compare the time to resolution of diarrhea (less than 3 loose stools/24 hours)

3.2 Study Outcome Measures

3.2.1 Primary Outcome Measures

1. Proportion of subjects with positive rapid EIA and positive antigen detection EIA for *E. histolytica* and resolution of diarrhea (less than 3 loose stools/ 24 hours) by Day 7.
2. Proportion of subjects with positive rapid EIA and positive antigen detection EIA for *Giardia* and resolution of diarrhea (less than 3 loose stools/24 hours) by Day 5.

3.2.2 Secondary Outcome Measures

1. *E. histolytica*:
    a. Proportion of subjects with positive rapid EIA and positive antigen detection EIA for *E. histolytica* and trophozoites on smear at enrollment with parasitological response (no detection of trophozoites on microscopic exam) by Day 7.
    b. Proportion of subjects with positive rapid EIA and positive antigen detection EIA for *E.* histolytica and trophozoites on smear at enrollment with parasitological response (no detection of trophozoites on microscopic exam) by Day 3 and 5.
    c. Rate of decrease of trophozoite/cyst load by qPCR in stools by Days 3, 5, and 7
    d. Proportion of subjects with negative stool antigen test by Days 3, 5, 7, and 14
    e. Proportion of subjects with sustained cure (no detection of trophozoites by microscopic exam) at 14 and 28 days
    f. Proportion of subjects with relapse (same strain) or re-infection (new strain) with positive stools at 14 and 28 days by genotyping the initial vs. subsequent strain
    g. Time to resolution of diarrhea (less than 3 loose stools/24 hours)

2. *Giardia*:
    a. Proportion of subjects with positive rapid EIA and positive antigen detection EIA for *Giardia* and trophozoites on smear at enrollment with parasitological response (no detection of trophozoites on microscopic exam) by Day 3 and 5
    b. Rate of decrease of trophozoite/cyst load by qPCR in stools by Days 3 and 5
    c. Proportion of subjects with negative stool antigens by days 3 and 5.
    d. Proportion of subjects with sustained cure (no detection of trophozoites by microscopic exam) at 14 and 28 days
    e. Proportion of subjects with relapse (same strain) or re-infection (new strain) with positive stools at 14 and 28 days by genotyping the initial vs. subsequent strain
    f. Time to resolution of diarrhea (less than 3 loose stools/24 hours)

4 STUDY DESIGN

This 30-day, Phase IIa, two-arm, randomized, placebo-controlled, single-blinded, superiority treatment study in 136 males and non-pregnant females ≥ 18 to 65 years of age will compare placebo to once daily doses of auranofin (Ridaura) for adults with amebiasis (68 subjects) or giardiasis (68 subjects).Potential Bangladeshi adult subjects will be identified by a health professional from otherwise stable patients presenting to the icddr,b or Rajshahi Medical College Hospitals with diarrhea (thought to be due to amebiasis or giardiasis). Rajshahi Medical College Hospital is being added as a new enrollment site because of their higher prevalence of amebic infections (Alam et al., 2014). Eligible subjects will be randomly assigned to a treatment group with auranofin (6 mg orally once daily for 5 days for giardiasis or 7 days for amebiasis) compared to a placebo group receiving similar but not identical placebo capsules. Projected duration of subject participation will be approximately 30 days of face to face visits, including the pre-enrollment screening period of up to 4 days. It is anticipated that it will take approximately 3.5 years to finish the study.

Primary and secondary outcomes to be measured during the course of the study are outlined in Section 3.2.

For additional details on study procedures and evaluations by study schedule and study visits see Section 7, 8, and Appendix A.

4.1 Sub Studies

No sub studies are planned.

5 STUDY ENROLLMENT AND WITHDRAWAL

One hundred thirty six males and non-pregnant females, aged 18-65 years, with amebiasis or giardiasis who are otherwise healthy and meet all eligibility criteria, will be chosen from adult patients presenting to the iccdr,b or Rajshahi Medical College Hospitals with mild diarrhea.

Subject Inclusion and Exclusion Criteria must be assessed by a study clinician licensed to make medical diagnoses and listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

No exemptions are granted on Subject Inclusion/Exclusion Criteria in DMID-sponsored studies. Questions about eligibility should be directed toward the DMID Medical Officer.

5.1 Subject Inclusion Criteria

Subjects eligible to participate in this study must meet all of the following inclusion criteria:

1. Provide written informed consent prior to initiation of any study procedures.

2. Able to understand and comply with planned study procedures and be available for all study visits.

3. Male or non-pregnant, non-lactating females 18-65 years of age, inclusive. Females of reproductive potential currently using effective contraceptive methods are eligible.

4. Amebiasis or giardiasis identified by rapid EIA and positive antigen detection EIA of stool[1].[1]

   [1]If a subject is infected with both *E. histolytica* and *Giardia,* they will be enrolled in the *E. histolytica* study arm. Once the *Entamoeba* study arm is fully enrolled, any subsequent dual infected subjects will be enrolled in the *Giardia* arm. If a subject is infected with both *Giardia* and *Cryptosporidium,* they will not be enrolled.

5. Has diarrhea (defined as three or more loose stools) in the past 24 hrs, but is assessed to be clinically stable and in otherwise good health[2].

   [2]As determined by medical history and targeted physical examination, if indicated based on medical history, to evaluate acute or currently ongoing chronic medical diagnoses or conditions that would affect the assessment of eligibility and safety of subjects. Existing

medical diagnoses or conditions (except those in the Subject Exclusion Criteria) must be deemed as stable chronic medical conditions. A stable chronic medical condition is defined as no change in prescription medication, dose, or frequency of medication in the last 3 months (90 days) and health outcomes of the specific disease are considered to be within acceptable limits in the last 6 months (180 days). Any change due to change of health care provider, insurance company, or that is done for financial reasons, as long as in the same class of medication, will not be considered a violation of this inclusion criterion. Any change in prescription medication due to **improvement** of a disease outcome, as determined by the site principal investigator or appropriate sub-investigator, will not be considered a violation of this inclusion criterion. Subjects may be on chronic or as needed (prn) medications if, in the opinion of the site principal investigator or appropriate sub-investigator, they pose no additional risk to subject safety. *Topical, nasal, and inhaled medications, vitamins, and contraceptives are permitted.*

6. Vital signs (oral temperature, pulse, and blood pressure) are all within normal protocol-defined ranges (abnormal criteria defined in Section 9.2.3 Additional Adverse Event Severity Grading).

7. Laboratory tests (blood urea nitrogen, creatinine, AST, ALT, white blood cells, platelets, hemoglobin) are all within protocol-defined ranges.

   Subjects will be eligible for enrollment with the following laboratory values:

   - Blood urea nitrogen less than or equal to 30 mg/dL
   - Creatinine less than or equal to 133umol/L
   - AST or ALT less than or equal to 70.0 U/L
   - White cell count between 3.5 and 13.0 inclusive(10^9/L)
   - Platelets between 131 and 550 (10^9/L) inclusive
   - Hemoglobin between 11.0 and 18.0 gm/dL inclusive

8. Urinalysis with no greater than trace protein. If a high protein is confirmed to be due to menstruation, it should be repeated.

9. Women of reproductive potential[4] must have a negative urine pregnancy test within 72 hours of starting study medications.

   [4]Female subjects who are surgically sterile via tubal sterilization, bilateral oophorectomy or hysterectomy, who have been postmenopausal for greater than 1 year are not considered to be of reproductive potential.

10. Female subjects participating in sexual activity that could lead to pregnancy must be using and continue to use highly effective[5] contraception for a total of 4 months after enrollment.

    [5]Highly effective methods of contraception are defined as having low failure rates (i.e. less than 1% per year) when used consistently and correctly and may include, but are not limited to, abstinence from intercourse, monogamous relationship with a vasectomized partner, male condoms with spermicide, diaphragm with spermicide, intrauterine devices, and licensed hormonal methods. Females on effective forms of birth control will continue while on the study and for the follow-up period of 4 months total. The method and compliance of birth control used will be confirmed and documented at all study visits.

5.2 Subject Exclusion Criteria

Subjects meeting any of the following exclusion criteria at baseline will be excluded from study participation within 1 week of the initial visit:

1. Known intolerance of auranofin or gold compounds.

2. Pregnant or breastfeeding women or women of reproductive potential not using effective contraception or who plan to become pregnant or breastfeed at any given time during the study or within 3 months of study completion.

3. Use of metronidazole within the past 7 days.

4. Has any condition that would, in the opinion of the site investigator, place the subject at an unacceptable risk of injury or render the subject unable to meet the requirements of the protocol.

5. Concurrent participation in other investigational protocols or receipt of an investigational product within the previous 30 days.

6. History of alcohol or drug abuse within the last five years.

5.3 Treatment Assignment Procedures

5.3.1 Enrollment and Randomization Procedures

Per International Conference on Harmonisation (ICH) guideline E6: Good Clinical Practice (GCP), screening records will be kept at the icddr,b or Rajshahi Medical College hospital site to document the reason why an individual was screened, but failed trial entry criteria. The reasons why individuals failed screening will be recorded in the Statistical and Data Coordinating Center's (SDCC) Advantage EDC[SM] (Electronic Data Capture System). No subject may be screened more than twice due to a screening failure result.

Randomization will be stratified by site to prevent confounding that could arise from imbalance in treatment allocations between sites. Because the relative pace of enrollment between the two sites is unknown, the number of participants to be enrolled at each site is not fixed in advance. With this approach, the faster enrolling site may enroll more subjects, allowing enrollment to complete as quickly as possible. Each site may enroll up to 68 subjects into each strata, so it is possible that one strata could be filled entirely with subjects from a single site. With the flexible approach, treatment assignments may not be perfectly balanced (overall or within each site), but they will be approximately balanced. With sample sizes of 68 in each strata, the impact of a small imbalance on power is negligible.

Once eligibility and consent are confirmed, subjects will be stratified by site and infection (a 2-level variable of either amebiasis vs. giardiasis), and then within each stratum, randomized 1:1 to receive auranofin or placebo on Day 1. Both disease groups will enroll concurrently. Enrollment will continue in the other arm after one arm reaches full enrollment. All subjects will receive doses of the assigned medication or placebo delivered orally on Days 1, 2, 3, 4, 5, 6, and 7 for amebiasis and Days 1, 2, 3, 4, and 5 for giardiasis.

The randomization code will be prepared by statisticians at the SDCC and included in the enrollment module for the trial. SDCC staff will generate two randomization lists and send corresponding documentation to the study site. The randomization lists will include the randomization codes that will be used to serially assign study treatment to subjects as they are assigned to the study (i.e., randomize subjects to study arm by study identification number). This table will not contain unblinded information; study staff will not be able to see the study arm assignment for each study identification number.

Eligible study subjects will be randomized under single-blind conditions by study staff members after consent. Since the placebo is similar but not identical to the study medication, only the lab

personnel are truly blind, however the participant will not be informed whether they receive study product or placebo. The randomization number will be recorded on the study CRFs.

A designated individual at icddr,b and Rajshahi Medical College hospital will be provided with the treatment key, which links the treatment code to the actual treatment assignment, which will be kept in a secure place.

5.3.2 Masking Procedures

This is a single-blind study because the placebo is similar, but not identical to the study drug. The laboratory personnel performing evaluations will be blinded to treatment assignment.

Subjects, investigators, and study personnel performing any study-related assessments following study product administration will not be informed of group allocation, but could detect differences in study and placebo capsules. They will be instructed to perform their clinical or AE assessments as though blinded to prevent potential bias.

The randomization scheme will be generated by the SDCC and provided to unblinded study personnel (i.e., study pharmacist).

The Data and Safety Monitoring Board (DSMB) may receive data in aggregate and presented by treatment group. The DSMB may also be provided with expected and observed rates of the expected AEs in an unblinded fashion. The DSMB may be unblinded to individual study treatment assignments, as needed, to adequately assess safety issues. Refer to the MOP for unblinding procedures.

5.3.3 Reasons for Withdrawal

Subjects may voluntarily withdraw their consent for study participation at any time and for any reason, without penalty. Subjects who have received study product, regardless of the number of doses received, or who developed an AE or SAE will be encouraged to remain in the study to be followed for safety purposes.

A subject may withdraw or be withdrawn from this study for any of the following reasons:

- Medical disease or condition, or any new clinical findings for which continued participation, in the opinion of the site principal investigator or appropriate sub-investigator, would compromise the safety of the subject, or would interfere with the subject's successful completion of the study, or would interfere with the evaluation of responses.

- Subject no longer meets eligibility criteria.
- As deemed necessary by the site principal investigator or appropriate sub-investigator for noncompliance or other reasons.
- Subject withdrawal of consent.
- Subject lost to follow-up.
- Termination of the study.
- New information becomes available that makes further participation unsafe.

A subject will be discontinued from further receipt of auranofin or placebo if:

- The subject misses more than one dose of drug or placebo.
- Any clinical adverse event, laboratory abnormality, intercurrent illness, other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject.

5.3.4 Handling of Withdrawals

The primary reason for withdrawal from the study will be recorded on the Study Status data collection form. Subjects will be encouraged to complete the Early Termination Visit. The Early Termination Visit procedures are listed in Section 7.4. Although subjects are free to withdraw at any time or may be withdrawn by the site principal investigator or appropriate sub-investigator at any time, subjects who receive at least one dose of study product will be encouraged to remain in the study for follow-up safety assessments and collection of stool specimens. Every attempt will be made to follow all adverse events, including systemic reactions, serious adverse events, and new-onset chronic medical conditions ongoing at the time of early withdrawal to resolution.

In the case of subjects who fail to appear for a follow-up safety assessment, extensive effort (i.e., three documented contact attempts via phone calls and/or in person visits to the household of the participants) will be made to locate or recall them, or at least to determine their health status. These efforts will be documented in the subject's records.

Subjects who sign the informed consent form and are randomized but do not receive study product may be replaced with new subjects who will receive the same treatment assignment. . Subjects who sign the informed consent form, are randomized and receive study product, and subsequently

withdraw, or are withdrawn or terminated from this study, or are lost to follow-up will not be replaced.

5.3.5 Termination of Study

Although the study sponsor has every intention of completing the study, it reserves the right to terminate the study at any time for clinical or administrative reasons. Reasons for termination include, but are not limited to, study closure due to DSMB review and recommendation and at the discretion of DMID.

6 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

6.1 Study Product Description

Auranofin (2,3,4,6-tetra-O-acetyl-1-thio-D-glucopyranosato-S) (triethyl-phosphine gold), is an orally available gold-containing compound with established pharmacology and is approved for human use by the FDA. It is available in an oral form as capsules called Ridaura, which contains 3 mg of auranofin. Auranofin 3 mg capsules are manufactured by Prometheus Laboratories, San Diego, CA.

6.1.1 Acquisition

Auranofin 3mg capsules will be purchased from a commercial supplier by Fisher BioServices.

The placebo will be manufactured by commercial supplier through contract with Fisher BioServices.

Upon request by DMID, Auranofin and placebo will be shipped to the following address:

DMID-Clinical Material Services (CMS)
Fisher BioServices
20439 Seneca Meadows Parkway
Germantown, MD 20876
Phone: 240-477-1350
Fax: 240-477-1360
Email: DMID.CMS@thermofisher.com

Auranofin and placebo will be stored at the DMID CMS- Fisher BioServices and will be provided to the clinical research site upon request and with prior approval from DMID.

6.1.2 Formulation, Packaging, and Labeling

6.1.2.1 Auranofin Capsules

Each capsule of auranofin has an opaque brown cap and opaque tan body, contains 3 mg of white crystalline powder, and is imprinted with the product name RIDAURA.[2] Inactive ingredients consist of benzyl alcohol, cellulose, cetylpyridinium chloride, D&C Red No. 33, FD&C Blue No. 1, FD&C Red No. 40, FD&C Yellow No. 6, gelatin, lactose, magnesium stearate, povidone, sodium lauryl sulfate, sodium starch glycolate, starch, titanium dioxide and trace amounts of other inactive ingredients. Auranofin 3 mg capsules are packaged in bottles of 60 count.

6.1.2.2 Placebo Capsules

The placebo capsules will be similar to auranofin capsules with opaque brown caps and an opaque tan body, but will not be imprinted with the product name RIDAURA. The placebo capsules will be manufactured by a contract supplier through Fisher BioServices. Measures will be taken to mitigate potential bias by the single-blinded nature of the trial. The specific measures will be detailed in the MOP.

6.1.2.3 Packaging and Labeling

The daily dose of placebo and Auranofin capsules will be identical in a tight, light-resistant container. The study products will be labeled to include FDA cautionary information: "Caution-New drug -Limited by Federal (or United States) Law to Investigational Use Only."

6.1.2.4 Storage and Stability

The study drugs will be sent first to icddr,b and then transported by icddr,b staff under continuously monitored, temperature controlled conditions to Rajshahi Medical College hospital for storage and dispensing as detailed in the MOP. Auranofin powder darkens slightly when exposed to strong light and also to some extent when stored at a temperature of 60°C or warmer; the darkening indicates a small degree of chemical degradation, but the effect on biologic activity is not known. The Auranofin capsules and the placebo will be stored between 15° and 30°C (59° and 86°F).

6.2 Dosage, Preparation and Administration of Study Intervention/Investigational Product

Auranofin 6mg (administered orally as two-3mg capsules) or similar placebo (administered orally as two capsules) once daily for 5 days for giardiasis or 7 days for amebiasis. All shipped study products will be initially stored at icddr,b hospital. Study products will subsequently be transported to Rajshahi Hospital for storage and dispensing.

Enrollment may occur any day of the week. The complete course of study capsules (7 days for amebiasis and 5 days for giardiasis) will be dispensed in individual daily containers upon randomization by the icddr,b or Rajshahi pharmacy and dispensed as follows:

For subjects enrolled at the icddr,b and Rajshahi Medical College Hospitals, the first doses will be dispensed until the patient is discharged, with all doses thereafter managed through home visits or at icddr,b or Rajshahi Medical College Hospitals or local site clinic. Refer to the protocol-specific MOP for additional information regarding dispensing procedures.

For all subjects: Friday is a holiday in Bangladesh so two days' supply will be given on Thursday and the Friday dose will be self-administered as instructed and documented on the next visit.

6.3 Modification of Study Intervention/Investigational Product for a Participant

Because auranofin is an FDA-approved drug, which has undergone extensive short term toxicity studies in a Phase I trial, there will be no dose modification of the drug, and subjects will be withdrawn if they miss more than one dose. If at baseline, a subject has any exclusionary abnormality in the screening blood and urine tests, they will be notified and not enrolled. The next safety lab testing time will be at Day 7 after completion of the study agent. Any laboratory abnormalities at that point will be evaluated as an AE, and may be reported to the DSMB as requested, and followed accordingly.

6.4 Accountability Procedures for the Study Intervention/Investigational Product(s)

After receipt of the study product, the site Principal investigator (PI) is responsible for distribution and disposition of these study products, and has ultimate responsibility for drug accountability. The site PI may delegate this responsibility to the site pharmacist for each site. Study product will be distributed to the health care workers to be dispensed during the subject's hospital stay and/or during daily subject visits at home or the Rajshahi Hospital or the local Rajshahi clinic. Study protocol records must be maintained and document logs of receipt, accountability, and storage temperature conditions at the icddr,b office or Rajshahi clinic. These study product accountability and dispensing logs must be maintained in the study file. Upon completion of the study and after the final monitoring visit, unused study product will be retained until monitored and released for disposition as per the Sponsor.

6.5 Assessment of Subject Compliance with Study Intervention/Investigational Product(s)

Subject compliance will be assessed through direct observation and pill counts.

6.6 Concomitant Medications/Treatments

Whenever a concomitant medication or study agent is initiated or the dose changed, investigators must review the concomitant medications' and study agents' most recent package inserts or investigator's brochure to obtain the most current information on drug interactions,

contraindications, and precautions. Administration of any medications, therapies, or vaccines will be recorded on the appropriate data collection form. Concomitant medications will include all current medications and medications taken within the previous 30 days through the end of the follow-up period and will be reported in the electronic case report form (eCRF). Prescription and over-the-counter drugs will be included as well as herbals, vitamins, and supplements.

Use of new medication should prompt evaluation for the presence of a new diagnosis or chronic medical disease or condition.

There are no drugs that are prohibited because of their interactions with Ridaura, but current systemic metronidazole are an exclusion criterion. There are no medications whose dose should be adjusted because of this short course of Ridaura treatment. Any patients with clinically significant underlying renal, hepatic, or immunologic abnormalities will be excluded before enrollment.

7 STUDY SCHEDULE

Complete study schedule details listed by study visit are described below. Any visit may occur at icddr,b or Rajshahi Medical College Hospitals or local site clinic, or home (except those requiring blood drawing, Visit 00B and Day 7). Refer also to Section 8 and Appendix A: Schedule of Events.

7.1 Screening Visits

7.1.1 Visit 00A, Day -4 to -1, Screening 1, icddr,b or Rajshahi Medical College Hospitals

Potential adult subjects from Bangladesh (between the ages of 18-65) will be chosen from adult patients presenting to the icddr,b or Rajshahi Medical College Hospitals with mild diarrhea. These potential subjects will be visited by a study team member or health professional from icddr,b or Rajshahi Medical College Hospitals. Screening may take place as many as 4 days prior to enrollment. A 5-minute screener will be administered at the first screening visit and will be used to identify eligible persons who will be referred to a study team member to discuss inclusion in the study. Written informed consent will be obtained from potentially eligible subjects after the 5-minute screener and prior to conducting any further screening or study procedures. A single ICF will be used to document the volunteer's consent to further screening and participation in the study.

For subjects who are potentially eligible and who have provided informed consent, the following procedures will be conducted at the first screening visit:

- Full eligibility criteria will be reviewed with subjects.
- Demographic data, including date of birth, sex, self-reported ethnicity, and residence location will be collected.
- A stool sample will be collected for detection of *Giardia* or *E. histolytica.*

7.1.2 Visit 00B, Day -4 to -1, Screening 2, icddr, b or Rajshahi Medical College Hospitals

Only eligible subjects who have provided informed consent and stool specimens that are positive for *Giardia* or *E. histolytica* will have this second screening visit for blood and urine testing. This visit may also occur on the same day at Visit 00A and must occur at the hospital or clinic.

- Medical history will be obtained by interview of subjects to assure eligibility.
- All concomitant medications taken within 30 days prior to signing the informed consent form will be recorded on the appropriate data collection form and eCRF. Subjects will be questioned specifically about any metronidazole in the past 7 days.
- A targeted physical examination will be performed, including vital signs (oral temperature, pulse (taken at the wrist or neck), blood pressure) observation for rashes or glossitis, and an abdominal exam.
- A urine pregnancy test will be performed on all female subjects of childbearing potential and must be negative.
- Approximately 17 mL of venous blood will be collected for CBC, ALT, AST, BUN, and creatinine tests. These values must be confirmed to meet the eligibility criteria as outlined in the Subject Inclusion Criteria (see Section 5.1) prior to randomization.
- A urine sample will be collected to test for the presence of protein. The result must be confirmed to meet the eligibility criteria as outlined in the Subject Inclusion Criteria (see Section 5.1) prior to randomization.

7.2 Visit 01, Day 1, Enrollment, Dose 1, icddr,b or Rajshahi Medical College Hospital or Clinic

- Enrollment and randomization of subjects
- Eligibility criteria, including results of all clinical screening laboratory evaluations, will be reviewed with subjects to assure continued eligibility.
- Targeted physical exam performed, if indicated
- 2 capsules of study product (6mg total) or placebo will be orally taken by the subject.
- Assessment of any new symptoms since Visit 00B before first dose is given.
- Medical history, including any concomitant medications, reviewed.
- Stool sample will be collected for ova and parasite testing. The window for stool specimens will be +1 day and the date of collection noted.

7.3 Follow-up Visits

This section summarizes the follow-up procedures conducted for each subject. Complete details for each Study Day are provided in Appendix A. Visit windows are specified around the target for each visit. Follow-up visits are scheduled in reference to dosing dates as indicated for each visit window. Subjects will be enrolled at the icddr,b or Rajshahi Medical College Hospitals and have follow-up visits in the Hospital until their diarrhea has resolved. Subsequent follow-up visits may be held at the icddr,b, Rajshahi Hospital, or clinic or their home except for Visit 07 which must be at icddr,b or Rajshahi Hospital or clinic because blood drawing is required. When a visit falls on a holiday Friday, two days' supply of study product will be given on Thursday and the Friday dose will be self-administered as instructed and documented upon the next visit. Staff will notify the subject on Friday to take the dose and document it was taken. The window for stool specimens will be +1 day and the date of collection noted. For visits that fall on a Friday, the study team member will confirm on the next scheduled visit the diarrhea status as noted on the Visit Documentation for the **missed** visit. This will be submitted in the Data System for study end-point analysis.

7.3.1 Visit 02, Day 2

- Targeted physical exam performed, if indicated.
- 2 capsules of study product (6mg total) or placebo will be orally taken by the subject.
- Assessment of any side effects or adverse events.
- Any subject with worsening diarrhea as assessed by the study physician, may have their dose discontinued and receive standard of care metronidazole.
- Medical history, including any concomitant medications, reviewed.

7.3.2 Visit 03, Day 3

- Targeted physical exam performed, if indicated.
- 2 capsules of study product (6mg total) or placebo will be orally taken by the subject.
- Assessment of any side effects or adverse events.
- Any subject with worsening diarrhea as assessed by the study physician, may have their dose discontinued and receive standard of care metronidazole.

- Medical history, including any concomitant medications, reviewed.
- Stool sample will be collected for ova and parasite testing. The window for stool specimens will be +1 day and the date of collection noted.

7.3.3 Visit 04, Day 4

- Targeted physical exam performed, if indicated.
- 2 capsules of study product (6mg total) will be orally taken by the subject.
- Assessment of any side effects or adverse events.
- Any subject with worsening diarrhea as assessed by the study physician, may have their dose discontinued and receive standard of care metronidazole.
- Medical history, including any concomitant medications, reviewed.

7.3.4 Visit 05, Day 5

- Targeted physical exam performed, if indicated.
- 2 capsules of study product (6mg total) or placebo will be orally taken by the subject.
- Assessment of any side effects or adverse events.
- Any subject with worsening diarrhea as assessed by the study physician, may have their dose discontinued and receive standard of care metronidazole.
- Medical history, including any concomitant medications, reviewed.
- Stool sample will be collected for ova and parasite testing. The window for stool specimens will be +1 day and the date of collection noted.

7.3.5 Visit 06, Day 6 (Amebiasis arm only)

- Targeted physical exam performed, if indicated
- 2 capsules of study product (6mg total) or placebo will be orally taken by the subject.
- Assessment of any side effects or adverse events.

- Any subject with worsening diarrhea as assessed by the study physician, may have their dose discontinued and receive standard of care metronidazole.
- Medical history, including any concomitant medications, reviewed.

7.3.6 Visit 07, Day 7 (Window + 1 day) icddr,b or Rajshahi Medical College Hospitals

- Targeted physical exam performed, if indicated.
- 2 capsules of study product (6mg total) or placebo will be orally taken by the subject (amebiasis arm only).
- Assessment of any side effects or adverse events.
- Any subject with worsening diarrhea as assessed by the study physician, may have their dose discontinued and receive standard of care metronidazole.
- Medical history, including any concomitant medications, reviewed.
- Stool sample will be collected for ova and parasite testing (amebiasis patients only) The window for stool specimens will be +1 day and the date of collection noted.
- Approximately 17 mL of venous blood will be collected for CBC, ALT, AST, BUN, and creatinine tests. The window for blood specimens will be +1 day and the date of collection noted.
- A urine sample will be collected to test for the presence of protein.

7.3.7 Visit 08, Day 14 (Window: +/- 3 days)

- Targeted physical exam performed, if indicated.
- Assessment of any side effects or adverse events.
- Any subject with worsening diarrhea as assessed by the study physician, may have their dose discontinued and receive standard of care metronidazole.
- Medical history, including any concomitant medications, reviewed.

- Stool sample will be collected for ova and parasite testing. The window for stool specimens will be +/- 3 days from the visit date and the date of collection noted.

7.3.8 Visit 09, Day 28 (Window: +/- 3 days)

The ninth study visit will occur on Day 28 when subjects will be assessed for medical history, targeted physical exam, concomitant medications review, and a final stool sample collected. Subjects will be instructed to contact the study staff, per the informed consent form, if any study product related adverse events develop in the following three months.

7.3.9 Visit 10, Day 30 (Window: +/- 3 days)

Visit 10 is a visit for any study patient who remains stool positive for Giardia or *E.* histolytica in the Day 28 stool specimen to receive the standard of care treatment for giardiasis or amebiasis. Subjects will be instructed to contact the study staff, per the informed consent form, if any adverse events develop in the following three months.

7.4 Early Termination Visit

Subjects may withdraw voluntarily from participation in the study at any time. Subjects may also withdraw voluntarily from receiving the study intervention for any reason. If early termination occurs and if the subject is willing, a final assessment for symptoms will be performed and a stool specimen obtained.

If voluntary withdrawal occurs, the subject will be asked to continue scheduled evaluations, complete an end-of-study evaluation, and be given appropriate care under medical supervision until the symptoms of any adverse event resolve or the subject's condition becomes stable.

7.5 Unscheduled Visit

The icddr,b and Rajshahi Medical College Hospital Study Teams have local clinic facilities that are open six days a week for unscheduled visits for subjects or their families. This has proven very successful for subject retention as well. If the subject needs to be seen on the one day a week the clinic is closed, there is a doctor on call for research studies or in Rajshahi or subjects also may come to the icddr,b or Rajshahi Medical College Hospitals or local Rajshahi site clinic.

Unscheduled visits may occur at any time during the study. Any of the following activities may be performed:

- Targeted physical exam, if indicated.
- Assessment of any side effects or adverse events.
- Medical history, including any concomitant medications, reviewed.
- If a subject complains of abdominal pain or diarrhea, a stool will be collected for ova and parasite to document whether *Giardia* or *Entamoeba* are present

7.6 Pregnancy Visit

If a subject should become pregnant at any time during the study, she will be asked to stay in the study for continued evaluation. If she leaves the study before the end of her pregnancy, the study staff will request permission for her to be contacted at the end of her pregnancy so that she and her baby can be evaluated.

8 STUDY PROCEDURES/EVALUATIONS

8.1 Clinical Evaluations

Medical History: Complete medical history will be obtained by interview of the subjects at the screening visit (Visit 00B) and will be reviewed and updated on Day 1 (Visit 01) prior to the first study product administration. Subjects will be queried regarding a history of significant medical disorders of the head, eyes, ears, nose, throat, mouth, cardiovascular system, lungs, gastrointestinal tract, liver, pancreas, kidney, urologic system, nervous system, blood, lymph nodes, endocrine system, musculoskeletal system, skin, and genital/reproductive tract. A history of any allergies, cancer, immunodeficiency, psychiatric illness, substance abuse, and autoimmune disease will be solicited. At follow-up visits after the first dose, an interim medical history will be obtained, when indicated, by interview of the subjects noting any changes since the previous visit.

Concomitant Medications: Concomitant medications will include a review of all current medications and medications taken within 30 days prior to signing the informed consent form and through approximately 30 days after the first study product administration. Prescription and over-the-counter drugs will be included as well as vitamins and supplements. Assessment of eligibility will also include a review of all permitted and prohibited medications per the Subject Inclusion and Exclusion Criteria (see Section 5).

Physical Examination and Vital Signs: A physical examination limited to vital signs (blood pressure, pulse, temperature), observation for rashes or glossitis, and an abdominal exam will be performed at screening (Visit 00B). On Day 1 (Visit 01) prior to the first study product administration and at follow-up visits after the first administration of study product, a targeted physical examination will be performed, if indicated, based on subject's interim medical history.

Adverse Events: Subjects will be interviewed to determine the occurrence of unsolicited AEs.

8.2 Laboratory Evaluations

8.2.1 Clinical Laboratory Evaluations

Laboratory Evaluations will include:

1. Stool examination: Stool samples will be obtained for ova and parasite testing. Subjects screened at icddr, b or Rajshahi Hospital will be given a stool cup for Day 1

sampling. Once discharged, all subjects will perform stool collections at icddr,b, Rajshahi Hospital, clinic or home on Days 3, 5, 7(window +1 day), 14 (window ±3 days) and 28 (window ±3 days).The specimen will be kept in a cooler with an ice pack at the subject's house until picked up by a Field Research Assistant and placed in a cooler with an ice pack for transport to the lab at icddr,b or Rajshahi Medical College. Initial screening involves having a positive stool sample test for the *E. histolytica* or *Giardia* parasites. All laboratory personnel will be blinded, and the specimens de-identified.

a. Testing of stool samples will be performed by the icddr,b laboratory or Rajshahi Hospital laboratory and will include:

i. Rapid EIA and antigen detection EIA to detect *E. histolytica* and *Giardia* antigens to identify protozoal infections;

ii. Wet mount for ova and parasite examination;

iii. PVA fixation for concentration and trichrome staining for ova and parasite examination

iv. Qualitative PCR to detect *E.* histolytica and *Giardia*

a. Off-site testing of stool samples collected at Rajshahi will be transported to icddr, b and shipped to UCSD to include quantitative *E. histolytica* or *Giardia* testing performed at UCSD on shipped aliquots of DNA extracted from positive stools at icddr,b. De-identified non-infectious DNA specimens labeled with the subject code will be obtained from stools on Days 1, 3, 5, 7, 14, and 28 following enrollment. Quantitative PCR assays have been developed based on the small subunit rRNA gene, which is highly conserved among *E. histolytica* (Taniuchi, 2011) and *Giardia* isolates (Haque, 2007).

2. Urine testing:

a. *Pregnancy testing at both hospital sites*. Upon screening: as a condition of enrollment, all women of child bearing age must agree to have a pregnancy test, receive the results, and use effective birth control for the next four months. Testing will be performed on urine using HCG test strip assays. If the test is positive or the woman is breastfeeding, they will not be enrolled, as auranofin is contraindicated in pregnant or breastfeeding women. Women who are enrolled in the study will continue their current effective birth control for 4 months after enrollment.

b. *Urine for proteinuria*. On Visit 00B and 7: urine will be collected from all participants for proteinuria assessment via dipstick testing. Proteinuria testing will be performed at the icddr,b

3. Blood testing: Blood will be drawn for a complete blood count, BUN, creatinine, ALT, and AST on Visit 00B and Day 7. Blood tests will be collected at the icddr,b Hospital or Rajshahi Hospital or Clinic and transported to the respective clinical laboratory.

4. *E. histolytica* and *Giardia* genotyping: Any stool specimen that is still positive for *Entamoeba* or *Giardia* cysts or trophozoites at Days 14 and 28 will have DNA genotyping of the parasite performed on de-identified specimens of extracted DNA. The genotypes from initial and final isolates will be compared to allow determination of the rate of relapse or re-infection without linking to individual patients. The genotyping will be performed off-site at the University of Virginia. There will be no human DNA genotyping performed.

8.2.2 Specimen Preparation, Handling and Shipping

Appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health. All potentially infectious specimens will only be transported locally to the laboratory using packing mandated in the Code of Federal Regulations, 42 CFR Part 72 and IATA guidelines.

8.2.3 Instructions for Specimen Preparation, Handling, and Storage

Any residual serum from safety screening, aliquots of stool, and parasite DNA extracted from the stool of enrolled subjects only will be stored in cryovials marked with the subject identification number and date of specimen collection at icddr,b or Rajshahi Hospital or Clinic following specific consent of the subjects. Long-term stored specimens will go to the Fisher Repository. Future studies may include measuring gold levels in the blood or stool. Other researchers may request access to these specimens for collaborative studies as well. Further instructions for specimen preparation, handling, and storage are included in the protocol-specific MOP as appropriate. There will be no human DNA collection or storage.

8.2.4 Specimen Shipment

Only noninfectious, parasite extracted DNA specimens will be shipped to UCSD for quantitative parasite loads or University of Virginia for genotyping. They can be sent at room temperature by standard international couriers, or designated members of the study team with completed specimen/Transport Receipt Logs. The residual long-term stored specimens will be shipped according to IATA guidelines to the Fisher Repository.

9 ASSESSMENT OF SAFETY

9.1 Specification of Safety Parameters

Because this is an FDA approved drug that has been in use for more than 25 years, the potential AEs have been well documented in subjects in longer term therapy for rheumatoid arthritis. Of the AEs listed on the Insert, the most serious reactions would be an allergic reaction to gold, which could include anaphylactic reactions, necrotizing enterocolitis, pulmonary fibrosis, exfoliative dermatitis, bone marrow aplasia or other severe hematologic disorders.

The adverse reactions incidences listed below are based on observations of 1) 4,784 Ridaura-treated subjects in clinical trials (2,474 U.S., 2,310 foreign), of whom 2,729 were treated more than one year and 573 for more than three years; and 2) post-marketing experience. The highest incidence is during the first six months of treatment; however, reactions can occur after many months of therapy. With rare exceptions, all subjects were on concomitant nonsteroidal anti-inflammatory therapy; some of them were also taking low dosages of corticosteroids. Reactions occurring in more than1% of Ridaura-treated patients:

- *Gastrointestinal:* loose stools or diarrhea (47%); abdominal pain (14%); nausea with or without vomiting (10%); constipation; anorexia*; flatulence*; dyspepsia*; dysgeusia.
- *Dermatological*: rash (24%); pruritus (17%); hair loss; urticaria.
- *Mucous Membrane*: stomatitis (13%); conjunctivitis*; glossitis.
- *Hematological:* anemia; leukopenia; thrombocytopenia; eosinophilia.
- *Renal:* proteinuria*; hematuria.
- *Hepatic:* elevated liver enzymes.
  *Reactions marked with an asterisk occurred in 3-9% of the patients. The other reactions listed occurred in 1-3%. Reactions occurring in less than 1% of Ridaura-treated patients
- *Gastrointestina*l: dysphagia; gastrointestinal bleeding†; melena†; positive stool for occult blood†; ulcerative enterocolitis.

9.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

9.2.1 Adverse Events

Adverse Event: International Conference on Harmonisation (ICH) E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

AEs not meeting the protocol-defined criteria for SAEs will be captured on the appropriate data collection form and electronic case report form (eCRF). Information to be collected for unsolicited AEs includes event description, date of onset, licensed clinician's assessment of severity and relationship to study product and alternate etiology (if not related to study product) (assessed only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator), date of resolution of the event, seriousness and outcome. AEs while on study will be documented appropriately regardless of relationship. AEs will be followed to resolution.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if the severity of any pre-existing medical condition increases, it will be recorded as an AE.

AEs must be graded for severity and assessed for relationship to study product (see definitions below). Adverse events characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on the appropriate data collection form and eCRF.

FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Severity of Event: AEs will be assessed by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or appropriate sub-investigator using a protocol-defined grading system (see Section 9.2.2 and 9.2.3). For events not included in the protocol-defined grading system, the following guidelines will be used to quantify severity:

- Mild (Grade 1): Events require minimal or no treatment and do not interfere with the subject's daily activities.

- Moderate (Grade 2): Events result in a low level of inconvenience or concern with therapeutic measures. Moderate events may cause some interference with functioning and daily activities.

- Severe (Grade 3): Events that prevent the subject's usual daily activities and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

The severity of systemic and clinical laboratory adverse events will be graded according to Section 9.2.2 and the toxicity tables included in the Appendices to this protocol.

Changes in the severity of any AE will be documented to allow an assessment of the duration of the event at each level of intensity. When AEs are intermittent, the onset and duration of each episode will be documented.

Relationship to Study Products: The study physician's assessment of an AE's relationship to study product is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. The relationship to study product must be assessed for AEs using the terms: related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used:

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

9.2.2 Solicited Events

Solicited events are AEs that are known to occur with this study product. Solicited AEs will not be collected that are related to any prescribed metronidazole. The below Toxicity Grading Scales will be used to grade events.

Adverse event data will be collected at each visit using specific questions and/or targeted physical examination. Based on the adverse reactions listed on the package insert, expected side effects include*:* loose stools or diarrhea, abdominal pain, nausea with or without vomiting, constipation, anorexia, flatulence, dyspepsia, dysgeusia, pruritus, hair loss, urticarial, stomatitis, conjunctivitis,

glossitis, and hematuria. Diarrhea, however, will not be recorded as a side effect because all participants will enroll with diarrhea, and because its resolution is the endpoint of the study. Physical examinations will look for abdominal pain, hair loss, urticaria, rashes, stomatitis, conjunctivitis, and glossitis. Hematologic, renal, or hepatic laboratory abnormalities will be picked up on testing at Day 7. Adverse event collection will continue until Day 28 (following three half-lives of the drug).

Event	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Loose stools or diarrhea	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity (incapacitating)
Abdominal Pain	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity (incapacitating)
Nausea with or without vomiting	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity (incapacitating)
Constipation	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity (incapacitating)
Anorexia	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity (incapacitating)
Flatulence	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity (incapacitating)
Dyspepsia	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity (incapacitating)
Dysgeusia	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity (incapacitating)
Pruritus	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity (incapacitating)
Hair loss	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity (incapacitating)
Urticaria	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity (incapacitating)
Stomatitis	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity (incapacitating)
Conjunctivitis	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity (incapacitating)
Glossitis	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity (incapacitating)

Event	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Hematuria	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity (incapacitating)
Rash	Does not interfere with daily activity	Interferes with daily activity	Prevents daily activity (incapacitating)

9.2.3 Additional Adverse Event Severity Grading

Vital signs will be graded as follows:

Vital Signs*			
Vital Signs	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Fever (°C) **	37.8-38.4	38.5-38.9	>= 39.0
Hypertension (systolic) mm Hg	141-150	151-155	>155 or an ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) mm Hg	91-95	96-100	>100 or an ER visit or hospitalization for malignant hypertension
Hypotension (systolic) mm Hg	85-89	80-84	<80 or an ER visit or hospitalization for hypotensive shock
Bradycardia – beats per minute ***	50-54 or>10 bpm less than baseline if baseline <60	45-49 or>15 bpm less than baseline if baseline <60	<45 or> 20 BPM or less than baseline if baseline <60, or an ER visit or hospitalization for arrhythmia
Tachycardia – beats per minute	101-115	116-130	>130 or an ER visit or hospitalization for arrhythmia

* Subjects should be at rest for at least 15 minutes prior to vital sign measurements

** Oral temperature; no recent hot or cold beverages or smoking

*** When resting heart rate is between 60-100 beats per minute. Pulse and Blood Pressure assessed at Visit 1 (Day 1) will be considered baseline. Sinus bradycardia among some healthy subject populations, for example, conditioned athletes maybe acceptable.

Abnormal laboratory findings that will trigger an Adverse Event report will include:

- Hematologic: WBC <3.5 or>13.0 (10^9/L), Hemoglobin <11.0 or >18.0.gm/dL, Platelets <130 or >550 (10^9/L)

- Hepatic: SGOT (AST) or SGPT (ALT)> 70.0
- Renal: Creatinine>133 umol/L, Blood Urea Nitrogen >30 mg/dL, ≥ 1+ proteinuria

Laboratory values that are outside of the normal range, but do not meet protocol defined criteria as adverse events will be evaluated by a clinician for clinical significance, recorded in the source document, and reported as laboratory AEs if clinically significant. Other laboratory parameters not specified in the protocol, but performed as part of the CBC and complete metabolic panel, need to be evaluated by a clinician, recorded in the source document, and reported as laboratory AEs if clinically significant.

Hematology	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
WBC 10^9/L (Decrease)	3.0-3.4	2.0-2.9	<2.0
WBC 10^9/L (Increase)	13.1-13.9	14.0-15.0	>15.0
Hgb g/dL (Decrease)	9.1-10.9	7.5-9.0	<7.5
Hgb g/dL (Increase)	18.1-18.5	18.6-19.0	>19.0
Platelets 10^9/L (Decrease)	100-130	50-99	<50
Platelets 10^9/L (Increase)	551-600	601-700	>700

Chemistry	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
BUN mg/dL	31-60	>60- and ≤70	>70
Creatinine umol/L[1] (Increase)	134-176	177-221	>221
AST IU/L (Increase)	70.1-200	201-300	>300
ALT IU/L (Increase)	70.1-200	201-300	>300

Urinalysis	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Protein	1+	2+	hospitalization or dialysis

[1]Conversion factor from mg/dL: http://onlinelibrary.wiley.com/doi/10.1002/97808138188825.app3/pdf
[2]Trace protein at screening (Visit 00B) is acceptable for inclusion into the study

9.2.4 Serious Adverse Events

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

- Death,
- a life-threatening adverse event*,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

* Life-threatening adverse event. An adverse event is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event, had it occurred in a more severe form, might have caused death.

All SAEs will be:

- recorded on the appropriate AE eCRF
- followed through resolution by a study clinician
- reviewed and evaluated by a study clinician

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

The site principal investigator or appropriate co-investigator is responsible for reporting all AE/SAEs that are observed or reported during the study, regardless of the relationship to study product. AE/SAEs, abnormal clinical laboratory test values, or abnormal clinical findings will

be documented, reported, and followed until levels return to baseline or until the abnormal value is determined to be not clinically significant.

9.3 Reporting Procedures

9.3.1 Serious Adverse Events

SAEs will be followed until resolution even if this extends beyond the study-reporting period. Resolution of an SAE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

Any AE that meets a protocol-defined serious criterion must be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group, at the following address:

DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20814, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Phone Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com

In addition to the SAE form, selected SAE data fields must also be entered into Emmes Advantage eClinical system. Please see the protocol-specific MOP for details regarding this procedure.

Other supporting documentation of the event may be requested by the DMID Pharmacovigilance Group and should be provided as soon as possible.

The site will notify the ISM when an SAE is provided to the DMID Pharmacovigilance Group. The DMID Medical Monitor and DMID Clinical Project Manager will be notified of the SAE by the DMID Pharmacovigilance Group. The DMID Medical Monitor will review and assess the SAE for regulatory reporting and potential impact on study subject safety and protocol conduct.

At any time after completion of the study, if the site principal investigator or appropriate sub-investigator becomes aware of an SAE that is suspected to be related to study product, the site principal investigator or appropriate sub-investigator will report the event to the DMID Pharmacovigilance Group.

9.3.2 Regulatory Reporting for Studies Conducted Under DMID-Sponsored IND

Following notification from the investigator, DMID, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. DMID will report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event. DMID will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. DMID will also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information. Relevant follow up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, DMID will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

All serious events designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

9.3.3 Reporting of Pregnancy

Pregnant women are not eligible to participate in the study. Women are counseled regarding prevention of pregnancy and encouraged to make every effort to avoid pregnancy during study participation. If a study subject becomes pregnant during study participation, no further doses of study agent will be given. The basic information about the pregnancy is recorded on the "Pregnancy" case report form via the Emmes Advantage eClinical system. If there are complications during the pregnancy, the complications are recorded as adverse events in the usual way. The subject is asked to report outcome of the pregnancy. If there is a congenital anomaly in the infant, this is recorded as a serious adverse event (SAE) in the data forms for the mother (i.e., the study subject). Efforts will be made to follow all pregnancies reported during the course of the study to pregnancy outcome pending the subject's permission.

9.4 Type and Duration of the Follow-up of Participants after Adverse Events

AEs and SAEs will be followed from the time of the first receipt of study drug Day 1 (Visit 01) through 28 days after receipt of study product.

AEs and SAEs will be followed until resolution even if this extends beyond the study-reporting period (3 months after the final study visit). Resolution of an AE/SAE is defined as the return to

pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

If the site principal investigator or appropriate sub-investigator becomes aware of a sign or symptom and the site principal investigator or appropriate sub-investigator decides to bring the subject in for an evaluation to determine etiology, then the site principal investigator or appropriate sub-investigator, at their own discretion, can determine what further testing is appropriate.

Follow-up procedures, evaluations, and outcomes will be recorded on the appropriate data collection form.

9.4.1. Halting Rules

Individual Halting Rules: Dosing for an individual subject will be discontinued if they develop any grade 3 drug-related event. The DSMB has requested to be notified of any individual subject who experiences a grade 3 drug-related event, however, the study will continue enrollment.

Study Halting Rules: Enrollment, dosing and study procedures will be halted for DSMB review/recommendation if any of the following are reported:

- Any death occurring after administration of study drug through the subject's last study visit that was not the result of trauma or accident, regardless of relatedness to study product.

- Any subject experiences a study drug-related SAE from the time of receipt of study drug through the subject's last study visit after relatedness is finalized by DMID.

- Occurrence of a severe allergic/hypersensitivity reaction (anaphylaxis) within 48 hours of dosing, requiring hemodynamic support with vasoactive medications or mechanical ventilation; the signs/symptoms will include any of the following: bronchospasm, dyspnea, wheezing, stridor, hypoxemia urticaria, angioedema, hives, and facial or oropharyngeal edema.

If any of the halting rules are met following any subject receipt of study drug, the study will not continue with the remaining enrollments without a review by and recommendation from the DSMB to proceed.

DMID retains the authority to suspend additional enrollment and study interventions/administration of study product during the entire study, as applicable.

9.4.2. Safety Oversight (DSMB)

9.4.2 Independent Safety Monitor (ISM)

The ISM is a physician with relevant expertise whose primary responsibility is to provide independent safety monitoring in a timely fashion. **For this study an ISM is not required**. However, at each site, and at the request of DMID, in real time, the local PI should be able to identify an independent physician to function as ad-hoc ISM. That person should have the privileges to examine the subject, review the subject medical and study record and provide an independent medical assessment and recommendation to DMID.

9.4.3 Data Safety Monitoring Board (DSMB)

Safety oversight will be conducted by a DSMB that is an independent group of experts that monitors subject safety and advises DMID. The DSMB members will be separate and independent of study personnel participating in this trial and should not have scientific, financial or other conflict of interest related to the study. The DSMB will consist of members with appropriate expertise to contribute to the interpretation of the data from this trial. The membership will include a chairperson and a statistician who are experienced in clinical trials conduct and have prior DSMB experience. The DSMB will review study progress and clinical and safety data at the following time points:

- Data review for safety at study specific time frames; at least annually.
- Data review for efficacy will be performed at the completion of enrollment. No interim analysis is planned.
- Study enrollment will be stopped for DSMB review of safety data when the study reaches 50% enrollment (i.e. 68 subjects enrolled). DSMB will review safety data after the 68th subject completed Visit 8.
- Final review meeting: 6 to 8 months after clinical database lock to review the cumulative unblinded safety and efficacy data for the study. The data will be provided in a standard summary format. The DSMB may be asked to provide recommendations in response to questions posed by DMID.
- Ad hoc review: may be in to an anticipated safety issue such as a halting rule being met.
- Additionally there will be ongoing DSMB notification of Grade 3 drug related events, after which the DSMB will then decide if an Ad Hoc DSMB meeting will be required

The DSMB will operate under the rules of a DMID-approved charter that will be written at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. Procedures for DSMB reviews/meetings will be defined in the charter. The DSMB will review applicable data to include, but not limited to, study progress and participant clinical and safety data which may include enrollment and demographic information,

medical history, concomitant medications, physical assessments, clinical laboratory values, dosing, AE/SAEs, protocol defined SAEs, and assays results. Additional data may be requested by the DSMB, and interim statistical reports may be generated as deemed necessary and appropriate by DMID. The DSMB may receive data in aggregate and presented by group. The DSMB will meet and review this data at scheduled time points or ad hoc as needed during the study as defined in the protocol and DSMB charter. As an outcome of each review/meeting, the DSMB will make a recommendation as to the advisability of proceeding with the study (as applicable), and to continue, modify, or terminate the study.

DMID or the DSMB chair may convene the DSMB on an ad hoc basis according to protocol criteria or if there are immediate concerns regarding observations during the course of the study. The DMID Medical Monitor is empowered to stop enrollment and dosing of the study drug if halting criteria are reported. The DMID Medical Monitor will be responsible for reviewing any protocol defined SAEs in real time. The DSMB will review SAEs on a regular basis and ad hoc during the study.

10. CLINICAL MONITORING STRUCTURE

10.1 Site Monitoring Plan

Site monitoring is conducted to ensure that the human subject protections, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet sponsor, ICH/GCP guidelines and applicable regulations, and that the study is conducted in accordance with the protocol, protocol-specific MOP and applicable sponsor standard operating procedures. DMID, the sponsoring agency, or its designee will conduct site-monitoring visits as detailed in the clinical monitoring plan.

Site visits will be made at standard intervals as defined by DMID and may be made more frequently as directed by DMID. Monitoring visits will include, but are not limited to, review of regulatory files, accountability records, eCRFs, informed consent forms, medical and laboratory reports, and protocol and GCP compliance. Site monitors will have access to the study site, study personnel, and all study documentation according to the DMID-approved site monitoring plan. Study monitors will meet with site principal investigators to discuss any problems and actions to be taken and document visit findings and discussions.

11. STATISTICAL CONSIDERATIONS

11.1 Study Hypotheses

This proposed clinical trial will test the hypothesis that oral auranofin is effective for the treatment of amebiasis and giardiasis, separately. Because this trial will re-profile an FDA-approved drug that is still in use, we propose a Phase IIa, randomized, single-blinded, placebo-controlled superiority clinical trial comparing auranofin (standard dose of 6 mg daily) for 7 days for treatment of amebiasis or 5 days for giardiasis to placebo in adults. The study will have two primary outcomes that will be tested on two different sets of 68 subjects (i.e. one set that has amebiasis and the other set has giardiasis). The amebiasis primary outcome will be the proportion of subjects with resolution of diarrhea (less than 3 loose stools/ 24 hours) by Day 7. The giardiasis primary outcome will be the proportion of subjects and resolution of diarrhea (less than 3 loose stools/24 hours) by Day 5. Additional secondary outcomes with *E. histolytica* include the proportion of subjects with positive EIA for *E. histolytica* and trophozoites on smear at enrollment with parasitological response of amebiasis (as determined by no detection of trophozoites of *E. histolytica* on microscopic exam day 3, 5, and 7; the rate of decrease of trophozoite/cyst load by qPCR in stools by Days 3, 5, and 7; the proportion of subjects with a negative stool antigen test by Days 3, 5, 7, and 14; the proportion of subjects with sustained cure (no detection of trophozoites by microscopic exam) at 14 and 28 days; the proportion of subjects with relapse (same strain) or re-infection (new strain) with positive stools at 14 and 28 days by genotyping the initial vs. subsequent strain. For patients with giardiasis, secondary outcomes include the proportion of subjects with positive EIA for *Giardia* and trophozoites on smear at enrollment with parasitological response (no detection of trophozoites on microscopic exam) by Days 3 and 5; the rate of decrease of trophozoite/cyst load by qPCR in stools by Days 3 and 5; the proportion of subjects with negative stool antigens by Days 3, 5, and 7; the proportion of subjects with sustained cure (no detection of trophozoites by microscopic exam) at 14 and 28 days; the proportion of subjects with relapse (same strain) or re-infection (new strain) with positive stools at Days 14 and 28 by genotyping the initial vs. subsequent strain.

11.2 Sample Size Considerations

The primary amebiasis outcome for this study is to compare the proportion of subjects with resolution of diarrhea (less than 3 loose stools/ 24 hours) by Day 7 of therapy, in which placebo is expected to have a clearance rate of approximately 15-20%. Sample size calculations for both primary endpoints are based on a two-sample, two-sided binomial test for proportions, with alpha set to 0.05. Calculations are performed using the R statistical package (Version 3.0.2; http://www.r-project.org). The primary amebiasis endpoint in this aim is to compare the resolution of diarrhea between the two study arms to demonstrate that auranofin increases

resolution of diarrhea. For this power analysis, we assume the following: a predicted diarrhea resolution rate of at least 80% (based on treatment results with current standard therapy with metronidazole, Pherson, 1984) in the auranofin treatment group.

With these assumptions, assuming 10% attrition (determined from the average of multiple other clinical trials in the same districts), a sample size of 68 with amebiasis (34 per arm; 60 completers in total) achieves greater than 95% power to detect a difference between the group proportions of 60%. The high statistical power for the primary endpoint is planned to also ensure adequate power for the secondary endpoints.

For the primary giardiasis endpoint of proportion of subjects with resolution of diarrhea by Day 5, a similar sample size of 34 per arm will achieve 85% power to detect a 40% difference between treatment arms with a predicted clearance rate of 70% (based on response rates to metronidazole or tinidazole, Gardiner, 2001) and a 30% clearance rates in placebo.

11.3 Planned Interim Analyses

There will be no planned interim analyses for efficacy or futility conducted for this study, but the DSMB may modify this during ongoing safety monitoring. The study team will review all adverse events by cumulative reports on a monthly basis. Adverse events will be graded using the protocol defined grading system. The DSMB, established by the study team, will monitor this study.

In the absence of any SAEs, the DSMB may review the safety data when the study reaches 50% enrollment (i.e. 34 subjects enrolled per parasite group). This review will include safety data after the 68th subject completes Visit 08.

11.3.1 Safety Review

Interim safety review may include enrollment and demographic information, medical history, concomitant medications, physical assessments, clinical laboratory values, dosing, and solicited and unsolicited AE/SAEs. Additional data may be requested by the DSMB, and interim statistical reports may be generated as deemed necessary and appropriate by DMID. The DSMB may receive data in aggregate and presented by group. The DSMB will meet and review this data at scheduled time points or ad hoc as needed during the study as defined in the DSMB charter. As an outcome of each review/meeting, the DSMB will make a recommendation as to the advisability of proceeding with study vaccinations (as applicable), and to continue, modify, or terminate the study.

Additionally, the study will be monitored to determine if any of the halting rules described in Section 9.5 are met.

11.4 Final Analysis Plan

Analysis Plan for Primary Outcomes. The primary amebiasis endpoint is resolution of diarrhea by Day 7. Parasite clearance rates between the placebo and auranofin arms for *Entamoeba* will be compared using a Fisher's exact test for proportions. Differences in the rates between the two groups, along with the odds ratio (OR) and their 95% confidence intervals will be reported. As a secondary analysis, multivariable logistic regression analysis will be performed to study the association between clearance rates and intervention arm, adjusting for baseline demographic, stratification variables, and clinical characteristics. Variables significantly associated with both treatment group and outcome ($p<0.10$) will be included in a multivariable logistic regression model as covariates. There will be no planned interim analyses for efficacy or futility conducted for this study, but the DSMB may modify this during ongoing safety monitoring.

Similarly, for the giardiasis primary endpoint, methods analogous to the amebiasis primary endpoint will be used to determine the resolution of diarrhea by Day 5.

Analysis Plan for Secondary Outcomes. The secondary outcome measures are: *Entamoeba*: Proportion of subjects with positive EIA for *E. histolytica* and positive smear for trophozoites on enrollment with parasitological response (no detection of trophozoites on microscopic exam) by Day 7; proportion of subjects with positive EIA for *E. histolytica* and positive smear for trophozoites on enrollment with parasitological response (no detection of trophozoites on microscopic exam or negative antigen detection) by Day 3 and 5; rate of decrease of trophozoite/cyst load by qPCR in stools by Days 3, 5 and 7; proportion of subjects with negative stool antigen test by Days 3, 5, 7, and 14; proportion of subjects with sustained cure (no detection of trophozoites by microscopic exam) at 14 and 28 days; and the proportion of subjects with relapse (same strain) or re-infection (new strain) with positive stools at Days 14 and 28 by genotyping the initial vs. subsequent strain, and time to resolution of diarrhea.

Giardia: Proportion of subjects with positive EIA for *Giardia* and positive smear for trophozoites on enrollment with parasitological response (no detection of trophozoites on microscopic exam) by Day 3 and 5; rate of decrease of trophozoite/cyst load by qPCR in stools by Days 3 and 5; proportion of subjects with negative stool antigens by days 3 and ; proportion of subjects with sustained cure (no detection of trophozoites by microscopic exam) at 14 and 28 days; the proportion of subjects with relapse (same strain) or re-infection (new strain) with positive stools at Days 14 and 28 by genotyping the initial vs. subsequent strain and time to resolution of diarrhea.

Methods analogous to the analysis of the primary outcomes, such as Fisher's exact test, will be applied for categorical secondary outcomes (proportion of trophozoite/cyst load in stools from enrollment by quantitative PCR at days 3, 5, and 7; and percentage of subjects who have relapsed at 14 and at 28 days). For continuous outcomes, such as count data, t-tests or equivalent non-parametric alternatives will be considered. No adjustments will be made for multiple testing of secondary outcomes. Longitudinal modeling, via mixed effects models, will be considered to compare the rate of change in loads between the two study arms. Appropriate treatment by time interaction terms will be included. Kaplan-Meier curves and log rank tests will be used to compare time to diarrhea resolution between the treatment and control arm for each disease group.

In general, analyses will incorporate the modified intent-to-treat (mITT) principle. Participants who are "randomized and receive at least one dose of study medication" will be included in the mITT population. Subjects who terminate their treatment regimen and receive metronidazole due to worsening symptoms will be imputed as treatment failures for the primary outcome. That is, they will be imputed as still symptomatic by Day 7 in the amebiasis group, and by Day 5 in the giardiasis group. Secondary outcomes measured at Days 3, 5, and 7 will be imputed analogously, with the exception of the quantitative trophozoite/cyst load. Multiple imputation may be used to adjust for missing data in the primary and secondary analyses on the mITT population. All results will be reported as point estimates (odds ratios or mean differences across groups, as appropriate) and interval estimates (95% confidence intervals). All tests of significance for the secondary outcomes will be 2-sided and no adjustments will be made for multiple comparisons. A p-value of 0.05 will be considered statistically significant. The Statistical Analysis Plan (SAP) will provide further details. In case the language in this section differs from the language in the SAP, the SAP takes precedence.

In the event that recruitment and follow-up for either the Giardiasis arm or the Amebiasis arm is completed ahead of the other, the primary and secondary endpoints may be analyzed for that arm using the specified methods. This report will be considered the interim CSR and will contain detailed unblinded information about the participants, their immunological response to treatment as well as their side effects and any laboratory abnormalities. The interim CSR will be followed with a release of data corresponding to the report. In order to maintain the blind and allow appropriate follow up of the remaining arm, no endpoint data for the remaining arm will be included in the interim CSR and no data related to that arm will be released. An amendment to the interim CSR will provide primary and secondary analysis of the remaining arm when all primary and secondary endpoint data are available for the second arm. The official reports will be drafted by Emmes and reviewed by the PI and DMID prior to finalization. Additional information regarding the methods will be provided in the study SAP.

The purpose of this study is to assess the efficacy of auranofin (Ridaura) for the treatment of amebiasis and giardiasis and the timely release of this information, once available, will support improved clinical care. It is anticipated that the results of this study will be presented to the

scientific community via oral presentations at meetings and written publications in scientific journals following finalization of the interim CSR. The data to be presented and the authorship will be discussed between partners before any official communication.

12 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

The site will maintain appropriate medical and research records for this trial, in compliance with ICH E6, Section 4.9, and regulatory and institutional requirements for the protection of confidentiality of subjects. The site will permit authorized representatives of the DMID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical study records for the purposes of quality assurance reviews, audits, monitoring and evaluation of the study safety and progress. These representatives will be permitted access to all source data, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial. Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Data collection forms will be derived from the eCRFs and be provided by the SDCC.

13 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site clinical quality management plan (CQMP), the investigational site is responsible for conducting routine quality assurance and quality control activities to internally monitor study progress and protocol compliance.

The PI will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The PI will ensure all study personnel are appropriately trained and applicable documentation is maintained on site. Staff Training will include Institution-specific Training, Protocol-specific Training, and DMID-specific training.

DMID-designated clinical monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, ICH/GCP guidelines, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to DMID.

The Statistical and Data Coordinating Center will implement quality control procedures beginning with the data entry system and generate data quality control checks that will be run on the database. Any missing data or data anomalies will be communicated to the site(s) for clarification and resolution.

14 ETHICS/PROTECTION OF HUMAN SUBJECTS

14.1 Ethical Standard

The principal investigator will ensure that this trial is conducted in full conformity with principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR 46, 21 CFR 50 and 56, and ICH E6; 62 Federal Regulations 25691 (1997), if applicable. The site principal investigator's Institution will hold a current Federal Wide Assurance (FWA) issued by the Office of Human Research Protection (OHRP) for federally funded research.

14.2 Institutional Review Board/Ethics Committee

UCSD is the lead institution in this project and where the PI is located. Quantitative PCR testing of positive stool specimens will be performed at UCSD. Although no direct human subjects research will occur at this institution, UCSD will be responsible for all regulatory matters in conjunction with our collaborators. This includes coordination of all IRB approvals, quality assurance, data safety and monitoring and protocol development. The International Centre for Diarrhoeal Research, Bangladesh (icddr,b) and Rajshahi Medical College Hospital and Clinic are the sites where human subjects research procedures will be performed, with the option for home visits also conducted as indicated. This includes screening, recruiting, and collecting all data/samples and following up subjects as detailed in Section 7. The icddr,b Ethics Committee will also review and approve study protocols which is also is used for approval of joint Rajshahi Medical College protocol. Dr. Petri's lab at the University of Virginia will perform off site molecular testing of specific strains of *Giardia* or *E.* histolytica to identify relapse or re-infection.

14.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the subjects and their families. Consent forms describing in detail the Study Agent, auranofin, and risks are given to the subject and written documentation of informed consent is required prior to starting study agent/intervention. Consent forms will be IRB approved and the subject will be asked to read and review the document. Upon reviewing the document, the investigator 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 any procedures being done specifically for the study. The subjects should have sufficient opportunity to discuss the study and process the information in

the consent process prior to agreeing to participate. The subjects may withdraw consent at any time throughout the course of the trial. 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 decline to participate in this study.

The informed consent procedure is designed to maximize the potential subject's comprehension of study procedures and to ensure that participation is voluntary. Before a subject is enrolled in the study, the purpose, the procedures to be followed, and the risks and benefits of participation will be explained by the clinic staff, and signed informed consent from the study subject will be obtained. For those subjects unable to read or sign the consent documents, a literate witness will be present to sign on behalf of the subject after the subject has given oral consent. In addition to the signature of a literate witness, agreement of subjects who are illiterate will be indicated by including his/her thumbprint on the consent form, in accordance with the WHO guidelines on the consent process for illiterate individuals (http://www.who.int/rpc/research_ethics/Process_seeking_IF_ printing.pdf).

Clinic staff will be trained to respond to the subject's questions and concerns. Details of study participation, including the need for follow-up contact, will be described in both the consent form and explained verbally. The research staff who will be responsible for obtaining informed consent will assess whether the potential subject has understood critical aspects of the study and consent form by asking key questions (e.g., "How much time will this take you?"; "What are the possible risks to you?"). Errors will be corrected and these potential subjects will then be asked if they need further clarification. If, after further attempts to clarify any misunderstandings, we determine that they may not fully comprehend the critical aspects of the study, they will not be enrolled. If a potential subject decides he/she does not wish to participate, his/her decision will be honored regardless of how well they comprehend the study information. The subject may refuse to participate or withdraw at any time without jeopardy to the medical care they regularly receive. The investigator will explain to the subject that only parasite DNA samples will be collected and analyzed and that no human genetic material will be collected.

A copy of the consent form, which includes a description of the study, will be provided to all subjects, which includes contact information of the main investigators in Dhaka and Rajshahi Bangladesh, and the U.S. Consent forms will be written in simple language at a fourth-grade reading level, and will be translated into Bengali. In the case of subjects with low or no literacy, the methods described above of: 1) Verbally reviewing the consent form; and 2) Giving a simple test about points made in each paragraph of the consent, should open enrollment to all regardless of literacy level. Participants will be encouraged to ask questions and retested if they do not fully understand any of the study protocols. Prior to study implementation we will receive approval from the UCSD Institutional Review Board (IRB) Committee on Human Research, the icddr,b

Ethical Review Committee in Bangladesh and the UVa Institutional Review Board (IRB) for Health Sciences Research.

Participation in this study is completely voluntary, and the alternative to study participation is not to participate. Participants may refuse to participate or withdraw at any time without jeopardy to the medical care they regularly receive. Treatment for diarrhea is available at health clinics in Bangladesh. If a subject chooses not to participate in this study or is deemed ineligible to participate, he/she may ask the study staff for a referral to their nearest clinic. Whether they join this study or not, subjects may also ask the study staff for information about infectious diseases, such as *Giardia* and *E. histolytica*.

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

This study will be inclusive of all adults who meet the inclusion/exclusion criteria, regardless of religion, sex, or ethnic background. Women who are pregnant or breastfeeding, women of childbearing potential who do not agree to practice effective contraception, and children are excluded for safety reasons. Should the outcome of this study be deemed acceptable, additional trials may be initiated in other populations.

14.5 Subject Confidentiality

Subject confidentiality is strictly held 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.

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.

To guard confidentiality, only the subject's identification number will appear on screening forms, questionnaires, databases and biological samples. The key to subjects' identification numbers will be encrypted in a computer file, which will be locked in the field coordinator's office. Only the site PI and field coordinator will be able to un-encrypt the computer file. Contact information for

the subject so that s/he can be located at follow-up visits will be stored in locked file cabinets in locked offices at the clinic, and will be destroyed once follow-up is completed.

14.6 Study Discontinuation

If the trial is discontinued, subjects who had previously signed the informed consent form, and were randomized and treated will continue to be followed for safety assessments. No further study treatments will be administered.

14.7 Future Use of Stored Specimens

At the time of informed consent, the Investigator will seek permission from subjects for use of their remaining sample for possible future research studies, investigating parasitic disease, such as the effect of parasitic infections and/or auranofin on the stool microbiome. All samples will be stored in a freezer at the icddr,b or Rajshahi Medical College Hospital for 3-4 days maximum, except for those that test positive for Giardia or Entamoeba. Samples from Rajshahi Medical College Hospital will be sent to icddr,b first , then to UCSD/UVA. These will be stored in the icddr, b freezer for the entire duration of the study, after which they will be stored indefinitely at the Fisher Repository Samples and may be shared with other investigators at other institutions. The samples will not be sold or used directly for production of any commercial product. No human genetic material will be collected or stored and no human genetic tests will be performed on any samples. Each sample will be de-identified and encoded (labeled) only with a barcode and a unique tracking number to protect subject's confidentiality.

There are no benefits to subjects in the collection, storage and subsequent research use of specimens. Reports about future research done with subject's samples will NOT be kept in their health records.

Subjects may be given the option to decide if they want their samples to be used for future research or have their samples destroyed at the end of the trial. The subject's decision can be changed at any time prior to the end of the trial by notifying the study doctors or nurses in writing. However, if the subject originally consents to future use and subsequently changes his/her decision, any data from a previously collected sample may still be used for this research.

15 DATA HANDLING AND RECORD KEEPING

The investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

Copies of the electronic CRF (eCRF) will be provided for use as source documents and maintained for recording data for each subject enrolled in the study. Data reported in the eCRF derived from source documents should be consistent with the source documents or the discrepancies should be explained

DMID and/or it designee will provide guidance to investigators on making corrections to the source documents and eCRF.

15.1 Data Management Responsibilities

All data collection forms and laboratory reports must be reviewed by the clinical team and data entry personnel, who will ensure that they are accurate and complete. Adverse events must be recorded on the appropriate data collection form, assessed for severity and relationship, and reviewed by the site principal investigator or appropriate sub-investigator.

Data collection is the responsibility of the study personnel at the participating sites under the supervision of the respective site principal investigator. During the study, the site principal investigator must maintain complete and accurate documentation for the study.

EMMES, The SDCC for this study will be responsible for data management, quality review, analysis, and reporting of the study data.

15.2 Data Capture Methods

Clinical (including, but not limited to, AE/SAEs, concomitant medications, medical history, physical assessments, and clinical laboratory values), data will be entered into a 21 CFR 11-compliant Internet Data Entry System provided by the SDCC. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the data collection forms completed by the study personnel.

15.3 Types of Data

Data for this study will include safety, laboratory, and outcome measures.

15.4 Timing/Reports

The study team will review all adverse events by cumulative reports of the treatment arms on a monthly basis.

Asymptomatic subjects (34) enrolled under Version 1-4 of the protocol will have their data reviewed and clinical database locked for analysis first and a separate report generated.

A final report will then be prepared following the availability of all the safety and outcome data for one study arm with symptomatic patients (Giardiasis or Amebiasis). An amendment to the final report will be produced following the availability of all safety and outcome data for the remaining study arm. Interim statistical reports may be generated as deemed necessary and appropriate by DMID. Safety and immunogenicity summary reports may be generated for the DSMB.

After full analysis and final reporting is complete, and upon request and DMID approval, the SDCC will provide the site with a summary of results by treatment group and/or subject treatment assignments. In this regard, the participating site requesting such information to share with study subjects must do so in compliance with their respective IRB guidelines.

15.5 Study Records Retention

Study records and reports, including, but not limited to, case report forms (CRFs), source documents, informed consent forms (except for future use informed consent forms), laboratory test results, and medication inventory records, shall be retained for 2 years after a marketing application is approved for the drug; or, if an application is not approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and FDA has been so notified. The site must contact DMID for authorization prior to the destruction of any study records. Informed consent forms for future use will be maintained as long as the sample exists.

15.6 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly. The site will use continuous vigilance

to identify and report deviations according to the guidelines of the IND sponsor, if applicable. Protocol deviations will be sent to the local IRB/IEC per their guidelines.

These practices are consistent with ICH E6:

4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, Section 5.1.1

5.20 Noncompliance, Sections 5.20.1, and 5.20.2

It is the responsibility of the site principal investigator and other study personnel to use continuous vigilance to identify and report deviations within five working days of identification of the protocol deviation, or within five working days of the scheduled protocol-required activity. All deviations must be promptly reported to DMID, via the SDCC's Emmes Advantage eClinical system.

All protocol deviations, as defined above, must be addressed in study subject data collection forms. A completed copy of the DMID Protocol Deviation Form must be maintained in the Regulatory File, as well as in the subject's chart. Protocol deviations must be sent to the local IRB/IEC per its guidelines. The site principal investigator and other study personnel are responsible for knowing and adhering to their IRB requirements.

16 PUBLICATION POLICY

All investigators funded by the NIH must submit or have submitted for them to the National Library of Medicine's PubMed Central (http://www.ncbi.nlm.nih.gov/pmc/) an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

Refer to:

- NIH Public Access Policy, http://publicaccess.nih.gov/
- NIH OER Grants and Funding, http://grants.nih.gov/grants/oer.htm

Following completion of the study, the lead principal investigator is expected to 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* (http://clinicaltrials.gov/), which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies.

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 I trials), would be exempt from this policy.

In the event that either the *Giardia* or *E. histolytica* portion of the trial is completed earlier than the other cohort, data from the completed cohort may be released after the database from that portion of the trial is locked.

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 on or before subject 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.

For trials in which DMID is not the IND/IDE sponsor, or there is no IND/IDE, and DMID does not provide data management services, it is the responsibility of the investigator to register the

trial and post results in compliance with Public Law 110-85, the Food and Drug Administration Amendments Act of 2007 (FDAAA).

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APPENDIX A: SCHEDULE OF EVENTS

Procedure	Visit* Number	00A	00B	01	02	03	04	05	06[2]	07	08	09	10[2]
	Study Day	**-4**	**-4**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**14**	**28**	**30**
	Window (days)	**-4 to1**	**-4 to -1**	**+1[6]---**	**---**	**--+1-**	**---**	**+1[6]---**	**---**	**+1**	**+/-3**	**+/-3**	**+/-3**
Review Eligibility		X		X									
Informed Consent		X											
Demographic Data		X											
Medical History			X	X	X	X	X	X	X	X	X	X	
Targeted Physical Exam[3]			X	X	X	X	X	X	X	X	X	X	
Vital signs[4]			X	X									
Concomitant Medications			X	X	X	X	X	X	X	X	X	X	
Randomization/ Enrollment				X									
Assessment for side effects of treatment					X	X	X	X	X	X	X	X	
AEs/SAEs				X	X	X	X	X	X	X	X	X	
Urine for pregnancy testing			X										
Treatment (giardiasis subjects)				X	X	X	X	X					

Treatment (E. histolytica)				X	X	X	X	X	X	X			
Procedure	**Visit Number**	**00A**	**00B**	**01**	**02**	**03**	**04**	**05**	**06[3]**	**07**	**08**	**09**	**10[4]**
	Study Day	**-4**	**-3**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**14**	**28**	**30**
	Window (days)	**-4 to 1**	**-3 to -1**	**+1[8]---**	**---**	**--+1[8]-**	**---**	**+1[8]---**	**---**	**+1**	**+/-3**	**+/-3**	**+/-3**
Urine Dipstick for Proteinuria			X							X			
Complete Blood Count[5]			X							X			
BUN			X							X			
Creatinine			X							X			
ALT			X							X			
AST			X							X			
Stool specimen[6]		X		X		X		X		X	X	X	
Blood volume			17							17			
Blood volume totals			17							34			
Standard of Care Treatment													X

*Subjects will be enrolled at the icddr,b or Rajshahi Hospitals and have follow-up visits until their diarrhea has resolved. Subsequent follow-up visits may be held at the icddr,b or Rajshahi Hospital, or local (to Rajshahi) clinic or their home except for Visit 07 which must be performed at icddr,b or Rajshahi Hospital or Clinic because blood drawing is required.

[1]Visit 06 will only be conducted for subjects receiving treatment for amebiasis.

[2]Visit 10 will only be conducted for subjects who remain positive for amebiasis or giardiasis at Day 28 and have not previously received standard of care treatment.

[3]Targeted physical exam may also include observations for rashes, hair loss, urticaria or glossitis, and an abdominal exam.

[4]Vital signs (oral temperature, pulse, blood pressure)

[5]Complete blood count includes white blood cells, hemoglobin, and platelet count.

[6]Window of + 1 day for stool specimens after (and including) Day 1. Date of collection will be noted. The window does not apply to treatment administration or other required assessments.

[7]Amebiasis patients only

APPENDIX B:LAB PROCESSING FLOW SHEET: CLINICAL/ SAFETY LAB TESTS

Protocol # 15-0015

Instructions- All specimens must be recorded on a form similar to the one below including the visit time/study ID number, tube type, specimen test, processing and storage instructions

Visit/Time of Draw	Tube Type/Quantity	Test/Assay/Derivative	Processing/Aliquot/Storage/Shipping
Screening Day -4 to -1, Visit 00A	Stool collection vial for Stool Evaluation	Rapid Stool EIA for *Giardia* or *E. histolytica* EIA stool antigen test if positive Wet mount Concentration and Trichrome staining Qualitative PCR for Giardia and *Entamoeba*	Stools kept cold and shipped daily to icddr,b or Rajshahi hospital- See Lab SOPs
Screening Day -4 to -1, Visit 00B	Urine collection vial	Dip stick for protein Pregnancy testing	Performed in icddr,b or Rajshahi Hospital or designated lab,
	Purple top tube (7mls)	Complete blood count	Kept at room temp and transferred same day to lab for analysis
	Serum separator tube (10 ml)	BUN, creatinine, SGOT, SGPT	Kept at room temp and transferred same day to lab for analysis Left over serum aliquoted and kept at -80ºC
Enrollment Day 1, Visit 01	Stool collection vial for Full Stool Evaluation (+ 1 Day)	Antigen testing for Giardia or *E. histolytica* Wet mount Concentration and Trichrome staining Qualitative PCR for Giardia and *Entamoeba* Quantitative PCR for Giardia or Entamoeba in positive stools	Specimens processed as above Processed as above Fixed stool stored at room temp Processed as above Extracted DNA from stool obtained before first auranofin dose. Shipped every 1-3months to UCSD for quantitative PCR
Day 3, Visit 03	Stool collection vial for Full Stool Evaluation (+1 day)	Refer to Day 1 (Not performed on patients who receive metronidazole prior to completing dosing regimen)	Refer to Day 1
Day 5, Visit 05	Stool collection vial for Full Stool Evaluation (+ 1 day)	Refer to Day 1 (Not performed on patients who receive metronidazole prior	Refer to Day 1

		to completing dosing regimen)	
Day 7, Visit 07	Stool collection vial for Full Stool Evaluation (+1 Day)	Refer to Day 1 (Not performed on patients who receive metronidazole prior to completing dosing regimen)	Refer to Day 1
	Urine Collection Vial	Urine Dip stick for protein	Refer to Screening Visit 00B
	Purple top tube (7mls)	Complete blood count and differential	Refer to Screening Visit 00B
	SST (10 ml)	BUN, Cr, SGOT, SGPT,	Refer to Screening Visit 00B
Day 14, Visit 08 +/- 3 days	Stool collection vial for Full Stool Evaluation	Refer to Day 1 If positive at Day 14, perform parasite genotyping. (Not performed on patients who receive metronidazole prior to completing dosing regimen)	Refer to Day 1 Stored DNA sent to UVA for parasite genotyping every 1-3 months
Day 28, Visit 09 +/- 3 days	Stool collection vial for Full Stool Evaluation	Refer to Day 1 If positive at Day 14, perform parasite genotyping. (Not performed on patients who receive metronidazole prior to completing dosing regimen)	Refer to Day 1 Stored DNA sent to UVA for parasite genotyping every 1-3 months

APPENDIX C: ICDDR,B NORMAL LABORATORY VALUES (APPLICABLE FOR BOTH SITES)

	Male	Female
White Blood cells (10^9/L)	4.0 –11.0	same
Platelets (10^9/L)	150.0 – 450.0	same
Hemoglobin (gm/dL)	12.5 –17.5	11.5 – 16.5
Blood urea nitrogen (mg/dL)	5 –24	same
Creatinine (umol/L)	53-106	44-97
AST/SGOT (U/L)	0.01 – 38.0	0.01 – 32.0
ALT/SGPT (U/L)	0.01 – 41.0	0.01 – 31.0