Histoplasmosis Induction and Consolidation Therapy Factorial Randomized Clinical Trial (Histo-FACT)

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STATEMENT OF ASSURANCE

Each Institution will hold a current Federal Wide Assurance (FWA) issued by the Office of Human Research Protections (OHRP) for federally-funded human subjects research. Each FWA will designate at least one Institutional Review Board (IRB)/Independent Ethics Committee (IEC) registered with OHRP, for which the research will be reviewed and approved by the IRB/IEC and will be subject to continuing review [45 CFR 46.103(b)]. The IRB/IEC designated under an FWA may include an institution's IRB/IEC, an independent IRB/IEC, or an IRB/IEC of another institution after establishing a written agreement with that other institution.

STATEMENT OF COMPLIANCE

The study trial will be carried out in accordance with Good Clinical Practice (GCP) and as required by the following:

- United States Code of Federal Regulations (CFR) 45 CFR Part 46: Protection of Human Subjects
- Food and Drug Administration (FDA) Regulations, as applicable: 21 CFR Part 50 (Protection of Human Subjects), 21 CFR Part 54 (Financial Disclosure by Clinical Investigators), 21 CFR Part 56 (Institutional Review Boards), 21 CFR Part 11, and 21 CFR Part 312 (Investigational New Drug Application), 21 CFR 812 (Investigational Device Exemptions)
- International Conference on Harmonisation: Good Clinical Practice (ICH E6); 62 Federal Register 25691 (1997); and future revisions
- Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, Report of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research
- National Institutes of Health (NIH) Office of Extramural Research, Research Involving Human Subjects, as applicable
- National Institute of Allergy and Infectious Diseases (NIAID) Clinical Terms of Award, as applicable
- Applicable Federal, State, and Local Regulations and Guidance

SIGNATURE PAGE

The signature below provides the necessary assurance that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH E6 Good Clinical Practice (GCP) guidelines.

I agree to conduct the study in compliance with GCP and applicable regulatory requirements.

I agree to conduct the study in accordance with the current protocol and will not make changes to the protocol without obtaining the sponsor's approval and IRB/IEC approval, except when necessary to protect the safety, rights, or welfare of subjects.

Site Investigator Signature:*

Signed: _____ Date: _____

Name

Title

*** The protocol should be signed by the local investigator who is responsible for the study implementation at his/her specific site; i.e., if it is an Investigational New Drug study, the individual who signs the Form FDA 1572 or, if it is a non-IND study, the individual who signs the Investigator of Record form.*

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ART	Antiretroviral Therapy
BUN	Blood Urea Nitrogen
CFR	Code of Federal Regulations
CI	Confidence Interval
CRF	Case Report Form
D	Day
DCC	Data Coordinating Center
DAIDS	Division of AIDS, NIAID, NIH, DHHS
DMID	Division of Microbiology and Infectious Diseases, NIAID, NIH, DHHS
DSMB	Data and Safety Monitoring Board
eCRF	Electronic Case Report Form
EORTC	European Organization for Research and Treatment of Cancer
FDA	Food and Drug Administration
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
HISTO-FACT	Histoplasmosis Induction and Consolidation Therapy Factorial Randomized Clinical Trial
HIV	Human Immunodeficiency Virus
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
IRIS	Immune Reconstitution Inflammatory Syndrome
IV	Intravenous
KDIGO	Kidney Disease Improving Global Outcomes
KUMC	University of Kansas Medical Center
LAmB	Liposomal Amphotericin B
LAR	Legally Authorized Representative
MIC	Mean Inhibitory Concentration
MOP	Manual of Procedures
MSGERC	Mycoses Study Group Education and Research Consortium

N	Number (typically refers to subjects)
NDC	National Drug Code
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
OHRP	Office for Human Research Protections
PCR	Polymerase Chain Reaction
PEG	Polyethylene Glycol
PK	Pharmacokinetics
PWH	People with HIV
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SAP	Statistical Analysis Plan
SOP	Standard Operating Procedure
SOC	Standard of Care
TDM	Therapeutic Drug Monitoring
UFCSPA	Universidade Federal de Ciencias da Saude de Porto Alegre
ULN	Upper Limit of Normal
UMN	University of Minnesota
US	United States
USP	United States Pharmacopeia
W	Week
WBC	White Blood Cell
WHO	World Health Organization

PROTOCOL SUMMARY

Title:	Histoplasmosis Induction and Consolidation Therapy Factorial Randomized Clinical Trial (HISTO-FACT)
Design of the Study:	A randomized, open label factorial design trial evaluating novel therapies for induction and consolidation in histoplasmosis.
Study Phase:	*3*
Study Population:	664 adult participants (≥18 years old) with histoplasmosis (confirmed or probable per European Organization for Research and Treatment of Cancer (EORTC)/Mycoses Study Group Education and Research Consortium (MSGERC) research definitions) with or without Human Immunodeficiency Virus (HIV). Approximately, 40 will be enrolled in the U.S. at the University of Kansas Medical Center (KUMC) and the remainder at 7 sites throughout Brazil. Distribution of biological sex is expected to be roughly 75% male based on prior enrollment in our phase 2 study and prior case distribution at sites not involved with our phase 2 study.[1]
Number of Sites:	8 (n=7 in Brazil, n=1 U.S.) Any additional sites will be added via notification to regulatory authorities.
Description of Study Product or Intervention:	● Aim 1: Liposomal amphotericin B (LAmB) single dose (10 mg/kg), intravenous vs standard of care (SOC) ● Aim 2: Oral posaconazole delayed-release tabs 300mg twice daily on day one then once daily versus SOC

- Aim 3 (separate consent at 26 weeks) in people with HIV on antiretroviral therapy (ART) with good HIV control only: completion of consolidation at the time of randomization versus SOC

Timing of Primary Endpoint

Aim 1 Induction Therapy: 2 weeks (10 week total study period)

Aim 2 Consolidation Therapy: 26 weeks
Aim 3 Duration of Consolidation Therapy: 52 weeks

Endpoints will be assessed for these study periods unless otherwise noted.

Study Endpoints:

Aims 1 and 2:

Aims 1 & 2

Primary Endpoint:

- Mortality (2-week for Aim 1, 26-week for Aim 2).

Key Secondary Endpoint

- Hierarchical composite end point. consisting of the following in a hierarchical order:

  5. Death within the study period (Aims 1 and 2) or lost to follow up in the first 1 week (Aim 1 only).

  4. Serious Adverse Event (SAE) in the study period.

  3. Grade 4 laboratory abnormality at two week visit (Aim 1) or through Week 26 visit (Aim 2) or discontinuation of medication due to intolerance during the study period (Aims 1 and 2).

  2. Grade 3 laboratory abnormality at Week 2 visit (Aim 1) or through Week 26 visit (Aim 2) or lost to follow up after the first week (Aim 2 only).

  1. Alive at the end of the study period without one of the above events.

Other Secondary Endpoints:

- Additional LAmB requirement by end of study period – need for 'rescue', re-initiation or extension of intravenous (IV) LAmB beyond the planned duration
- Discontinuation of study medication due to side effects or suspected failure (measured separately and in total) by end of study period

Safety and Other Endpoints:

- Laboratory events: mean percentage change in hemoglobin, kidney function, liver function at 2 week visit (Aim 1 only), grade 2, 3, 4 liver function test abnormalities (Aim 1 at 2 week visit, Aim 2 at 26 week visit)
- Grade 3-5 clinical Adverse Events (AE) (Aim 1 through 10 weeks, Aim 2 through 26 weeks)
- Clinical Response at week 10 visit defined as resolution of fever and signs/symptoms attributable to histoplasmosis, except for manifestations expected to last 10 weeks such as some skin lesions and hepatosplenomegaly .
- Rehospitalization through the study period
- Duration of hospitalization (Aim 1): The duration of the initial hospitalization will be recorded. The initial analysis will include all enrolled participants and for those who die in the hospital, the day of death will be the last day of hospitalization. A sensitivity analysis will exclude those who died in the hospital (e.g., what was the duration of hospitalization for those who survived to discharge).
- Paradoxical Immune Reconstitution Inflammatory Syndrome (IRIS) incidence through 10 weeks
- Mean change in *Histoplasma* urine antigen concentration at 2 week visit (Aim 1), 10 weeks visit (Aims 1 and 2), 26 week visit (Aim 2)

	● Blood PCR change at 2 week visit (Aim 1), 4 week visit (Aims 1 and 2)
Study Endpoints: **Aim 3**	Primary: ● SAE-free survival between 26 and 52 weeks from induction therapy among those who survived 26 weeks Secondary: ● Discontinuation of study medication due to side effects or suspected failure (measured separately and in total) for the SOC arm at 52 weeks. Other: ● Grade 3-5 clinical AEs, SAEs between 26 and 52 weeks ● Mean decrease urine antigen concentration at 52 week visit ● Lost to follow up between 26 and 52 weeks
Duration of Individual Subject Participation:	56 weeks.
Interim Analyses	Data Safety Monitoring Board (DSMB) will meet at least annually to review interim data on safety. In the event that one randomization closes, the other randomizations would continue with future participants receiving the best available care. For example, if induction therapy is proven non-inferior, the induction therapy would close to further randomization, but the consolidation therapy randomization would continue.
Estimated Time to Last Subject/Last Study Day:	6 years.

Table 1: Treatment Arms by Randomization/Treatment Stage

Factorial Randomization 1 - Induction	Single high-dose (10mg/kg) liposomal amphotericin B	332 (n=>50 from ongoing trial)	Intervention
	Daily liposomal amphotericin B (3mg/kg)	332 (n=>50 from ongoing trial)	Standard of care

Factorial Randomization 2 - Consolidation	Posaconazole	282	Intervention arm
	Itraconazole	282	Standard of care

Randomization 2 – Duration of Consolidation *	26 weeks total consolidation	234	Intervention
	52 weeks total consolidation	234	Standard of care

*Only people with HIV in Brazil, separate consent at Week 26

Figure 1: Study Schematic and Randomization Schedule

End of therapy, week 52. Last study visit week 56.

1 KEY ROLES

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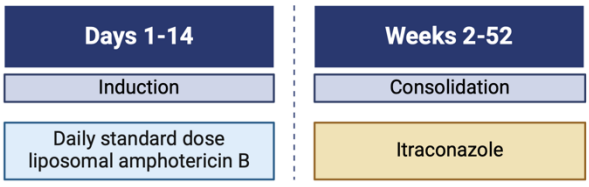
2 BACKGROUND AND SCIENTIFIC RATIONALE

2.1 Background

Investigational products are all FDA approved for other fungal infections and include 1) Liposomal amphotericin B, 2) posaconazole.

The dimorphic fungus *Histoplasma capsulatum* causes histoplasmosis in humans via inhalation and subsequent immune evasion. Histoplasmosis is the most common opportunistic infection (tied with tuberculosis) in Central and South America in people with HIV (PWH).[2] The distribution of histoplasmosis is now recognized to be nearly worldwide although with higher risk in some areas.[3] In the U.S. alone, >500,000 cases are estimated to occur per year.[4] Histoplasmosis can range in its effects from asymptomatic or mildly symptomatic pulmonary infection to uniformly fatal without treatment in its disseminated form in PWH.[4] Moderate to severe histoplasmosis is treated with 14 days of liposomal amphotericin B (3mg/kg daily) induction therapy followed by at least a year of itraconazole consolidation therapy.[4] (**Figure 2**) Yet, these therapies cause frequent toxicities and even with prompt treatment, two-week mortality is still ~10-20% and six-month mortality, 20-30%.[1] These mortality numbers are the best case scenarios as part of clinical trials. In routine care, mortality rates as high as 71% have been cited for HIV-associated disseminated histoplasmosis.[5-7]

Figure 2: Current Standard of Care Therapy for Moderate to Severe Histoplasmosis



Until 2023, no clinical trial of histoplasmosis had been published since 2002 .[8] The 2002 study enrolled 81 participants with moderate-severe disseminated histoplasmosis and HIV to evaluate the safety and efficacy of liposomal amphotericin B compared to amphotericin deoxycholate for induction therapy. In 2023 we published a larger trial evaluating single high-dose liposomal amphotericin B-based induction therapy compared to 14 days of standard dose liposomal amphotericin B for disseminated histoplasmosis among 118 PWH despite having been enrolled during the worst of the COVID-19 pandemic from February 2020 to April 2022.[1] This study showed similar safety and 2-week mortality but requires a large phase III study for confirmation.[1]. Notably, Kidney Disease Improving Global Outcomes (KDIGO) stage 3 kidney toxicity occurred in 30% of participants in the standard of care arm and only 12% of those who received single high-dose liposomal amphotericin.[1, 9]

Although itraconazole is the standard of care for consolidation, side effects are common and, drug-drug interactions are numerous and therapeutic drug monitoring (TDM) is necessary. In one open label randomized controlled trial published in 2024, 87% (39/46) of persons who took itraconazole had an adverse event, 50% (23/46) had a serious adverse event, and 24% (11/46) did not complete treatment.[10] As a result, alternatives are needed. Posaconazole is a medication of interest given its

improved toxicity profiles compared to itraconazole, *in vitro* activity (median mean inhibitory concentration (MIC) <0.007 in 17 isolates) against *Histoplasma capsulatum* and has been reported as clinically effective in individual cases and cases series'.[11-16] Regarding tolerability, one retrospective study in persons with hematologic malignancy using antifungal prophylaxis, 28% (41/149) prematurely discontinued posaconazole due to tolerability compared to 46% (43/115) with itraconazole.[17] Yet, there are no clinical trials regarding efficacy for histoplasmosis for posaconazole with clinical reports largely coming from case reports or small case series'. A large, phase III clinical trial would determine whether posaconazole is acceptable treatments for histoplasmosis. Moreover, if 6-month survival time is non-inferior in at least one of the novel treatment arms, tolerability and safety (where we believe posaconazole is likely an improvement compared to itraconazole) will be thoroughly evaluated for superiority.

2.2 Scientific Rationale

Purpose of Study

The purpose of the study is threefold:

1) Assess the safety and efficacy of a single high-dose intravenous (LAmB 10mg/kg) compared to the SOC daily dosing (3mg/kg) of the same medication for induction therapy in moderate to severe histoplasmosis.
2) Assess the safety and efficacy of oral posaconazole 300mg delayed-release tablets twice daily on day 1 then once daily for consolidation therapy compared to SOC oral itraconazole 200 mg capsules three times daily for three days then twice daily in moderate to severe histoplasmosis
3) Assess the safety and efficacy of 26 weeks of consolidation therapy compared to the SOC 52 weeks of consolidation therapy in PWH on appropriate antiretroviral therapy.

We *hypothesize* that in Aim 1, single high-dose liposomal amphotericin B will show non-inferior two-week survival and further *hypothesize* superior performance via the hierarchical composite end point compared to standard daily dosing liposomal amphotericin be given for 2 weeks. We *hypothesize* that in Aim 2 posaconazole will show non-inferior 26 week survival when compared to itraconazole and further *hypothesize* that posaconazole will be superior to itraconazole via the hierarchical composite end point. Lastly, we *hypothesize* that in Aim 3, 26 weeks of consolidation therapy will be non-inferior to 52 weeks for serious adverse event free survival from 26 to 52 weeks among those who survived the initial 26 weeks of therapy.

Study Population

664 participants hospitalized with moderate to severe histoplasmosis (confirmed or probable) with or without HIV (first n= >100 to be enrolled in the first randomization (Induction therapy) which has already secured funding from the Brazilian government and Gilead, NCT05814432).

We will utilize a total sample size to recruit n=664 persons with confirmed or probable histoplasmosis, per the EORTC/MSGERC research definitions.[18] Of these 664, approximately 100 will come from randomization of Aim 1,[19] and ~554 will be enrolled for randomization into Aims 1, 2, and 3. For Aim 3, a smaller number of participants may be enrolled from the ongoing trial if they are still available for Aim 3 (e.g., have not yet passed 26 weeks of consolidation therapy). Please see the statistical plan for sample size justification.

Participants may have an underlying immune deficiency or not. Participants will be enrolled at 7 sites in Brazil and one site in the United States. Most participants from Brazil will be PWH, most patients from the U.S. site will have other immune compromising conditions. The overall study population reflects persons who have moderate to severe histoplasmosis, globally.

Pregnant women or women who are breastfeeding and not able to stop breastfeeding for the duration of study will not be eligible due to azole-related toxicity. Women are otherwise eligible. Women of childbearing age must take birth control while on azole therapy as is standard of care. Children are not eligible as histoplasmosis presents differently and is uncommon in children. Prisoners will not be eligible as there is not a scientific reason to include this vulnerable population. All racial and ethnic groups are eligible. Demographic data will include self-reported race/ethnicity according to site- specific categories harmonized for study reporting purposes.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

The study drugs are U.S. FDA approved for other indications.

Potential risks should be considered in the context that moderate to severe histoplasmosis carries at least a 15% mortality risk in the first 2 weeks and a 25% mortality risk in the first 6 months in our phase II clinical trial with some cohorts reporting mortality as high as 71% on current SOC therapy.[1, 5-7] These outcomes are, of course, unacceptable and so some risk in trials for new therapies is reasonable.

- Lack of efficacy: This is a primary risk with any new treatment strategy. Though current treatment outcomes are inadequate a worse treatment is possible.
  Mitigation of risk:
  - A phase II trial suggests similar outcomes in the use of single high-dose LAmB versus SOC LAmB dosing for disseminated histoplasmosis.[1]
  - Posaconazole has excellent *in vitro* activity for *Histoplasma capsulatum* and a number of clinical reports suggesting efficacy although no formal clinical trial.[11, 15, 16]
- Liposomal amphotericin B: Risks associated with the IV LAmB include nephrotoxicity, life-threatening hypokalemia (common), life-threatening hypomagnesemia (common),

severe anemia (common), nausea (common), vomiting (common), diarrhea (common), severe infusion reactions including rigors, local inflammation, and thrombophlebitis (common), and many other less common side effects. LAmB is an FDA pregnancy category B (no evidence of risk in humans); Liposomal amphotericin B has a better safety profile than amphotericin B deoxycholate, which is among the most toxic medicines used in medicine. The strategy of single high-dose liposomal amphotericin B has been found to be less toxic than standard daily dose liposomal amphotericin B for persons with cryptococcal meningitis, particularly for nephrotoxicity.[20]

Mitigation of risk:

- Hydration is used to mitigate nephrotoxicity risk, typically 1L of normal saline. Infusion reactions can be mitigated with diphenhydramine and/or acetaminophen in most cases. Electrolytes (potassium, sodium, magnesium) will be monitored on enrollment, day 4, day 7 and day 14 at minimum and more often with any abnormality or concern. Platelet count and hemoglobin will be monitored at the same intervals.

- Itraconazole is the SOC for histoplasmosis consolidation therapy and carries an FDA indication for histoplasmosis. consolidation therapy and carries a black box warning for congestive heart failure, cardiac effects and drug interactions. Intravenous itraconazole given to anesthetized dogs results in dose-related negative ionotropic affect. In healthy human volunteers, asymptomatic left ventricular ejection fraction decreases were observed using gated single-photon emission computed tomography imaging; these resolved prior to the next dose, 12 hours later. Other notable side effects include life threatening hepatotoxicity (rare, including persons without underlying hepatic abnormalities), abnormal hepatic function tests (common), drug-drug interactions (common), neuropathy (rare), nausea (common), vomiting (common), diarrhea (common), fatigue (common), rash (common), QT interval prolongation, a measure of delayed ventricular repolarization that can increase the risk of arrhythmias (common), pseudo hyperaldosteronism (rare) and headache (common).

Mitigation of Risk:

- Persons on medications with significant drug-drug interactions with itraconazole will not be included in the trial. Electrocardiograms will be completed for all participants. Anyone with a QTc interval consistently >450milliseconds will not be enrolled. Additional QTc measurements will be completed after starting the medication if the QTc was >400milliseconds after a week of enrollment and as needed. Electrolytes (potassium, sodium, magnesium) will be monitored on enrollment, day 7 and day 14 at minimum and more often with any abnormality or concern. Similarly, liver function tests will be checked at enrollment, day 7, day 14, week 4, week 10 and as needed. Medications for symptomatic treatment for nausea, headache and diarrhea may be used per provider discretion.

- Posaconazole is the study drug being compared to itraconazole. Posaconazole is generally considered safer than itraconazole.[17] Side effects include life threatening hepatotoxicity (rare, NOT including persons without underlying hepatic abnormalities, QT interval prolongation, a measure of delayed ventricular repolarization that can increase the risk of arrhythmias (common), abnormal hepatic function tests (common), nausea (common), headache (common), pseudohyperaldosteronism (rare), anemia (common), thrombocytopenia (common), fatigue (common).

Mitigation of Risk:

  - Persons on medications with significant drug-drug interactions with posaconazole will not be included in the trial. Electrocardiograms will be completed for all participants. Anyone with a QTc interval consistently >450milliseconds will not be enrolled. Additional QTc measurements will be completed after starting the medication if the QTc was >400milliseconds after a week of enrollment and as needed. Electrolytes (potassium, sodium, magnesium) will be monitored on enrollment, day 7 and day 14 at minimum and more often with any abnormality or concern. Platelet count and hemoglobin will be monitored at the same intervals. Similarly, liver function tests will be checked at enrollment, day 7, day 14, week 4, week 10 and as needed. Medications for symptomatic treatment for nausea, headache and diarrhea may be used per provider discretion.

- Pregnancy: Both itraconazole and posaconazole are pregnancy category C.

Mitigation of Risk:

- No pregnant persons will be enrolled. Pregnancy tests will be completed on all participants of child-bearing potential prior to enrollment. Participants who have the potential to become pregnant must use effective forms of contraception to avoid becoming pregnant while on either drug.

2.3.2 Potential Benefits

- While there are potential theoretical benefits, the known benefits are limited.
- Participants will have access to *Histoplasma* antigen testing which is not uniformly available in routine care at all sites.
- Participants will have care provided by expert physicians who have extensive experience treating persons with histoplasmosis. This is not the case in every setting for routine care and is likely to have some survival benefit.
- Theoretical (as yet, unproven) benefits include
  - Single high-dose liposomal amphotericin B is likely less toxic than daily dosing based on prior studies and studies of the regimen in other diseases without worsening mortality.

- Posaconazole is likely less toxic and easier to tolerate than itraconazole based on comparative studies in other conditions. This may additionally lead to survival benefit, decreased rehospitalization and decreased costs for the patient and for society.
- Twenty six weeks of consolidation therapy is likely equivalent to 52 weeks, if proven this would benefit the patient with less costs and less toxicities. Persons seeking to become pregnant would have 26 additional weeks that they could pursue pregnancy.
- There is significant potential benefit to society by adding generalized knowledge to all facets of treatment for moderate to severe histoplasmosis.

3 STUDY DESIGN

3.1 Study Design Description

- Open label, factorial design trial involving participants hospitalized with moderate to severe histoplasmosis.
- The factorial design will involve experimental treatment arms for induction therapy, consolidation therapy and duration of consolidation therapy.
- Randomization will take place on enrollment for induction and consolidation therapy.
- Randomization for duration of consolidation therapy will take place near the completion of 26 weeks of consolidation therapy after a second informed consent process.
- For induction therapy there will be one experimental arm testing a single high-dose (10mgkg) of LAmB B vs. SOC daily standard dose (3mg/kg) LAmB.
- For consolidation therapy there will be one experimental arm testing posaconazole vs. SOC itraconazole.
- For duration of therapy there will be one experimental arm testing 26 weeks of consolidation therapy vs. SOC 52 weeks of consolidation therapy.
- This trial will take place in at least 7 centers in Brazil and at least 1 center in the United States.
- The expected duration of subject participation 52 weeks of therapy plus 4 weeks of monitoring after completion of study medication (e.g., 56 weeks total).
- Induction treatment domain (open label), randomizing between:
    - Single-high dose intravenous liposomal amphotericin B (10 mg/kg)
    - World Health Organization (WHO)-recommended SOC (daily intravenous liposomal amphotericin B 3mg/kg, for 2 weeks or at least 7 days if felt stable for discharge per the clinician)
- Consolidation treatment domain (open label), randomizing between:
    - Posaconazole delayed-release tabs, 300mg twice daily on day 1 then once daily
    - WHO-recommended SOC Itraconazole 200mg capsules three times daily x 3 days then twice daily
- 500mL-1L of 0.9% normal saline or similar intravenous fluids to be given prior to administration of liposomal amphotericin B to minimize nephrotoxicity
- Induction phase (Aim 1) will be a single day of therapy for the experimental arm and 14 days for the SOC arm. Endpoints are up to 10 weeks.

- Consolidation phase (Aims 2 and 3) will be 26 weeks or 52 weeks of treatment and follow up for 4 weeks after that last medication is given.
- Liposomal amphotericin B is given in weight-based dosing. Doses may be held for nephrotoxicity per physician discretion. This will be tracked.
- Itraconazole dose adjustments are not needed for renal or liver impairment. Itraconazole levels will be obtained locally after 1 week of therapy when available. Target levels for itraconazole should be at least 1.0mcg/mL. Combined hydroxyitraconazole and itraconazole levels >1.0mcg/mL are acceptable assuming itraconazole level is >0.5mcg/mL but require repeat measurement a week later. Doses may be increased per clinician discretion based on these parameters.
- Itraconazole capsules should be given with acidic drinks such as cola when possible.
- Posaconazole dose adjustment is not required for renal or hepatic impairment. Posaconazole level measurement is not routine at the study sites
- Blood will be stored for transportation to Universidade Federal de Ciencias da Saude de Porto Alegre (UFCSPA) for PCR testing at 2 weeks and 4 weeks.
- Blood will be stored for transportation to University of Minnesota (UMN) for itraconazole, posaconazole and antiretroviral therapy levels. One sample will be stored after 1 week of azole therapy, for 12.9% of those in Aim 3 at 26 weeks and at sick visits. 12.9% (n=86) was chosen because the expected event rate is 4.3% and we plan to test levels in a case control design using 2 controls per case. These plasma samples will be stored in EDTA tubes at -80°C.

Schematic of Study Design - See **Figure 1**

3.2 Study Objectives

Primary Objectives

- Assess the safety and efficacy of a single high-dose LAmB (10mg/kg) compared to the SOC daily dosing (3mg/kg) for induction therapy in moderate to severe histoplasmosis.
- Assess the safety and efficacy of posaconazole for consolidation therapy compared to SOC itraconazole in moderate to severe histoplasmosis.
- Assess the safety and efficacy of 26 weeks of consolidation therapy compared to the SOC 52 weeks of consolidation therapy in PWH on appropriate antiretroviral therapy.

  Participants will be actively followed through 56 weeks (4 weeks after completion of 52 weeks, the latest therapy arm). Efficacy will be determined by 2 weeks survival (Aim 1) or 26 weeks survival (Aim 2), performance using a hierarchical composite end-point which takes into account mortality, safety and tolerability (Aims 1 and 2) or SAE-free survival at 52

weeks (Aim 3). Safety will be monitored closely throughout the trial and include monitoring for SAEs, liver injury, kidney injury, electrolyte abnormalities, hemoglobin, platelet count and QTc interval prolongation over the follow up period.

Secondary Objectives

- Assess the need for additional LAmB by 10 weeks – includes the use of 'rescue' liposomal amphotericin B, extension of LAmB beyond the planned duration or re-initiation of IV LAmB by 10 weeks (Aim 1) and week 26 (Aim 2)
- Assess the frequency of discontinuation of study medication due to side effects or suspected failure (measured separately and in total) at 10 weeks (Aim 1), 26 weeks (Aim 2) and 52 weeks (Aim 3, SOC arm only)

Exploratory Objectives

- Assess the mean change in *Histoplasma* urine antigen concentration at 2 weeks visit (Aim 1), 10 week visit (Aims 1 and 2), 26 week visit (Aim 2) and 52 week visit (Aim 3) with the latter two time points being only in a subset of high enrolling sites
- Assess blood PCR change at 2 week visit (Aim 1), 4 week visit (Aims 1 and 2)
- Assess the incidence of paradoxical IRIS incidence through 10 weeks (Aims 1 and 2)
- Assess the duration of hospitalization (Aim 1): The duration of the initial hospitalization will be recorded. The initial analysis will include all enrolled participants and for those who die in the hospital, the day of death will be the last day of hospitalization. A sensitivity analysis will exclude those who died in the hospital (e.g., what was the duration of hospitalization for those who survived to discharge).
- Assess frequency of participants lost to follow up between 26 and 52 weeks (Aim 3).
- Assess the frequency of rehospitalization through 10 weeks (Aim 1) and 26 weeks (Aim 2).

3.3 Trial Endpoints

3.3.1 Timing of Endpoint Assessment

- Aim 1 Induction Therapy: 2 weeks (2 weeks for mortality, 10 weeks for the hierarchical composite endpoint)
- Aim 2 Consolidation Therapy: 26 weeks
- Aim 3 Duration of Consolidation Therapy: from 26 to 52 weeks

Endpoints will be assessed for these study periods.

3.3.2 **Induction (Aim 1) and Consolidation (Aim 2) Therapy Endpoints:**

Primary End Point

- Mortality at Week 2 for Aim 1, and Week 26 for Aim 2

Key Secondary End Point

- Hierarchical composite end point consisting of the following in a hierarchical order*:
    5. Death within the study period (Aims 1 and 2); or lost to follow up within the first one week (Aim 1 only),
    4. Serious Adverse Event (SAE) in the study period, e.g. rehospitalization ,
    3. Grade 4 laboratory abnormality at two weeks (Aim 1) or through Week 26 (Aim 2) or discontinuation of medication due to intolerance during the study period (Aims 1 and 2)
    2. Grade 3 laboratory abnormality at 2 week visit (Aim 1) or through Week 26 visit (Aim 2) or lost to follow up after 1 week** (Aim 2) ,
    1. Alive without an above event to end of the study period

*Within category tiebreakers are the number of events or the time-to-event, as relevant.

**For Aim 2, this will be modified intention to treat, if the participant does not receive the azole, they will be excluded

Other Secondary Endpoints

- Additional LAmB requirement during the study period, i.e. need for 'rescue', re-initiation or extension of IV LAmB beyond the planned duration
- Discontinuation of study medication due to side effects or suspected failure (measured separately and in total) during the study period

Safety and Other Endpoints

- Laboratory Anomalies events:
    a. mean percentage change in hemoglobin, kidney function, liver function at Week 2 visit (Aim 1)
    b. Grade 2, 3, 4 liver function test abnormalities through Week 10 visit (Aim 1) or Week 26 visit (Aim 2)
- Grade 3-5 clinical AEs through study period
- Clinical Response at week 10 (Aim 1) and week 26 (Aim 2) defined as resolution of fever and signs/symptoms attributable to histoplasmosis, except for manifestations expected to last 10 weeks such as some skin lesions and hepatosplenomegaly
- Rehospitalization through study period
- Duration of hospitalization (Aim 1)

The duration of the initial hospitalization will be recorded. The initial analysis will include all enrolled participants and for those who die in the hospital, the day of death will be the last day of hospitalization. A sensitivity analysis will exclude those who died in the hospital (e.g., what was the duration of hospitalization for those who survived to discharge).

- Paradoxical IRIS incidence through 10 weeks (Aim 1) and 26 weeks (Aim 2)
- Mean change in *Histoplasma* urine antigen concentration at Week 2 visit (Aim 1), 10 weeks visit (Aims 1 and 2), 26 weeks (Aim 2)
- Blood PCR change at 2 week visit (Aim 1), 4 week visit (Aims 1 and 2)

3.3.3 Duration of Consolidation Therapy Endpoints (Aim 3)

Primary:

- SAE-free survival from 26-52 weeks from induction therapy among those surviving at 26 weeks

Secondary:

- Lost to follow up between 26 and 52 weeks
- Grade 3-5 clinical AEs from 26 to 52 weeks
- Mean change in *Histoplasma* urine antigen concentration from 26 to 52 weeks
- Discontinuation of study medication due to side effects or suspected failure at 52 weeks (SOC arm only)

4 STUDY INVESTIGATIONAL PRODUCTS

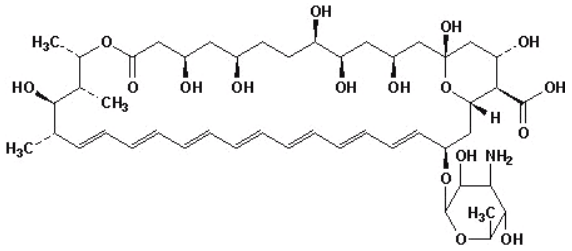
Adapted from package inserts which are included with the application.

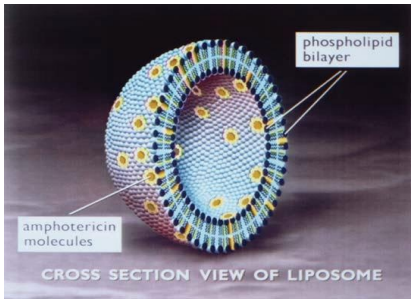
4.1 Liposomal Amphotericin B (LAmB, Aim 1 SOC and Experimental Arm):

Amphotericin B is a macrocyclic, polyene, antifungal antibiotic produced from a strain of *Streptomyces nodosus*. Amphotericin B is designated chemically as: [(1R,3S,5R,6R,9R,11R,15S,16R,17R,18S,19E,21E,23E,25E,27E,29E,31E,33R,35S,36R,37S)]-33-[(3-Amino-3,6-dideoxy-beta-D-mannopyranosyl)oxy]-1,3,5,6,9,11,17,37-octahydroxy-15,16,18- trimethyl-13-oxo-14,39 dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid (CAS No. 1397-89-3).

Amphotericin B has a molecular formula of $C_{47}H_{73}NO_{17}$ and a molecular weight of 924.09.

The structure of amphotericin B is shown below (Left), a liposome depiction is shown (right):





LAmB is a single bilayer liposomal drug delivery system. Liposomes are closed, spherical vesicles created by mixing specific proportions of amphophilic substances such as phospholipids and cholesterol so that they arrange themselves into multiple concentric bilayer membranes when hydrated in aqueous solutions. Single bilayer liposomes are then formed by microemulsification of multilamellar vesicles using a homogenizer. LAmB consists of these unilamellar bilayer liposomes with amphotericin B intercalated within the membrane. Due to the nature and quantity of amphophilic substances used, and the lipophilic moiety in the amphotericin B molecule, the drug is an integral part of the overall structure of the LAmB liposomes. LAmB contains liposomes that are less than 100 nm in diameter.

Liposomal encapsulation or incorporation into a lipid complex can substantially affect a drug's functional properties relative to those of the unencapsulated drug or non-lipid associated drug. In addition, different liposomal or lipid-complex products with a common active ingredient may vary from one another in the chemical composition and physical form of the lipid component. Such differences may affect the functional properties of these drug products.

4.1.1 LAmB Formulation, Packaging, and Labeling

(LAmB, Aim 1 SOC and Experimental Arm)

LAmB for Injection is a sterile, non-pyrogenic lyophilized product for intravenous infusion. Each vial contains 50 mg of amphotericin B, United States Pharmacopeia (USP), intercalated into a liposomal membrane consisting of approximately 213 mg hydrogenated soy phosphatidylcholine; 52 mg cholesterol, NF; 84 mg distearoylphosphatidylglycerol; 0.64 mg alpha tocopherol, USP; together with 900 mg sucrose, NF; and 27 mg disodium succinate hexahydrate as buffer. Following reconstitution with Sterile Water for Injection, USP, the resulting pH of the suspension is between 5-6.

Dosage will be 10mg/kg in the single high-dose arm and 3mg/kg given daily for at least 7 days (less only when medically indicated or if the investigator feels the patient is ready for discharge from the hospital and can safely transition to consolidation) with the SOC being 14 days in the SOC arm.

How supplied: LAmB for Injection is available as single cartons (equivalent to 50mg amphotericin B) and in packs of ten individual vial cartons (National Drug Code (NDC) 0469-3051-30). Each carton contains one pre-packaged, disposable sterile 5 micron filter.

Liposomal amphotericin B must by reconstituted in sterile water for injection, USP (without a bacteriostatic agent) as follows.

- Aseptically add 12 mL of Sterile Water for Injection, USP to each LAmB vial to yield a preparation containing 4 mg amphotericin B/mL. CAUTION: DO NOT RECONSTITUTE WITH SALINE OR ADD SALINE TO THE RECONSTITUTED CONCENTRATION, OR MIX WITH OTHER DRUGS. The use of any solution other than those recommended, or the presence of a bacteriostatic agent in the solution, may cause precipitation of LAmB.
- **Immediately after the addition of water, SHAKE THE VIAL VIGOROUSLY** for 30 seconds to completely disperse the LAmB. LAmB forms a yellow, translucent suspension. Visually inspect the vial for particulate matter and continue shaking until completely dispersed.
- Calculate the amount of reconstituted (4 mg/mL) LAmB to be further diluted.
- Withdraw this amount of reconstituted LAmB into a sterile syringe.
- Attach the 5-micron filter, provided, to the syringe. Inject the syringe contents through the filter, into the appropriate amount of 5% Dextrose Injection. (Use only one filter per vial of LAmB.)
- LAmB must be diluted with 5% Dextrose Injection to a final concentration of 1 to 2 mg/mL prior to administration. Lower concentrations (0.2 to 0.5 mg/mL) may be appropriate for infants and small children to provide sufficient volume for infusion. **DISCARD PARTIALLY USED VIALS**.

The study product will be labeled according to manufacturer or regulatory specifications.

4.1.2 Product Storage and Stability: Liposomal Amphotericin B

- **Storage of Reconstituted Product Concentrate**

Do no freeze. The reconstituted product concentrate may be stored for up to 24 hours at 2-8 C (36-46 F) following reconstitution with Sterile Water for Injection, USP.

- **Storage of Diluted Product**

Injection of LAmB should commence within 6 hours of dilution with 5% Dextrose Injection.
As with all parenteral drug products, the reconstituted LAmB should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use material if there is any evidence of precipitation or foreign matter. Aseptic technique must be strictly observed in all handling since no preservative or bacteriostatic agent is present in LAmB or in the materials specified for reconstitution and dilution.

4.1.3 Acquisition/Distribution: Liposomal Amphotericin B

This medication is being donated by Gilead (see Gilead Letter of Support) for all sites.

Normal saline to be given prior to the dose will be obtained locally from each hospital's typical supplier.

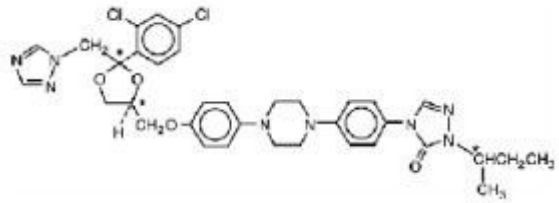
4.2 Itraconazole (Aim 2, SOC)

Itraconazole is represented by the following structural formula and nomenclature:

(±)-1-[(R)-sec-butyl]-4-[p-[4-[p-[[(2R*,4S*)-2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1- ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-Δ^2-1,2,4-triazolin-5-one mixture with (±)-1-[(R)-sec-butyl]-4-[p-[4-[p-[[(2S,4R)-2-(2,4-dichlorophenyl)-2-(1H- 1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-Δ^2-1,2,4-triazolin-5-one

or

(±)-1-[(RS)-sec-butyl]-4-[p-[4-[p-[[(2R,4S)-2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1- ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]- Δ^2-1,3,4-triazolin-5-one



4.2.1 Itraconazole Formulation, Packaging, and Labeling

Itraconazole is a 1:1:1:1 racemic mixture of four diastereomers (two enantiomeric pairs), each possessing three chiral centers. Capsules contain 100 mg of itraconazole coated on sugar spheres (composed of sucrose, maize starch, and purified water). Inactive ingredients are hard gelatin capsule, hypromellose, polyethylene glycol (PEG) 20,000, titanium dioxide, FD&C Blue No. 1, FD&C Blue No. 2, D&C Red No. 22 and D&C Red No. 28.

Itraconazole has a molecular formula of $C_{35}H_{38}Cl_2N_8O_4$ and a molecular weight of 705.64. Itraconazole is a white to slightly yellow powder, insoluble in water, slightly soluble in alcohols and soluble in dichloromethane.

Itraconazole capsules and oral solution should not be used interchangeably. This is because drug exposure is greater with the oral solution than with the capsules when the same dose of drug is given. In addition, the topical effects of mucosal exposure may be different between the two formulations. Only the oral solution has been demonstrated effective for oral and/or esophageal candidiasis.

Itraconazole capsules should be administered after a full meal. Under fasted conditions, itraconazole absorption was decreased in the presence of decreased gastric acidity. The absorption of itraconazole may be decreased with the concomitant administration of antacids or gastric acid secretion suppressors. Studies conducted under fasted conditions demonstrated that administration with 8 ounces of a non-diet cola beverage resulted in increased absorption of itraconazole in PWH with relative or absolute achlorhydria. This increase relative to the effects of a full meal is unknown. Capsules must be swallowed whole.

The recommended dose is 400 mg once daily (2 capsules). If there is no obvious improvement, or there is evidence of progressive fungal disease, the dose should be increased in 100-mg increments to a maximum of 800 mg daily. Doses above 200 mg/day should be given in two divided doses. Although clinical studies did not provide for a loading dose, it is recommended, based on pharmacokinetic (PK) data, that a loading dose of 200 mg (2 capsules) three times daily (600 mg/day) be given for the first 3 days of treatment.

Itraconazole capsules are available containing 100 mg of itraconazole, with a blue opaque cap and pink transparent body, imprinted with “JANSSEN” and “SPORANOX 100.” The capsules are supplied in unit-dose blister packs of 3 × 10 capsules (NDC 50458-290-01), bottles of 30 capsules (NDC 50458-290-04) and in the *Pulse*Pak® containing 7 blister packs × 4 capsules each (NDC 50458-290-28).

4.2.2 Product Storage and Stability Itraconazole

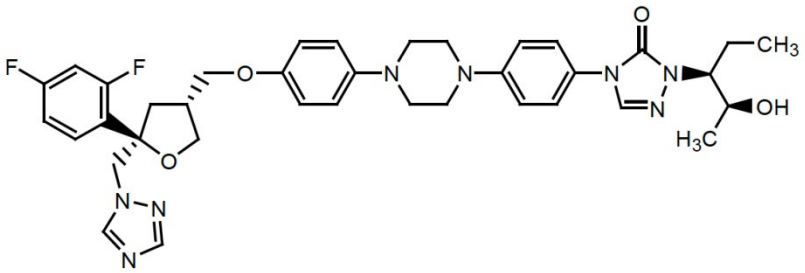
Store at controlled room temperature 15°-25°C (59°-77°F). Protect from light and moisture.

4.2.3 Acquisition/Distribution: Itraconazole

Itraconazole will be obtained locally. Suppliers are well established at all sites for this product. Based on current prices this is the cheapest way to obtain reliable product. If unexpected shortages occur or if prices rise, another option is Cost Plus Drugs who is willing to supply the medication at their current price, if needed. During shipping will be stored at a controlled room temperature 15°-25°C (59°-77°F) and protected from light and moisture.

4.3 Posaconazole (Aim 2 Experimental Arm)

Posaconazole is designated chemically as 4-[4-[4-[4-[[(3*R*,5*R*)-5- (2,4-difluorophenyl)tetrahydro-5-(1*H*-1,2,4-triazol-1-ylmethyl)-3-furanyl]methoxy]phenyl]-1-piperazinyl]phenyl]-2-[(1*S*,2*S*)-1-ethyl-2-hydroxypropyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one with an empirical formula of $C_{37}H_{42}F_2N_8O_4$ and a molecular weight of 700.8. The chemical structure is:



4.3.1 Posaconazole Formulation, Packaging, and Labeling

Posaconazole is an azole antifungal agent available as concentrated solution to be diluted before intravenous administration, delayed-release tablet, or suspension for oral administration. Posaconazole is a white powder with a low aqueous solubility.

Posaconazole delayed-release tablet is a yellow, coated, oblong tablet containing 100 mg of posaconazole. Each delayed-release tablet contains the inactive ingredients: hypromellose acetate succinate, microcrystalline cellulose, hydroxypropylcellulose, silicon dioxide, croscarmellose sodium, magnesium stearate, and Opadry® II Yellow (consists of the following ingredients: polyvinyl alcohol partially hydrolyzed, Macrogol/PEG 3350, titanium dioxide, talc, and iron oxide yellow). This will be the formulation primarily used for this study, only using other formulations if this form is not an option.

Posaconazole injection is available as a clear colorless to yellow, sterile liquid essentially free of foreign matter. Each vial contains 300 mg of posaconazole and the following inactive ingredients: 6.68 g Betadex Sulfobutyl Ether Sodium (SBECD), 0.003 g edetate disodium, hydrochloric acid and sodium hydroxide to adjust the pH to 2.6, and water for injection.

Noxafil oral suspension is a white, cherry-flavored immediate-release suspension containing 40 mg of posaconazole per mL and the following inactive ingredients: polysorbate 80, simethicone, sodium benzoate, sodium citrate dihydrate, citric acid monohydrate, glycerin, xanthan gum, liquid glucose, titanium dioxide, artificial cherry flavor, and purified water.

Administration:
Delayed-Release Tablets

Take delayed-release tablets with food. Tablets must be swallowed whole and not divided, crushed, or chewed.
If patients they miss a dose, they should take it as soon as they remember. If they do not remember until it is within 12 hours of the next dose, they should be instructed to skip the

missed dose and go back to the regular schedule. Patients should not double their next dose or take more than the prescribed dose.

Oral Suspension
Patients should take each dose of oral suspension during or immediately (i.e., within 20 minutes) following a full meal. In patients who cannot eat a full meal, each dose of oral suspension should be administered with a liquid nutritional supplement or an acidic carbonated beverage (e.g., ginger ale) in order to enhance absorption. If patients miss a dose, they should take it as soon as they remember. However, if it is almost time for the next dose, they should skip the missed dose and resume the regular schedule. Patients should not double their dose or take more than the prescribed dose.
How supplied:

Injection is available in Type I glass vials closed with bromobutyl rubber stopper and aluminum seal (NDC 0085-4331-01) containing 300 mg per 16.7 mL of solution (18 mg of posaconazole per mL). Store refrigerated at 2-8°C (36-46°F).

Delayed-Release Tablets 100 mg delayed-release tablets; yellow, coated, oblong, debossed with "100" on one side. Bottles with child-resistant closures of 60 delayed-release tablets (NDC 0085-4324-02). Store at 20-25°C (68-77°F), excursions permitted to 15-30°C (59-86°F) [see USP Controlled Room Temperature].

Dosing for this study: Posaconazole delayed-release tabs, 300mg twice daily on day 1 then once daily

Oral Suspension Noxafil oral suspension is available in 4-ounce (123 mL) amber glass bottles with child-resistant closures (NDC 0085-1328-01) containing 105 mL of suspension (40 mg of posaconazole per mL). Supplied with each oral suspension bottle is a plastic dosing spoon calibrated for measuring 2.5-mL and 5-mL doses.

4.3.2 Product Storage and Stability Posaconazole

- Store posaconazole injection refrigerated at 36°F to 46°F (2°C to 8°C).
- Store posaconazole delayed-release tablets and oral suspension at room temperature between 68°F to 77°F (20°C to 25°C).
- **Do not** freeze posaconazole oral suspension.
- Safely throw away medicine that is out of date or no longer needed.

4.3.3 Acquisition/Distribution Posaconazole

Posaconazole will be supplied by Cost Plus Drugs (see Cost Plus Drugs Letter of Support) at the price which they sell posaconazole at the time of purchase. Currently this is much cheaper than the alternative options. Posaconazole will be shipped to UMN at room temperature between 68°F to 77°F (20°C to 25°C). If needed this will be briefly stored at the UMN research pharmacy but this is not expected to be needed. UMN will ship onward to the research pharmacies at KUMC and UFCSPA, the latter will distribute further to Brazilian sites.

4.4 Dosage/Regimen, Preparation, Dispensing and Administration of Investigational Product

Product Name	Dose	Route	Frequency of Administration	Duration of Therapy
Liposomal Amphotericin B*	10mg/kg	IV	Once	Once
Liposomal Amphotericin B*	3mg/kg	IV	Daily	14 days (at least 7 days if ready for hospital discharge earlier than 14 days)
Posaconazole	300mg	Oral	Twice daily x 1 day then once daily	26-52 weeks (duration randomized)
Itraconazole	200mg	Oral	Three times daily x 3 days then twice daily	26-52 weeks (duration randomized)

*This product is shipped lyophilized. When ready for use, the product is reconstituted in sterile water. Directions: Aseptically add 12 mL of Sterile Water for Injection, USP to each LAmB vial to yield a preparation containing 4 mg liposomal amphotericin B/mL. One must not use saline or mix with other medications. Immediately after adding the water, the vial should be shaken vigorously for 30 seconds to completely disperse the medication to form a yellow, translucent solution. The vial should be inspected for particulate matter. If any is present shaking should be continued until there is no particulate matter remaining. Filtration and dilution: Calculate the amount of reconstituted (4mg/mL) medication to be further diluted. Withdraw the amount needed into a sterile syringe. Attach the 5-micron filter, provided, to the syringe. Inject the syringe contents through the filter, into the appropriate amount of 5% Dextrose Injection. (Use only one filter per vial of LAmB.) LAmB must be diluted with 5% Dextrose Injection to a final concentration of 1 to 2 mg/mL prior to administration. Lower concentrations (0.2 to 0.5 mg/mL) may be appropriate for infants and

small children to provide sufficient volume for infusion. **DISCARD PARTIALLY USED VIALS**.

- Storage of Reconstituted Product Concentrate: The reconstituted product concentrate may be stored for up to 24 hours at 2-8 C (36-46 F) following reconstitution with Sterile Water for Injection, USP. Do not freeze.
- Storage of Diluted Product: Injection of LAmB should commence within 6 hours of dilution with 5% Dextrose Injection.
- As with all parenteral drug products, the reconstituted LAmB should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use material if there is any evidence of precipitation or foreign matter. Aseptic technique must be strictly observed in all handling since no preservative or bacteriostatic agent is present in LAmB or in the materials specified for reconstitution and dilution.

4.5 Pre-determined Modification of Investigational Product for an Individual Subject

Liposomal amphotericin B: This product is known to cause acute kidney injury. Yet, the dose is not adjusted with acute kidney injury. However, most often doses are held until stabilization or improvement of kidney function, or in some cases until the planned end of amphotericin therapy when an alternative agent could be used. For this trial, in the single dose arm, there will not be a need to repeat dosing if acute kidney injury occurs. For the daily dosing arm, liposomal amphotericin may be held until kidney function has stabilized or improved per the physician's discretion. In the event that kidney function necessitates holding the medication until the time when consolidation would be started, transition to consolidation would be appropriate. There is no adjustment for poor hepatic function.

Infusion reactions can be treated or pre-treated with agents such as acetaminophen or diphenhydramine per physician discretion. Most often a dose can be tolerated once these medications are used.

Itraconazole: No dose adjustment is needed for renal or hepatic impairment. Doses can be increased based on drug levels after 1 week of therapy to a maximum of 800mg daily. Ondansetron or other anti-emetic agents may be used as needed for nausea. If emesis occurs <1 hour after the initial dose, a repeat dose with prior anti-emetic would occur.

Posaconazole: No dose adjustment is needed for renal or hepatic impairment. Doses can be increased based on drug levels after 1 week of therapy with a maximum dose of 600mg per day in divided doses for tablets, or if oral suspension is used, up to 800mg per day in divided doses. The target trough concentration should be >1mcg/mL. Ondansetron or other anti-emetic agents

may be used as needed for nausea. If emesis occurs <1 hour after the initial dose, a repeat dose with prior anti-emetic would occur. For the IV formulation (which we do not plan to use) there is sometimes caution around using at creatinine clearances less than 50 mL/min due to concerns about the vehicle (cyclodextrin) but whether this is warranted is not clear. If IV posaconazole is used per provided discretion (e.g., only if oral cannot be tolerated), then we would not routinely recommend stopping for low creatinine clearance, but this would be allowed if the local physician desired to do so.

4.6 Accountability Procedures for the Investigational Product(s)

Liposomal amphotericin B will be stored and shipped from the Gilead to the Clinical Sites. Posaconazole will be stored and shipped from Cost Plus Drugs to UMN and then onto the clinical sites. Once received, liposomal amphotericin B, posaconazole and itraconazole will be stored in and dispensed by the Investigational Pharmacy. Unused product may be destroyed.

The FDA requires accounting for the disposition of all investigational products. The Investigator is responsible for ensuring that a current record of product disposition is maintained and product is dispensed only at an official study site by authorized personnel as required by applicable regulations and guidelines. Records of product disposition, as required by federal law, consist of the date received, date administered, quantity administered, and the subject number to whom the drug was administered.

The Investigational Pharmacist will be responsible for maintaining accurate records of the shipment and dispensing of the investigational product. The pharmacy records must be available for inspection by the Division of Microbiology and Infectious Diseases (DMID) monitoring contractors, and is subject to inspection by a regulatory agency (e.g., FDA) at any time. An assigned Study Monitor will review the pharmacy records.

5 SELECTION OF SUBJECTS AND STUDY ENROLLMENT

Subject Inclusion and Exclusion Criteria must be confirmed by a study clinician licensed to make medical diagnoses.

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

5.1 Eligibility Criteria

5.1.1 Subject Inclusion Criteria [a]

- Age ≥18 years
- Hospitalized with suspected histoplasmosis [b]
- Diagnosis of confirmed or probable histoplasmosis (via positive *Histoplasma* antigen test, culture, histopathology or microscopy)
- Provision of Informed Consent by participant or surrogate [c]
- Aim 3 only
    - PWH at Brazil sites only [c]
    - Attend week 26 study visit with no clinician concern related to ART adherence OR with clinician concern for ART adherence but with CD4 ≥ 100 cells/mcL [c]

[a] Consenting for randomization of induction and consolidation (Aims 1 and 2)

[b] Should have one of fever, weight loss (>10% of usual body weight), diarrhea, miliary pattern or consolidation on thorax imaging, pancytopenia, lymphadenopathy, splenomegaly, or hepatomegaly.

[c] PWH who are near completion of 26 weeks of consolidation therapy will be invited to consent to Aim 3 and be randomized at Week 26 visit. CD4 counts will be completed but may not return until after the time of consent. Those participants where the clinicians have no concerns related to ART adherence will be eligible regardless of CD4 counts. Those participants where clinicians do have concerns for ART adherence will be eligible conditional on CD4 results. If CD4 is <100 cells/mcL and there is clinician concern for ART adherence, the participant will be administratively withdrawn from Aim 3, evaluated by the study team and offered appropriate antifungal therapy as per the local standard of care. Of note, persons referred from the induction trial that is currently taking place which has only 10 week follow up may be eligible for Aim 3 if all inclusion/exclusion criteria for the study are met – they will not be included in Aim 2.

5.1.2 Subject Exclusion Criteria

- Previous diagnosis of histoplasmosis
- Pregnant persons (all persons who could potentially be pregnant will have a pregnancy test prior to enrollment, and if negative, must agree to contraception for the duration of the study)
- Breastfeeding and unable to stop for the duration of the study
- Renal impairment (serum creatinine or blood urea nitrogen (BUN) >2.0x upper limit of normal)
- Allergy or contraindication to a study medicine
- More than one dose of an amphotericin product in the prior 7 days
- Suspected central nervous system involvement of histoplasmosis
- Likely to die in the next 48 hours in the judgment of the investigator
- Unlikely to follow up for the duration of the study in the judgement of the investigator
- Significant drug-drug interaction with itraconazole or posaconazole (such as rifampin in persons with TB)
- Current diagnosis of tuberculosis (e.g., considered active and on treatment)
- Current diagnosis of cryptococcosis or leishmaniasis
- QTc interval consistently >450 milliseconds
- Prisoners
- Unable to take oral medications
- Brazilian indigenous populations considered to be high risk for inclusion in research studies

5.2 Withdrawal from the Study or Discontinuation of Study Product

5.2.1 Withdrawal from the Study or Discontinuation of the Study Product

Subjects may voluntarily withdraw their consent for study participation at any time and for any reason without penalty or loss of benefits to which they are otherwise entitled.

An investigator may also withdraw a subject from receiving the study product for any reason. Follow-up safety evaluations will be conducted if the subject agrees. If a subject withdraws consent prior to completion of the study, the reason for this decision must be recorded in the case report forms (CRFs).

The reasons an investigator might withdraw study treatment might include, but are not limited to, the following:

- Subject no longer meets eligibility criteria – particularly, those initially thought to have histoplasmosis but later diagnosed an excluding condition such as leishmaniasis
- Subject meets individual halting criteria (reference to section 8.5.2)
- Medical disease or condition, or new clinical finding(s) for which continued participation, in the opinion of the investigator might compromise the safety of the subject, interfere with the subject's successful completion of this study, or interfere with the evaluation of responses
- Subject placed in prison (given our study does not include prisoners)
- Subject becomes pregnant, see Pregnancy standard operating procedure (SOP)
- Determined by a physician's discretion to require additional therapy not indicated in the protocol to ensure subject's health and well-being (or treatment failure, if applicable)

Participants who are withdrawn from study treatment will continue to be followed in the study per the schedule of events.

Individuals failing induction therapy with a single high-dose of liposomal amphotericin B will be allowed to receive a full course of liposomal amphotericin B, for two weeks. All decisions regarding therapy for these patients will be as per physician discretion, after discussion with the study medical team. Requirement for additional liposomal amphotericin b will be recorded as a study endpoint.

All clinical AEs recorded during the study will be closely monitored, until resolution. SAEs will be reported to the Ethical Committee within 24 hours of site awareness.

The investigator will inform the subject that already collected data will be retained and analyzed even if the subject withdraws from this study. Data from subjects lost to follow up will be analyzed up to the date of the last visit.

5.2.2 Subject Replacement

Early Administrative Withdrawal Criteria

Disseminated histoplasmosis is a medical emergency, thus participants may be enrolled and randomized with pending laboratory values which require verification within ≤72 hours. In recognition that in histoplasmosis, a pending diagnostic work up may be common, participants may be withdrawn if an alternative infection is confirmed within 14 days and with a decision to stop histoplasmosis therapy. Participants administratively withdrawn will be replaced. This early withdrawal is expected to be <3% of participants so as to not delay emergent intervention.

For Aim 3, when the clinician has concerns for participant ART adherence, persons can be enrolled pending CD4 testing. Among this group, those who are found to have CD4 < 100 cells/mCL will be administratively withdrawn (see section 5.1.1)

Other withdrawal
Participants who withdraw, are withdrawn for non-administrative reasons or are lost to follow up after signing the informed consent form (ICF) and administration of the study product will not be replaced.

Subjects who withdraw, or are withdrawn from this study, or are lost to follow-up after signing the ICF but before administration of the study product may be replaced.

5.2.3 Study Termination

If the study is prematurely terminated by the sponsor, any regulatory authority, or the investigator for any reason, the investigator will promptly inform the study subjects and assure appropriate therapy or follow-up for the subjects, as necessary. The investigator will provide a detailed written explanation of the termination to the IRB/IEC.

If the consolidation randomization (Aim 2) is completed or stopped early, and a new medication with proper rationale could be investigated and would sufficiently powered to do so, an alteration to the study protocol and regulatory approvals could be considered with NIH approval. This would only be considered if a sound candidate medication was available.

6 STUDY PROCEDURES

6.1 Screening

Adults with suspected histoplasmosis will be identified via active surveillance at 7 centers in Brazil and 1 center in Kansas, U.S. will be invited to provide informed consent to obtain blood and urine as part of the screening process.

Patients will be considered eligible if they meet inclusion criteria described in section 5.1. Patients will be screened at baseline for histoplasmosis, as previously described, and also for conditions that will prevent subjects from entering the study – pregnancy and renal insufficiency (must be within 3 days of screening). Participants who qualify will be invited to provide informed consent for the clinical trial.

6.2 Enrollment

Patients diagnosed with histoplasmosis on screening who fit eligibility criteria will be invited to sign an informed consent form for the first randomization (Aims 1 and 2). Results from pregnancy tests (when applicable) and renal function tests must be available before consent can be obtained.

After obtaining consent, randomization will be carried out by study staff at each site via a central electronic interface. Baseline signs and symptoms, a targeted physical examination, as well as complete blood counts, kidney function and electrolytes, must be obtained on the day of Aim 1 and 2 randomization before study drug administration.

The pharmacy staff will instruct the medical staff regarding study drug dosage and regimen for Aim 1 and Aim 2.

For consolidation therapy duration, patients who still fit in eligibility criteria at the week 26 visit will be invited to sign an additional consent form for different durations of therapy (26 vs. 52 weeks). The randomization will be conducted by study staff at each site via a central electronic interface.

6.3 Planned Study Visits

Study visits will occur at days (D) 1, 2, 4, 7 and weeks (W) 2, 4, 10, 18, 26, 40, 52 and study completion (W56 for participants who complain Aim 3 and W30 for persons who do not enroll in Aim 3). For patients randomized for single high-dose liposomal amphotericin for induction therapy, D1 study visit will include administration of liposomal amphotericin and study visits from D2 onwards will include the reporting of administration of oral antifungal agent for

consolidation therapy (either itraconazole or posaconazole). For patients randomized for standard induction therapy, D1, D2, D4, D7 and W2 visits will include the reporting of administration of liposomal amphotericin and study visits from W4 onwards will include reporting of administration of oral antifungal agent.

Additional tests or study visits will be performed as needed at the investigator's discretion. This section highlights the minimum follow-up that will be undertaken.

6.3.1 Follow-up

Expected duration for study visits is around 2 hours for D1 visit and 1 hour for following visits.

Allowable windows for study visits are as follows:
D1: no window
D2: +1 day
D4: +2 days
D7: +3 days
W2: -3 days, + 7 days
W4: -6 days, + 7 days

Weeks 10, 18, 26, 40 have a ±7 day window.
Weeks 52 and 56 will have a ±14 day window.
Week 56 will be conducted virtually or over the phone.

Week 30 will be conducted virtually or over the phone as an end of study visit for those who complete Aims 1 and 2 but do not enroll in Aim 3.

Patients who are not hospitalized will be invited for clinic visits in all time points; if a patient fails to attend a study visit, telephone contacts will be attempted at least three times.

In all study visits, possible reactogenicity and other adverse events will be assessed and information regarding concomitant medications will be collected, as well as laboratory testing with complete blood counts, kidney function and electrolytes through week 2 and liver function tests through week 10. For all patients under consolidation therapy, adherence will be assessed in every visit.

A thorough description of study procedures including laboratory tests and physical examination findings is provided in Section 7.1.1 – Study Procedures.

6.3.2 Final Study Visit

Patients will be followed up for one year from the start of antifungal therapy plus 4 weeks of follow up after completion of therapy for SAE reporting, per FDA standards. At the final study visit vital status and adverse events will be recorded.

6.3.3 Early Termination Visit

In case of early termination, a reason for study termination will be documented. Reasons for early termination include withdrawal of consent, death, or transfer of care. An attempt will be made to schedule a termination visit. Evaluations for an early termination visit will be the same as the Week 10 visit.

Participants enrolled in the study but choosing to leave the hospital early against medical advice will continue to participate in the study, unless they choose to withdraw. Additional phone calls by study personnel will encourage the participant to seek follow up care and to rejoin the trial per the ongoing schedule of events. Assessment of vital status will continue via telephone calls at a minimum, unless consent is completely withdrawn.

All analysis is by intention to treat, thus anyone voluntarily discontinuing study medications may continue study participation.

For persons involuntarily defaulting from study participation due to circumstances beyond their individual control (e.g., moving residence due to financial reasons, etc.), appropriate referral to local care will be arranged and a request made for a Week 10 visit. Actual transport expenses may be reimbursed in these rare occasions with prior approval of the site PI. Alternatively, phone visits may be conducted as an outpatient to assess interval history and vital status.

6.4 Unscheduled Study Visits

Additional tests or study visits will be performed as needed at the investigator's discretion. Unscheduled study visits can occur either because of suspected new adverse events, suspected clinical failure (including the need for additional induction course) or poor adherence to consolidation therapy.

All patients who are readmitted due to suspected histoplasmosis, other opportunistic infection (in PWH) or suspected immune reconstitution syndrome will be submitted to at least one unscheduled study visit at readmission.

Unscheduled study visits may include inpatient visits, clinic visits or telephone contact.

6.5 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or protocol-specific requirements. The noncompliance may be either on the part of the subject, the site principal investigator, or the site personnel. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

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 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 per the Data Coordinating Center (DCC) protocol deviation reporting procedures.

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 their guidelines. The site principal investigator and personnel are responsible for knowing and adhering to their IRB requirements.

7 DESCRIPTION OF CLINICAL AND LABORATORY EVALUATIONS

7.1 Clinical Evaluations

Clinical evaluations and treatment outside of the care of histoplasmosis will be conducted according to the local standard of care.

7.1.1 Research Procedures

- Baseline visit for the Induction phase of the study will include an evaluation of demographic characteristics (including sex, age, weight, body mass index, being homeless, use of crack cocaine in the last 12 weeks), data on HIV infection (including date of diagnosis, ART therapy, CD4 cell count, HIV viral load (when available)), signs and symptoms, including weight loss (>15% body weight), respiratory symptoms, presence of dyspnea, need for mechanical ventilation, WHO performance status, Karnofsky scale, Glasgow coma scale, and analysis of concomitant medications.
- Physical examination at baseline (Induction phase) will include presence of fever (>37.8^{o}C), respiratory frequency, systolic blood pressure, hepatosplenomegaly, skin lesions, oral lesions, and lymphadenopathy.
- Laboratory tests performed at baseline (Induction phase) will include biochemistry tests (serum aminotransferases, total bilirubin, creatinine, electrolytes (potassium, sodium, magnesium), complete blood count, and pregnancy test for woman. Lactate dehydrogenase, ferritin, fibrinogen, tricglycerides and C-reactive protein are considered optional per the local standard of care – these will be recorded if completed. At study baseline, patients will be tested for *Histoplasma* antigen in the urine. Blood sample will be taken for *Histoplasma* PCR (PCR in Brazil only). Additional procedures to diagnose fungal disease will also be performed, if needed/available: fungal culture, microscopy, histopathology, and culture by lysing centrifugation. Blood and urine will also be taken for storage.
- Imaging of the chest will be obtained for all patients entering the study (either chest X-ray or computed tomography).

The second day of therapy will include obtaining maximum temperature, Glasgow coma scale, concomitant medications, Physical examination including presence of fever (>37.8^{o}C), respiratory frequency, systolic blood pressure, hepatosplenomegaly, skin lesions, oral lesions, and lymphadenopathy. All AEs and SAEs, vital status, grade 3-4 laboratory events, lost to follow up and medication intolerance will be recorded.

- On the fourth and seventh days of therapy, the following variables will be obtained: maximum temperature, Glasgow coma scale, concomitant medications, biochemistry tests

(liver and kidney function, electrolytes) and complete blood count. Ferritin and lactate dehydrogenase may be done (optional) per the local standard of care and will be recorded if completed. A blood sample will be obtained for *Histoplasma* PCR (Brazil only). All AEs and SAEs will also be recorded. Blood for an itraconazole level will be collected for those taking intraconazole after seven days of therapy (this is per the local SOC in the U.S., but is often not available in Brazil and so will be a study lab test in Brazil), when testing is available. Blood will also be stored for itraconazole/posaconazole levels for those in the single high-dose LAmB arm (after 7 days of therapy). All AEs and SAEs, vital status, grade 3-4 laboratory events, lost to follow up and medication intolerance will be recorded.

- On the 14th day of therapy, the following will be observed: physical examination, maximum temperature, need for further course of liposomal amphotericin B, presence of dyspnea, respiratory frequency, need for mechanical ventilation, systolic blood pressure, WHO performance status, Karnofsky scale, Glasgow coma scale, biochemistry tests (liver and kidney function, electrolytes), complete blood count, and concomitant medications. Optional tests (ferritin, lactate dehydrogenase, C-reactive protein) may be done per the local standard of care, if done, these will be recorded. Urine sample will be tested for *Histoplasma* antigen, and whole blood will be taken for *Histoplasma* PCR. All AEs and SAEs will be recorded. Vital status and components of the hierarchical endpoint (death, SAE, grade 3-4 laboratory events, lost to follow up, medication intolerance) will be recorded. Blood for an itraconazole level will be collected for those taking itraconazole after at least seven days of therapy as part of standard of care, when available. Blood will also be taken for storage including PK studies if not done at day 7. Also, survival status will be determined at this visit (primary endpoint for Aim 1)
- At week four, we will obtain vital status, physical examination including temperature and other vital signs, need for further course of liposomal amphotericin B, presence of dyspnea, respiratory frequency, WHO performance status, Karnofsky scale, Glasgow coma scale, liver function tests and concomitant medications. Whole blood will be taken for *Histoplasma* PCR. For those in the daily LAmB arm on itraconazole, blood will be taken for an itraconazole level where the test is available. Blood will be taken for PK in those who were part of the daily LAmB arm. All AEs and SAEs, vital status, grade 3-4 laboratory events, lost to follow up and medication intolerance will be recorded.
- On the 10th week of therapy, survival status will be determined (secondary endpoint for the study). The following variables will also be obtained on W10 visit: body weight, need for an additional course of antifungals, dialysis requirement, adherence to antifungal consolidation therapy and ART, occurrence of inflammatory immune reconstitution syndrome, and diagnosis of tuberculosis. All AEs and SAEs will be recorded. Liver function tests will be obtained. Urine sample will be tested for *Histoplasma* antigen. All AEs and SAEs, vital

status, grade 3-4 laboratory events, lost to follow up and medication intolerance will be recorded.

- On W18, W26, W40 and W52, the following variables will be obtained: vital status, body weight, need for an additional course of antifungals, dialysis requirement, adherence to antifungal consolidation therapy and ART, *Histoplasma* antigen quantification in the urine (optional for W18 and W40, only a sub-group of sites at W40 and W52), occurrence of inflammatory immune reconstitution syndrome, and diagnosis of tuberculosis. All AEs and SAEs, vital status, grade 3-4 laboratory events, lost to follow up and medication intolerance will be recorded. Sick visits will occur when necessary. Data and tests collected will depend on the clinical presentation but in general we will be as above. We will obtain urine *Histoplasma* antigen at each of these visits (optional for W18 and W40), W52 only for a sub-set of sites. Blood CD4 will be obtained at W26. Chest and other imaging and well as other blood lab studies will only be obtained if indicated. Survival status will also be determined. Blood will be stored for PK at W26.
- At the end of study visit (virtual or by phone) we will assess vital status, need for an additional course of antifungals, dialysis requirement, adherence to antifungal consolidation therapy and ART, occurrence of inflammatory immune reconstitution syndrome, and diagnosis of tuberculosis. All AEs and SAEs, vital status, grade 3-4 laboratory events, lost to follow up and medication intolerance will be recorded. Sick visits will occur when necessary.
- For all instances where blood is drawn for storage (PK and future diagnostic research studies), one tube serum and one tube plasma should be collected (~20mL). Blood tubes are 5-10mL based on local standards, See Appendix 1.
- Excess urine will be stored for future research including diagnostic tests.

7.1.2 Assessment of Concomitant Medications

Itraconazole and posaconazole are known to interact with several other drugs having hepatic metabolism. Therefore, investigators should identify patients receiving one of the following medications:

Aliskiren, Alprazolam, Apixaban, Astemizole, Axitinib, Atazanavir, Atorvastatin, Bepridil, Carbamazepine, Cisapride, Cimetidine, Colchicine, Conivaptan, Cyclosporine, Dabrafenib, Darifenacin, Darunavir, Dasatinib, Digoxin, Dihydroergotamine, Diltiazem, Disopyramide, Dofetilide, Domperidone, Dronedarone, Efavirenz, Eplerenone, Ergometrine (ergonovine), Ergotamine, Esomeprazole, Everolimus, Felodipine, Fentanyl, Fesoterodine, Fosamprenavir, Glipizide, Halofantrine, Ibrutinib, Irinotecan, **Isoniazid**, Ivabradine, Lercanidipine, Levacetylmethadol

(levomethadyl) and Methadone, Loperamide, Lovastatin, Lurasidone, Metoclopramide, Methylergometrine (methylergonovine), Midazolam (oral), Mizolastine, Nevirapine, Nicardipine, Nifedipine, Nilotinib, Nisoldipine, Phenytoin, Phenobarbital, Pimozide, Quinidine, Ranolazine, Rifabutin, **Rifampin,** Ritonavir, Rivaroxaban, Salmeterol, Sertindole, Sibutramine, Sildenafil, Simeprevir, Simvastatin, Solifenacin, Sunitinib, Sirolimus, Tacrolimus, Tamsulosin, Telithromycin, Terfenadine, Ticagrelor, Tolvaptan, Trabectedin, Triazolam, Vardenafil, Verapamil, Vincristine, Vinblastine.

All medications will be recorded with special attention paid to the medications above.

7.1.3 Assessment of Subject Compliance with the Study Visit Schedule

Participants will be informed of the study visit schedule at enrollment. The next study visit date/time will be discussed at each visit. If visits are missed the study team will contact the participant and/or next of kin if permission is given to allow for ongoing study participation. On rare occasions where there is concern for the patient's well-being or when the patient cannot present in person, the study team may elect to conduct a home visit. This is, however, expected to be a rare occurrence.

7.1.4 Assessment of Subject Compliance with Investigational Product

Regarding induction therapy, subjects will be directly observed at the time of dosing by a member of the clinical research team who is licensed to administer the study product. Any infusion reactions will be recorded. Administration will be documented on the pharmacy log and the participant's medical chart and entered into the eCRF in REDCap.

For consolidation therapy, the 4-item Morisky Medication-Taking Adherence Scale will be applied in order to assess adherence. It involves the following 4 questions:

1. Do you ever forget to take your medication?
2. Do you ever have problems remembering to take your medication?
3. When you feel better, do you sometimes stop taking your medication?
4. Sometimes if you feel worse when you take your medication, do you stop taking it?

This scale will be applied in every visit performed after induction therapy.

7.1.5 Non-Research Standard of Care

Patients who are classified as clinical failure during consolidation therapy may be submitted to additional induction courses with amphotericin B. This definition will occur at the investigator's discretion. Other required medical care will occur according to the local standard of care and at the discretion of the treating physician.

7.2 Laboratory Evaluations

7.2.1 Clinical Laboratory Evaluations

All laboratory studies will be done locally except PCR testing which will be done centrally at UFCSPA and non-patient care itraconazole and posaconazole levels which will be done at UMN. Local sites will complete itraconazole levels for therapeutic drug monitoring when available. Posaconazole therapeutic drug monitoring may be done per local convention but is not required by the study.

- Hematology: hemoglobin, hematocrit, white blood cells (WBC) with differential count, platelet count.
- Biochemistry: creatinine, blood urea nitrogen, sodium, potassium, magnesium, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase.
- Serology: HIV.
- Pregnancy test, usually to be done within 24 hours prior to study intervention and results must be available prior to administration of study product.
- Itraconazole drug concentrations: Itraconazole levels will be obtained locally after 1 week of therapy as part of the local standard of care for itraconazole use when available, samples are 5mL of plasma/serum. Target levels for itraconazole should be at least 1.0mcg/mL. Combined hydroxyitraconazole and itraconazole levels >1.0mcg/mL are acceptable assuming itraconazole level is >0.5mcg/mL but require repeat measurement a week later. See additional details in section 7.1.1 and appendix 1.

7.2.2 Research Assays

- *Histoplasma* urinary antigen: The IMMY Clarus *Histoplasma* Galactomannan Enzyme Immunoassay (HGM201) will be performed according to manufacturer instructions using 100µL of urine per patient. This test will be performed in all Brazilian medical centers participating in the study, when not available locally, samples will be shipped to Santa Casa de Porto Alegre for batch testing. KUMC will use the local standard of care *Histoplasma* antigen test, currently the MiraVista antigen EIA.
- Some sites will use the MiraVista lateral flow assay antigen test for screening. Testing will be performed according to manufacturer instructions using 100µL of urine per patient. Any positives will be confirmed by the IMMY enzyme immunoassay (and negative tests will also be tested by the IMMY enzyme immunoassay). This will be only for initial screening – not for following quantitative values.

- *Histoplasma* PCR: Brazil only. Blood samples will be collected at D1, D4, D7, W2 and W4, stored and transported to Santa Casa de Porto Alegre, Porto Alegre, Brazil, to perform *Histoplasma* PCR utilizing 500µL of whole as described previously.[21]
- Given the MiraVista lateral flow assay antigen test has potential for false positive results and is not FDA approved, only cases confirmed with another test (PCR, IMMY immunoassay antigen test, culture, microscopy/other visualization) will be included in the patient population for the primary analysis. An exploratory analysis will consider results positive only by the lateral flow assay. See the SAP.
- PK studies: Samples will also be stored, separately at study entry, after one week of azole therapy and at the week 26 visit for additional PK studies at UMN. These studies will require ~10mL of plasma/serum and will test simultaneously for itraconazole, posaconazole, tenofovir, lamivudine and dolutegravir. Medications are combined in one assay to improve throughput and decrease assay validation cost. See additional details above, section 7.1.1 and appendix 1.

7.2.3 Laboratory Specimen Preparation, Handling, and Storage

After local disinfection of the site from which blood will be drawn, blood samples will be collected using sterile techniques and appropriate blood collection tubes based on the required assays.
Blood samples should be gently inverted to mix anticoagulants if applicable and samples should be promptly processed in order to separate plasma or serum. After centrifugation (when necessary), aliquots will be prepared in clean, sterile tubes to avoid multiple freeze-thaw cycles. Each aliquot should contain the minimum volume required for analysis, based on the number of assays expected to be performed.
Urine samples will be collected by assigned professionals in sterile vials and will be promptly processed for *Histoplasma* testing.
Plasma, serum and urine aliquots should be stored at -80°C for long-term storage and 4°C for short-term processing. Samples will be stored in secured freezers located at the medical sites and should be labeled with Participant ID, date and time of collection, type of specimen and name of the medical site.

7.2.4 Laboratory Specimen Shipping

In Brazil, blood specimens collected at enrollment, D4, D7, W2 and W4 visits should be shipped to Santa Casa de Porto Alegre for *Histoplasma* PCR. Blood samples should be transported frozen (-20°C) and shipping will occur in batches.

Blood specimens collected after 7 days of itraconazole or posaconazole therapy will be shipped to UFCSPA and then centrally from UFCSPA to UMN for pharmacokinetic studies.

Shipments for this sample will be transported frozen (-80°C). Proper export and import permits will be in order. Our team has experience shipping samples at these temperatures globally.

Contact information for laboratory personnel and detailed shipping information is described in the study laboratory manual.

8 ASSESSMENT OF SAFETY

8.1 Assessing and Recording Safety Parameters

Safety will be assessed by the frequency and severity of: SAEs, grade 2, 3 and 4 laboratory abnormalities, grade 3-5 AEs and discontinuation, interruption ≥ three days or dose reduction of therapy due to intolerability or AEs. All of these parameters will be recorded throughout the study period (e.g., from study day one through up to 56 weeks).

8.2 Adverse Events (AEs)

Table 2 Types and Definitions of Adverse Events

Event	Abbreviation	Definition
Adverse Event	AE	An AE is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment
Adverse Reaction	AR	Any untoward and unintended response to an investigational medicinal product related to any dose administered
Unexpected Adverse Reaction	UAR	An adverse reaction, the nature and severity of which is not consistent with the information about the investigational medicinal product in question set out in the Summary of Product Characteristics (SPC)
Serious Adverse Event Serious Adverse Reaction Serious Unexpected Adverse Reaction	SAE SAR SUSAR	Respectively any adverse event, adverse reaction or unexpected adverse reaction that is: ● Fatal ● Life-threatening (defined as a participant at immediate risk of death at time of the AE, e.g. anaphylaxis) ● Requires re-hospitalization (after hospital discharge for the initial, pre-existing histoplasmosis) or significant prolongation of the initial hospitalization. ● Results in persistent or significant disability or incapacity ● Results in congenital anomaly or birth defect; ● An important medical event that may jeopardize the subject or may require immediate intervention to prevent one of the other outcomes listed in the definition above

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

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of 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.

All AEs, including solicited local (injection site) and systemic (subjective and quantitative) reactions, will be captured on the appropriate data collection form and eCRF. Information to be collected for AEs includes event description, date of onset, assessment of severity, relationship to study product and alternate etiology (assessed only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as an investigator), date of resolution, seriousness and outcome. AEs occurring during the trial collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through 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 should be recorded as an AE.

If an event meets both the criteria of a study endpoint and an adverse event, the event will be reported either as a study endpoint or as an adverse event (not both).

8.3 Adverse Events Grading

All AEs (laboratory and clinical symptoms) will be graded for severity and assessed for relationship to study product (see definitions). AEs 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. **Laboratory AEs do not need to be individually collected on eCRFs as these will be automatically categorized by AE grade via the statistical database, unless they result in a SAE.** Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity.

Severity of Event:

AEs will be assessed by the investigator using a protocol-defined grading system. For events not included in the protocol-defined grading system, the following guidelines will be used to quantify severity:

- Mild (Grade 1): Events that are usually transient and may require only minimal or no treatment or therapeutic intervention and generally do not interfere with the subject's usual activities of daily living.
- Moderate (Grade 2): Events that are usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.

- Severe (Grade 3): Events interrupt usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention. Severe events are usually incapacitating.
- Life Threatening (Grade 4)
- Death (Grade 5)

Relationship to Study Product: The assessment of the AE's relationship to study product will be done by the licensed study physician indicated on the Form FDA 1572 or if an Investigational New Drug (IND) application is not deemed necessary by FDA, by a study investigator at the site and the assessment will be part of the documentation process. Whether the AE is related or not, is not a factor in determining what is or is not reported in this trial. If there is any doubt as to whether a clinical observation is an AE, the event should be reported.

In a clinical trial, the study product must always be suspect. The relationship to study product will be assessed for AEs using the terms related or not related:

- Related – There is a reasonable possibility that the study product caused the AE. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the AE.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.3.1 Solicited Events

Solicited events are AEs that are common and known to occur following administration of study product. We will be particularly collecting incidence of:

- liver injury, kidney injury hypomagnesemia, hypokalemia, anemia and infusion reactions for liposomal amphotericin B.
- Hepatotoxicity will be solicited for posaconazole and itraconazole.

See Section 7.1.1 for timing of laboratory monitoring and AE solicitation.

8.3.2 Unsolicited Events

Unsolicited events are any other AEs that occur following administration of study medication. See section 8.1.1 for schedule of obtaining this information on a scheduled basis. Notably, AEs will be documented whenever they occur including at sick visits outside of planned study visits.

8.3.3 Dose Escalation Criteria

Itraconazole levels will be checked after one week as above, when available. Dose may be escalated per clinician discretion when levels are checked if levels are below standard levels as specified in Section 7.2.1.

8.4 Serious Adverse Events (SAEs)

An SAE is a new untoward medical occurrence that:

- Results in death.
- Is immediately life-threatening. Any adverse experience that places the subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred (i.e., it does not include a reaction that, had it occurred in a more serious form, might have caused death; or an illness which if untreated could have been life threatening).
- Requires in-patient hospitalization or prolongation of existing hospitalization due to the new SAE. (A prolonged hospitalization due to pre-existing histoplasmosis is not a SAE).
- Results in persistent or significant disability or incapacity.
- Is a congenital anomaly/birth defect.
- An event that required immediate intervention to prevent permanent impairment or damage.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 (once completed) or by the Institution as the site Principal Investigator or Sub-Investigator.
- Recorded on the appropriate SAE data collection form and eCRF.
- Followed through resolution by a licensed study physician.
- Reviewed and evaluated by, an Independent Medical Monitor, the DSMB and the IRB/IEC.

8.5 Reporting Procedures

8.5.1 Reporting Serious Adverse Events

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

8.5.2 Regulatory Reporting Procedures

The coordinating center should be notified of all SAEs within 72 hours of the investigator becoming aware of the event, see reporting flowchart. The Adverse Event Reporting CRF must be completed. Investigators should notify the coordinating center of all SAEs occurring from the time of informed consent until study termination.

The coordinating center is undertaking a number of duties as delegated by the trial sponsor and is responsible for the reporting of SAEs to the regulatory authorities and the research ethics committees, as appropriate. The Medical Monitor will review all SAE reports. Per national guidelines, reportable SAEs must be then reported to the regularity authorities within 7 days of the coordinating center becoming aware of the event. Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's regulatory binder.

At the time of the initial report, the following information will be provided:

- Study identifier
- Study Site
- Participant identification number
- Date of event
- A description of the event
- Relationship to Study Agent
- Medical treatment given / discontinued
- Working diagnosis
- Current vital status
- Name of reporting investigator

Within the 7 days of the initial report (or concurrently), the investigator will provide further information on the AE Reporting CRF in the form of a written narrative of the SAE. This will be documented along with any other diagnostic information that will assist understanding of the event. Significant new information on on-going serious AEs will be provided promptly to the study sponsor and local IRB of record. SAEs that are still on-going at the end of the reporting period must be followed up to determine the final outcome.

8.5.3 Expected Disease Related Events

This study population, with histoplasmosis and a subset with advanced HIV, are critically ill and will have a complicated clinical course. Opportunistic infections and nosocomial infections are expected. Rare events such as motor vehicle accidents, though unrelated to histoplasmosis or HIV,

are also expected within this population. Disease-related events will be frequent and include but are not limited to:

- Death due to histoplasmosis.
- Opportunistic infections / illnesses – *Pneumocystis jirovecii* pneumonia, bacteremia, Herpes simplex virus, cryptococcosis, Kaposi's sarcoma, candidiasis, prurigo.
- Nosocomial infections in neurologically obtunded patients - hospital acquired pneumonia or aspiration pneumonia.
- Common ART side-effects e.g. anemia, sleep disturbance, diarrhea, mild renal dysfunction
- Drug induced liver injury is expected with transient elevation of liver transaminases.
- Re-hospitalization due to recurrent symptoms. This is captured separately as the SAE secondary endpoint.

Expected disease-related events from pre-existing conditions will be recorded in study CRFs but will not require reporting to regulatory authorities unless the severity of the event was considered to be unexpected.

8.5.4 Regulatory Reporting for Investigator-Sponsor FDA IND

Following notification from the investigator, the IND investigator sponsor will report events that are both serious and unexpected that are related to study product(s) to the FDA within the required timelines as specified in 21 CFR Part 312.32: fatal and life-threatening events within 7 calendar days (by telephone or fax) All written reports will be sent to the FDA within 15 calendar days. All serious events designated as "not related" to study product(s), will be reported to the FDA at least annually in a summary format.

8.5.5 Reporting of Pregnancy

Pregnant persons are not eligible for the study. Persons who have the potential to become pregnant must agree to use an effective contraceptive method for the duration of the study. If persons do become pregnant during the study, that will be recorded and reported to the IRB and sponsor. For further details related to this situation, refer to the pregnancy SOP.

8.6 Type and Duration of Follow-up of Subjects after Adverse Events

AEs will be assessed and followed from initial recognition of the AE through end of the protocol defined follow-up period.
SAEs will be followed up through resolution even if duration of follow-up goes beyond the protocol-defined follow-up period.
Resolution of an AE is defined as the return to pre-treatment status or stabilization of the condition with the expectation that it will remain chronic.

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

Collection of laboratory data will be limited to those laboratory parameters that are relevant to safety, study outcome measures, and/or clinical outcome.

Reporting will be dependent on the abnormality, the study intervention, and the study. This will be a hospitalized population, most persons will have significant immune suppression. As such, a number of expected SAEs and AEs are outlined above (see Section 8.3.3) We will record laboratory AEs as noted in Section 8.1. The NIH Division of AIDS (DAIDS) definitions will be used for severity. Laboratory abnormalities will only be classified as SAEs when SAE criteria as outline in Section 8.1.2 are met. Appropriate documentation as described in Section 8.3.2 will be completed. The medical monitor will follow the SAE to resolution. The Clinical Advisory Committee is available for management advice as needed.

8.8 Halting Rules

DSMB guidelines are given below. Yet, ultimately, the DSMB will be empowered to make their own decision, irrespective of stopping guidelines. Each aim will have an independent interim analysis. If one aim is stopped early, moving forward, all persons will be given the SOC if the randomization is stopped for futility or harm or the experimental treatment if stopped for superiority – this change would be for the Aim that is stopped only. Aims that were not halted would continue to enroll.

8.8.1 Stopping for Efficacy or Harm

A DSMB will review interim safety and efficacy data at least annually. Primary endpoints will be reviewed. Early stopping for efficacy will use a Haybittle-Peto stopping rule ($P<0.001$). The purpose of the early DSMB reviews is to assess the trends in safety and efficacy and allow for the DSMB to determine if more frequent than annual DSMB reviews are appropriate. If more interim analyses are desired by the DSMB, the Haybittle-Peto stopping rule will be recalculated and provided with each closed report.

8.8.2 Stopping for Futility

Early stopping for futility will be recommended if the conditional power is less than 20%, or if the observed mortality rate in the experimental arm exceeds the non-inferiority margin after 50% of the participants have reached the study endpoint. If the experimental arm closes early after DSMB review, we would either give all patients SOC (if stopped for safety) or the experimental regimen (if stopped for superiority) for that treatment component. Enrollment for the other study components would continue.

8.8.3 Actions if a Stopping Boundary is Crossed

We recommend that the DSMB consider early termination or protocol modification only when the Haybittle-Peto boundary is crossed for the difference in the primary end point for each aim. Should a stopping boundary be crossed, we would recommend a sub-group analysis to determine whether the entire study should be stopped or a pre-specified subgroup excluded from further enrollment. Subgroups to consider include clinical site, CD4 count and ART use at baseline (naïve or experienced) and site of disease.

8.8.4 Individual Halting Criteria

The study intervention will be discontinued in an individual if:

1. ALT or AST > 10x Upper limit normal (ULN) (in this case, if therapy is paused, levels return to within normal limits, and the participant and investigators wish to re-challenge, that is permissible). Such participants would remain in the analysis.

2. Confirmed diagnosis explaining their illness other than histoplasmosis occurs. For persons enrolled pending culture and/or antigen testing, if an alternative diagnosis is made that explains the illness, histoplasmosis specific testing is negative, and the decision is made to stop antifungal therapy. Such participants would be excluded from the analysis population. If antifungal therapy is continued, such participants would remain in the analysis.

3. The participant becomes consistently non-compliant and there is concern about their continued participation from a safety standpoint. Such participants would remain in the analysis.

The preference would be to continue data collection even if the intervention is stopped for analysis.

8.9 Safety Oversight (ISM, SMC, DSMB, as applicable)

8.9.1 Independent Safety Monitor (ISM)

For this clinical trial an Independent Safety Monitor is <u>not</u> required. However, at each participating site, **upon Medical Monitor request,** the principal investigator (PI) will identify a physician with relevant expertise, to act as a Secondary Medical Assessor. The Secondary Medical Assessor will examine a subject and/or medical records and provide a medical assessment (or second medical opinion) to the Medical Monitor of the safety event in question. The PI will send a summary of the event to the PI, sponsor and regulatory bodies.

8.9.2 Data and Safety Monitoring Board

DSMB Description

- This DSMB will be independent of the study sponsor, IRB, and investigators.

- The DSMB will function in accordance with the principles of the ICH GCP guidelines.

Reporting

- The biostatisticians and data managers responsible for the trial, will provide data to be reviewed by the DSMB.
- Details of closed session deliberations (e.g., minutes) will be considered privileged and not subject to disclosure, except as required by law.
- The study team will submit DSMB summary reports of the open sessions to the relevant oversight IRBs.

DSMB Membership

The DSMB will consist of at least 4 members, with a quorum of 3 members to meet. The DSMB members have been selected by the study PI in consultation with co-investigators.

As characteristic qualifications, members will:

- Work professionally and meet qualifications for their respective professions
- Comply with accepted practices of their respective professions.
- Be independent from the sponsor, IRB/EC, regulatory agencies, principal investigator, co-principal or sub-principal investigator, site investigator, site sub-investigator, clinical care of the study subjects, or any other capacity related to trial operations.
- Not be on the list of FDA Notice of Initiation of Disqualification Proceedings and Opportunity to Explain and/or debarred list of investigators www.fda.gov/ora/compliance_ref/debar
- Not have a financial conflict of interest.

Although each DSMB member will be expected to serve for the duration of the trial, in the unlikely event that a member is unable to continue participation, the reason will be documented. The study sponsor, in consultation with the Histo-FACT primary investigators, will select a replacement.

DSMB Roles and Responsibilities

This DSMB will meet periodically (see DSMB Meetings) to review aggregate and individual subject data related to safety, data integrity and overall conduct of the trial.

More details as to the DSMB tasks, meeting frequencies, and study stopping rule are specified in the study DSMB charter.

9 HUMAN SUBJECTS PROTECTION

9.1 Institutional Review Board/Independent Ethics Committee

Each site principal investigator will obtain IRB approval for this protocol to be conducted at their research site(s) and send supporting documentation to NIH before initiating recruitment of subjects. Brazil sites will all run through the UFCSPA IRB. KUMC and UMN will have a reliance agreement in place for this study with Advarra. Advarra will serve as the IRB of record. The investigator will submit applicable information to the IRB/IEC on which it relies for the review, to conduct the review in accordance with 45 CFR 46, ICH E6 GCP, and as applicable, 21 CFR 56 (Institutional Review Boards) and 21 CFR 50 (Protection of Human Subjects), other federal, state, and local regulations. Brazilian sites will obtain ethical approval in accordance with Federal Resolutions 466/12 and 441/12. The IRB/IEC must be registered with OHRP as applicable to the research. NIH must receive the documentation that verifies IRB/IEC-approval for this protocol, associated informed consent documents, and upon request any recruitment material and handouts or surveys intended for the subjects, prior to the recruitment and enrollment of subjects.

Any amendments to the protocol or consent materials will be approved by the IRB/IEC before they are implemented. IRB/IEC review and approval will occur at least annually throughout the enrollment and follow-up of subjects and may cease if annual review is no longer required by applicable regulations and the IRB/IEC. The investigator will notify the IRB/IEC of deviations from the protocol and reportable SAEs, as applicable to the IRB/IEC policy.
Each institution engaged in this research will hold a current Federalwide Assurance (FWA) issued by the OHRP for federally funded research.

9.2 Informed Consent Process

Informed consent is a process that is initiated prior to an individual agreeing to participate in a trial and continuing throughout the individual's trial participation. Before any study procedures are performed, informed consent will be obtained and documented. Subjects will receive a concise and focused presentation of key information about the clinical trial, verbally and with a written consent form. The explanation will be organized, and presented in lay terminology and language that facilitates understanding why one might or might not want to participate.

An investigator or designee will describe the protocol to potential subjects face-to-face. The key information about the purpose of the study, the procedures and experimental aspects of the study, risks and discomforts, any expected benefits to the subject, and alternative treatment will be presented first to the subject.

Subjects will also receive an explanation that the trial involves research, and a detailed summary of the proposed study procedures and study interventions/products. This will include aspects of the trial that are experimental, the probability for random assignment to treatment groups, any expected benefits, all possible risks (including a statement that the particular treatment or procedure may involve risks to the subject or to the embryo or fetus, if the subject is or may become pregnant, that are currently unforeseeable), the expected duration of the subject's participation in the trial, alternative procedures that may be available and the important potential benefits and risks of these available alternative procedures.

Subjects will be informed that they will be notified in a timely manner if information becomes available that may be relevant to their willingness to continue participation in the trial. Subjects will receive an explanation as to whether any compensation and any medical treatments are available if injury occurs, and, if so, what they consist of, or where further information may be obtained. Subjects will be informed of the anticipated financial expenses, if any, to the subject for participating in the trial, as well as any anticipated prorated payments, if any, to the subject for participating in the trial. They will be informed of whom to contact (e.g., the investigator) for answers to any questions relating to the research project.

Information will also include the foreseeable circumstances and/or reasons under which the subject's participation in the trial may be terminated. The subjects will be informed that participation is voluntary and that they are free to withdraw from the study for any reason at any time without penalty or loss of benefits to which the subject is otherwise entitled.
The extent of the confidentiality of the subjects' records will be defined, and subjects will be informed that applicable data protection legislation will be followed. Subjects will be informed that the monitor(s), auditors(s), IRB, NIAID, and regulatory authority(ies) will be granted direct access to the subject's original medical records for verification of clinical trial procedures and/or data without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations, and that, by signing a written informed consent form, the subject is authorizing such access.
Subjects will be informed that records identifying the subject will be kept confidential, and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available and, if the results of the trial are published, the subject's identity will remain confidential. Subjects will be informed whether private information collected from this research and/or specimens will be used for additional research, even if identifiers are removed.

Subjects will be allowed sufficient time to consider participation in this research trial, and have the opportunity to discuss this trial with their family, friends or legally authorized representative, or think about it prior to agreeing to participate.

Informed consent forms will be IRB-approved and subjects will be asked to read and review the consent form. Subjects must sign the informed consent form prior to starting any study procedures being done specifically for this trial.
Once signed, a copy of the informed consent form will be given to the subject(s) for their records. The subject(s) may withdraw consent at any time throughout the course of the trial. The rights and welfare of the subject(s) 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.

Study personnel may employ recruitment efforts prior to obtaining study consent if a patient-specific screening consent is on record or if the IRB has agreed that chart review is allowed without a fully executed screening consent. In cases where there is not a patient-specific screening consent on record, site Clinical staff may pre-screen via chart review and refer potential subjects to the Research staff. Research staff would obtain written consent per the standard informed consent process before conducting protocol-specific screening activities.

New information will be communicated by the site principal investigator to subjects who consent to participate in this trial in accordance with IRB requirements. The informed consent document will be updated and subjects will be re-consented per IRB requirements, if necessary. Subjects will be given a copy of all informed consent forms that they sign.

Three different consent forms (one for each randomization) will be applied at two time points in the study: first, a consent form will be applied before induction therapy. After patients have finished induction therapy, two additional ICFs will be applied for consolidation therapy: one for different durations of therapy and another for different antifungals. Once signed, a copy of each informed consent document will be given to the subjects for their records.

When the consent interview is conducted in English, the consent document should be in English. In Brazilian centers, both the ICF and consent interview will be conducted in Portuguese. When the study subject population includes non-English, non-Portuguese speaking people or the clinical investigator or the IRB anticipates that the consent interviews will be conducted in a language other than English or Portuguese, the IRB should require a translated consent document to be prepared and assure that the translation is accurate.

Identical (aside from ICF language) procedures will occur for the second informed consent conducted at 26 weeks for Aim 3.

9.2.1 Other Informed Consent Procedures

Use of a Legally Authorized Representative (LAR)

Potential subjects for this study are adults but may be unable to provide legally effective informed consent due to their health status (*i.e.*, dementia, intubated, sedated). The subjects may be enrolled in the study if consent is obtained from the LAR. The investigator will be familiar with the local IRB/IEC policy regarding the priority list of LAR and whether enrollment in a study is permitted by an advanced directive *(e.g.*, living will, durable power of attorney for proxy consent). Additionally, subjects will be informed about the study to the extent compatible with the person's understanding and assent may be obtained when applicable, and enrollment declined if the subject refuses participation.
If a LAR originally provides legally effective informed consent and the subject's condition improves, the subject will also be informed about the study as soon as is feasible and will be re-consented. The subject may continue in the study only if the subject's consent is provided. This is not technically required but we feel it is the ethically responsible procedure to put in place.

Illiterate Subjects
If illiterate**,** subjects will be consented according to the following procedures: information regarding the study will be provided in an understandable form and oral consent from the participant will be obtained. Additionally, agreement of illiterate participants should be indicated by including his/her thumb print on the ICF and a literate witness should sign the ICF.

9.3 Consent for Future Use of Stored Specimens and Data

Residual samples/specimens are those that are left over after protocol-specified testing and this study has been completed. Residual samples derived from venous blood samples or urine samples may be stored for possible use in future research studies, such as examining additional immunological assessments or testing for antibodies against other viruses or bacteria. These residual specimens will be stored coded indefinitely at UFCSPA or UMN. Brazilian specimens will preferentially be stored at UFCSPA unless specific experiments are to be conducted at UMN. Specimens may be shared with UMN or UFCSPA (from the other sites) depending on the scientific plan for those specimens. Transportation will occur with proper permits.

Additional venous blood samples specimens will be collected during this study specifically for the purpose of future immunologic and diagnostic testing. This is considered part of the study and is not optional. These samples/specimens collected during this trial specifically for the purpose of future research will be stored coded indefinitely at UFCSPA (Brazil) or UMN (KUMC samples). Specimens may be shared with UFCSPA or UMN depending on who will conduct the additional experiments.

There are no benefits to subjects in the collection, storage and subsequent future use of their samples/specimens. Future use samples/specimens will not be sold or used directly for production of any commercial product. Each sample/specimen will be encoded (labeled) only with a barcode and/or a unique tracking number that connects to a code key at the study site.

Restricted access to the code key is maintained by the principal investigator to protect subject confidentiality. Reports from future research studies performed using subjects' samples/specimens will NOT be kept in their health records.

When Protocol Purpose Includes Extra Samples
Subjects will **not** be given the option to decide if they want extra samples/specimens collected during this trial **specifically for the purpose of future research**. These samples/specimens are protocol-required; thus, subjects will be asked to consent to the future use of these samples/specimens as a condition of their study participation.

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

The study will enroll participants ≥18 years of age regardless of biological sex, sexual orientation, race or ethnicity. Disseminated histoplasmosis in immunosuppressed adult patients has a high mortality rate. Our expertise and prior work have focused on adult populations, and the need remains. There will be no upper age limit on potential study participants.

Children have different immune systems as compared to adults and the epidemiology of pediatric histoplasmosis is different from that of adults. Children also have different risk factors and clinical presentations of disease compared to adults. All of these considerations could affect patient selection and diagnostic performance, thus potentially biasing each aim of the proposed study. Second, the relative paucity of HIV-infected children due to the effective rollout of preventing mother-to-child HIV transmission contributes to the rarity of disseminated histoplasmosis in children. Those infants who do become HIV-infected generally receive effective ART. Thus, histoplasmosis is now rare in pediatric populations.

Prisoners will not be included due to ethical concerns related to their ability to give voluntary consent.

9.5 Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality includes documentation, investigation data, subject's clinical information, and all other information generated during participation in the study. No information concerning the study or the data generated from the study will be released to any unauthorized third party without prior written approval of NIAID and the subject. Subject confidentiality will be maintained when study results are published or discussed in conferences. The study monitor or other authorized representatives of the sponsor or governmental regulatory agencies 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.
All records will be kept locked and all computer entry and networking programs will be carried out with coded numbers only and with password protected systems. All non-clinical specimens, evaluation forms, reports, and other records that leave the site will be identified only by a coded number.

9.6 Costs, Subject Compensation, and Research Related Injuries

There is no cost to subjects for the research tests, procedures, and study product while taking part in this trial. Procedures and treatment for clinical care may be billed to the subject, subject's insurance or third party. Subjects may be compensated for their participation in this trial. Compensation will be in accordance with the local IRB's policies and procedures, and subject to IRB approval.

If it is determined by the site principal investigator that an injury occurred to a subject as a direct result of the tests or treatments that are done for this trial, then referrals to appropriate health care facilities will be provided to the subject. Study personnel will try to reduce, control, and treat any complications from this trial. Immediate medical treatment may be provided by the participating site. There is an insurance policy in place for the induction trial in Brazil to provide financial compensation for participants in the event harm is directly related to trial participation. Similar policies will be sought for this proposal.

10 STATISTICAL CONSIDERATIONS

10.1 Study Hypotheses

10.1.1 Study Hypotheses Aim 1:

We hypothesize that single high-dose LAmB will show non-inferior 2-week mortality compared to SOC, daily LAmB for moderate to severe histoplasmosis.

We also hypothesize Single high-dose LamB arm will be superior to SOC by the hierarchical composite end point analysis, driven by more frequent grade 3 and 4 laboratory abnormalities at 2 weeks and SAEs through 10 weeks with similar 10 week survival time.

10.1.2 Study Hypotheses Aim 2:

We hypothesize that Posaconazole will show non-inferior 26-week mortality compared to itraconazole for moderate to severe histoplasmosis consolidation.

We also hypothesize that Posaconazole will be superior to itraconazole by hierarchical composite end point analysis, driven by more frequent discontinuation of itraconazole due to intolerance.

10.1.3 Study Hypotheses Aim 3:

We hypothesize twenty-six weeks of consolidation therapy will show non-inferior SAE-free survival (i.e. alive without rehospitalization)compared to 52 weeks of consolidation therapy.

10.2 Sample Size Considerations

10.2.1 Sample Size Considerations Aim 1:

The sample size consideration for Aim 1 is mainly powered for non-inferiority comparison: Assuming 2-week mortality in the SOC group is 10% and using a two-sample Score test (Gart & Nam), a sample size 664 (332 in each arm) provides 90% power to assess the non-inferiority hypothesis with a 8% non-inferiority margin and a two-sided type 1 error rate of 0.05. This estimate includes up to 20 dropouts per arm. FDA suggests basing the non-inferiority margin on the effect size of the active control compared to non-treatment.[23] In this case, mortality of disseminated histoplasmosis in an immunocompromised host is 100% with no treatment. [24, 25]

Hypotheses: $H0: P1 - P2 \geq \delta 0$ vs. $H1: P1 - P2 < \delta 0$

Table 3: Sample Sizes Required for Various Mortality Rates

Power	N Per Arm	N Total	SOC Mortality	Experimental Group Mortality	Non-Inferiority Margin δ0	Actual Difference δ1	Alpha (1-sided)

				Upper 95%CI			
0.9002	164	328	0.04	0.12	0.08	0	0.025
0.9003	189	378	0.05	0.13	0.08	0	0.025
0.9004	214	428	0.06	0.14	0.08	0	0.025
0.9005	239	478	0.07	0.15	0.08	0	0.025
0.9008	264	528	0.08	0.16	0.08	0	0.025
0.9003	288	576	0.09	0.17	0.08	0	0.025
0.9001	312	624	0.10	0.18	0.08	0	0.025

An additional 20 participants are added per arm in the event of dropouts (i.e. withdrawal of consent) for a sample size of 332 per arm and 664 overall.

10.2.2 Sample Size Considerations Aim 2:

Only participants randomized at baseline of HISTO-FACT will be included for Aim 2. Sample size for Aim 2 is powered for non-inferiority comparison in mortality*:* Assuming the death event rate in the SOC arm is 25% at 26 weeks(based on the phase II trial) and the non-inferiority margin to be Δ_0 =12%, and the statistical significance level to be one-sided alpha = 0.025, a total sample size of 550 (275 in each arm) provides 90% power for the non-inferiority assessment (Table). If the 26-week mortality is lower, the necessary sample size to show non-inferiority decreases.

Table 4: Sample Sizes Required for Various Mortality Rates

Power	N Per Arm	N Total	SOC 26- week Mortality	Experimental Group Mortality Upper 95%CI	Non-Inferiority Margin δ0	Actual Difference δ1	Alpha (1-sided)
0.900	170	340	0.125	0.245	0.12	0.0	0.025
0.901	194	388	0.150	0.270	0.12	0.0	0.025
0.900	216	432	0.175	0.295	0.12	0.0	0.025
0.900	237	474	0.200	0.320	0.12	0.0	0.025
0.901	257	514	0.225	0.345	0.12	0.0	0.025
0.901	**275**	**550**	**0.250**	**0.370**	**0.12**	**0.0**	**0.025**
0.900	291	582	0.275	0.395	0.12	0.0	0.025
0.900	306	612	0.300	0.420	0.12	0.0	0.025

10.2.3 Sample Size Considerations Aim 3:

Using a parallel, two-group design where the groups are 26 versus 52 weeks of consolidation therapy with a 5% non-inferiority margin with 75% power and a one-sided type 1 error rate of 0.025 and assuming 4.3% incidence of serious adverse event or death between 26 and 52 weeks with SOC based on the phase II trial would require a total of 468 evaluable persons (234 persons in each arm) at the time of randomization, which will occur at Week 26.[1] Assuming 25% mortality by 26 weeks based on the phase II trial, a total of at least 624 persons would need to be

enrolled at the start of the trial to retain 468 persons total (234 per group) to be randomized at Week 26 for Aim 3.This aim will include only persons enrolled in Brazil. We will include patients still in follow up for the ongoing trial of induction therapy to increase the size of those enrolled in Aim 1 in this R01 making the enrollment goal of 624 feasible (combining n=524 enrolled in R01 Aim 1 in Brazil and n=~100 enrolled in ongoing trial to be reconsented for required Aim 3 follow up).

10.2.4 Hierarchical Testing Strategy and Power Assessment

A hierarchical testing strategy will be used to assess superiority of the primary endpoint and key secondary endpoint so that the type-one-error rate can be maintained. Details of the hiearchical testing order will be specified in the SAP. The power assessment of the hierarchical testing strategy and how that may be impacted by event rates and correlations among the endpoints will also be assessed and specified in the SAP.

The power and type-one-error assessment of the potential impact of dropping one of the randomization is also assessed in the SAP.

10.3 Treatment Assignment Procedures

10.3.1 Randomization Procedures

The trial will be randomized. Randomization will be carried out by staff at each site using automated software. The first randomization (factorial assignment of study arms for Aims 1 and 2) will occur at enrollment. The second randomization (Aim 3) will occur after 26 weeks of consolidation are completed among survivors and will be stratified by their Aims 1 and 2 randomization assignments.

Patients will be equally allocated to each study invention or control for each aim (1:1 ratio). Comparisons will be to concurrently randomized controls, only. Randomization will be permutated blocks of unequal size. The factorial randomization will be stratified by country. The second randomization, which only occurs in Brazil, will be stratified by the randomization assignment from Aim 1 and 2.

The research pharmacist shall maintain the scientific integrity of the trial by managing access to randomization-assignment records. The dispensing pharmacists will maintain the scientific integrity by ensuring that the prescribed drugs are correctly dispensed. All pharmacy accountability shall be maintained in logs.

Copies of prescriptions (drug disbursement forms) for which trial subjects have been issued, log books pertaining to the trial shall be kept in a drug cabinet which will be locked at all times and accessed only by the Pharmacist, Study Coordinator, or other essential clinic personnel for patient clinical care until the study is completed.

10.3.2 Masking Procedures
There will be no masking procedures as this is an open label trial.

10.4 Planned Interim Analyses
A DSMB will review interim safety and efficacy data at least annually.

10.4.1 Interim Safety and Efficacy Review
Primary endpoints will be reviewed. Early stopping for efficacy will use a Haybittle-Peto stopping rule (P<0.001). Early stopping for futility will be recommended if the conditional power is less than 20%, or if the observed mortality rate in the experimental arm exceeds the non-inferiority margin after 50% of the participants have reached the study endpoint. If the experimental arm closes early after DSMB review, we would either give all patients SOC (if stopped for safety) or the experimental regimen (if stopped for superiority) for that treatment component. Enrollment for the other study components would continue. There will be no impact of the interim analyses on the final analyses unless a study arm is stopped early. The possibility of Type I or Type II error, in that case, will be mitigated by firm stopping rules.

10.5 Analysis Plan
Additional details are detailed in the Statistical Analysis Plan (SAP). The SAP supersedes the protocol for analysis purposes.

For each aim, there will be three analysis datasets:

- Efficacy analysis dataset: all participants randomly assigned to study intervention by intent-to-treat (or modified intent-to-treat* for Aim 2) with confirmed/probable histoplasmosis**
- Full dataset: all participants randomly assigned to a study intervention by intent-to-treat (or modified intent-to-treat for Aim 2)
- Safety analysis dataset: all participants randomly assigned to study intervention and who take at least 1 dose of the study intervention. Participants will be analyzed according to the study intervention they received.

The primary endpoint, key secondary endpoint and secondary efficacy endpoints will be evaluated using the efficacy analysis dataset. The full analysis dataset may be used for conducting sensitivity analysis for the primary endpoints. Safety endpoints will be evaluated using the safety analysis dataset.

*Analysis datasets for Aims 1 and 3 are defined on the basis of intention-to-treat . Analysis datasets for Aim 2 are defined on the basis of modified intention-to-treat, which only includes participants who are alive and still in follow-up upon the initiation of consolidation therapy..

**Confirmed histoplasmosis is defined as positive test by PCR or other molecular test for

Histoplasma species, culture, histopathology. Probable is positive by EIA antigen test. Some persons will have positive results for the non-FDA approved lateral flow assay antigen test (MiraVista) but negative results by other testing. Those participants will not be included in the primary analysis but will be considered as part of a sensitivity analysis. Those positive by the lateral flow assay must also have a positive result by another test.

10.5.1 Analysis Plan Aim 1:

Baseline characteristics and patient outcomes (rehospitalization, IRIS, clinical response, use of additional amphotericin, duration of hospitalization, percentage urine antigen and PCR decreases) of the SOC and experimental groups will be compared using the Mann-Whitney U test for continuous variables and Cohran-Mantel-Hanzel test for categorical variables. Survival time will be calculated as the days from enrollment. For the primary endpoint of 26-week mortality, the primary analysis will be based on the absolute risk difference between the experimental vs SOC group (regardless of consolidation therapy assignment) versus the standard-of-care (SOC) control group for all-cause mortality and its corresponding two-sided 95% confidence interval (CI) will be calculated using Miettinen-Nurminen method. The two-week all-cause mortality in the experimental group will be declared non-inferior to controls if the upper bound of the two-sided 95% confidence interval (CI) of the difference ($p_{experimental} - p_{soc}$) is less than 8%. If non-inferiority is met, the superiority hierarchical composite end point analysis will be conducted via Win Ratio.

Given that the randomized consolidation therapies start at Day 2 for the single high dose LAmB arm, the two-week mortality for single high dose LAmB combined with Posaconazole or Itraconazole will also be reported. Additional comparisons between single high dose LAmB + itraconazole and single high dose LAmB + posaconazole vs. SOC will be assessed. Two-week and ten-week survival will be assessed via time-to-event analyses summarized by Kaplan-Meier curves, mean survival estimates and 95%CI. Mortality data for sub-groups of interest including age, HIV status, ART status, CD4 count, setting, underlying risk factor, site of disease (local vs. disseminated) and sex as a biological variable will be considered hypothesis generating. Refer to the SAP for more details including type one error control strategies.

Paradoxical IRIS will be defined as clear improvement of symptoms from histoplasmosis, new start or change in ART usage or regimen with subsequent worsening of symptoms and no microbiological evidence of worsening infection or concurrent infection with another pathogen.[28, 29] For PCR performance, invalid tests will be counted as negative results (i.e., not excluded). Sensitivity, specificity, negative predictive value and positive predictive values for PCR will be completed at baseline using MSGERC/European Organization for Research and Treatment of Cancer definitions of definite and probable histoplasmosis.[18]

SAEs, laboratory AEs, and clinical AEs as per the NIAID DAIDS toxicity grading table will be separately compared between the experimental and SOC groups for the frequency of having at least one event and the mean number of events with linear and logistic regression models. If the cumulative incidence of a lab AE for any given anomaly (e.g., anemia) is >10%, they will be individually summarized, and AE incidence tested between groups by Fisher's Exact test. Laboratory values specifically considered as endpoints above such as hemoglobin and creatinine will be summarized regardless of incidence.

10.5.2 Analysis Plan Aim 2:

Survival time will be calculated as the days from enrollment.
The absolute risk difference between the experimental group versus standard-of-care control group in all-cause mortality and its corresponding two-sided 95% confidence interval (CI) will be calculated using the Miettinen-Nurminen method, stratified by the induction treatment arm. The all-cause mortality in the experimental arm will be declared non-inferior to standard of care (SOC) controls if the upper bound of a two-sided 95%CI of the absolute risk difference of mortality ($p_{experimental} - p_{soc}$) is less than 12%.
As a sensitivity analysis, we will use a time dependent Cox model to account for the different starting time of consolidation therapies in the single high-dose liposomal amphotericin arm's transition to consolidation therapy at day 2 and the SOC induction arm's transition to consolidation at after day 14. Pre-specified baseline variables that will impact the prognosis (HIV status, ART status, CD4 counts, site of disease (local vs. disseminated) will be adjusted as covariates in the time-dependent Cox model to account for potential imbalances.

If non-inferiority is met, the superiority hierarchical composite end point analysis will be conducted via Win Ratio. Additional comparisons between posaconazole and itraconazole will also be made within the LAmB treatment arms guarded with appropriate type-one-error adjustments. Sub-group analysis will be hypothesis generating. Baseline characteristics and patient outcomes (clinical response, rehospitalization by 26 weeks, study medication discontinuation due to treatment failure, percentage urine antigen and PCR decreases) of the SOC and experimental groups will be compared using the Mann-Whitney U test for continuous variables and Fisher's exact test for categorical variables. AEs including SAEs will be analyzed as in Aim 1 by grade through 10 weeks with attention to liver function tests and incidence of AEs associated with study medication discontinuation, interruption, or dose reduction will be noted. The same will be recorded through 26 weeks for Aim 2.

10.5.3 Analysis Plan Aim 3:

The primary end point of SAE-free survival from 26 to 52 weeks among those who survived 6 months will be conducted comparing 26 to 52 weeks of consolidation. The absolute risk

difference between the experimental group versus standard-of-care control group in SAE and all-cause mortality and its corresponding one-sided 95% (or two-sided 90%) confidence interval (CI) will be calculated using Miettinen-Nurminen method stratified by induction and consolidation treatment groups. The absolute risk difference of SAE or death in the 26 week arm will be non-inferior to SOC if the upper bound of the one-sided 95%CI of the difference ($p_{experimental}$ - p_{soc}) is less than 5%.

Sub-group analysis will be hypothesis generating. Risk factors for SAE or death from 26 to 52 weeks evaluated. Possible risk factors of interest a priori include antifungal and antiretroviral drug concentrations at 26 weeks, CD4 and HIV viral load at 26 weeks. Baseline characteristics and patient outcomes (lost to follow up and restart/switch in antifungal) of the SOC and experimental groups will be compared using the Mann-Whitney U test for continuous variables and Fisher's exact test for categorical variables. AEs including SAEs will be analyzed as in Aim 1. Discontinuation of study medication due to AE will be tracked for the SOC arm.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Each participating site will maintain appropriate medical and research records in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. Each site will permit authorized representatives of the NIAID, its designees, and appropriate regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. These representatives will be permitted access to all source data and source documents, which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, subjects' memory aid or 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.

12 QUALITY CONTROL AND QUALITY ASSURANCE

Following a written DMID-accepted site quality management plan, each participating site and its subcontractors are responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The site principal investigator will provide direct access to all study-related sites, source data/data collection forms, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities. The site principal investigator will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

The DCC 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 participating site(s) for clarification and resolution.

13 DATA HANDLING AND RECORD KEEPING

13.1 Data Management Responsibilities

All personal identifiers will be removed from collected data and replaced with unique subject identifiers. Data will be stored securely in password-protected REDCap databases with restricted access limited to authorized personnel and all research staff will undergo training on confidentiality protocols.

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 or blue permanent 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 eCRF will be provided for use as source data collection forms and maintained for recording data for each subject enrolled in the study. Data reported in the eCRF derived from source data collection forms should be consistent or the discrepancies should be explained.

To ensure that data collected are accurate, consistent, complete and reliable, CRFs will be designed to minimize errors, with clear definitions for each data point. Standardized formats will be used to ensure consistency across all study sites. Step-by-step instructions for completing forms will be provided in SOPs, which will outline how to accurately capture data.

All data entry personnel will receive training on data handling procedures and regular data entry audits will be performed in order to identify discrepancies. Metrics for monitoring compliance and consistency include completeness checks, query resolution rates and adherence to protocol requirements.

The sponsor and/or its designee will provide guidance to the site principal investigators and other study personnel on making corrections to the data collection forms and eCRF.

13.2 Data Coordinating Center/Biostatistician Responsibilities

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

The data coordinating center (UMN) is responsible for performing regular data audits and queries for the study sites when necessary, as well as training data entry personnel.

The data coordinating center for this study will be responsible for data management, quality review, analysis, and reporting of the study data. At the end of the study, a copy of all datasets will be provided to NIAID.

13.3 Data Capture Methods

Clinical (including, but not limited to, AE/SAEs, concomitant medications, medical history, physical assessments, and clinical laboratory values) data will be collected on data collection forms by study personnel then entered into eCRFs via a 21 CFR Part 11-compliant internet data entry system provided by the study data coordinating center. The data system (REDCap) includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate.

13.4 Types of Data

Data for this trial will include clinical, safety, and outcome measures (e.g., clinical laboratory values, and pharmacokinetic data) in one database

13.5 Study Records Retention

Study records and reports including, but not limited to, eCRFs, source documents, ICFs, laboratory test results, and study drug disposition records will be retained for 2 years after a marketing application is approved for the study product for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for the study product, until 2 years after the investigation is discontinued and the FDA has been notified. These documents will be retained for a longer period, however, if required by local regulations. ICFs for future use will be maintained as long as the sample/specimen exists.
No records will be destroyed without the written consent of the sponsor. It is the responsibility of the sponsor to inform the site principal investigator when these documents no longer need to be retained.

14 CLINICAL MONITORING

Site monitoring is conducted to ensure that the human subject protection, study and laboratory procedures, study intervention administration, and data collection processes are of high quality and meet the sponsor, ICH E6, GCP and regulatory guidelines. Site monitoring will also ensure that this trial is conducted in accordance with the protocol, protocol-specific MOP and applicable sponsor standard operating procedures. A separate remote monitoring plan will be developed by to describe who will conduct the monitoring, at what frequency, in what level of detail, and who will be responsible for ensuring that monitoring findings are addressed. Monitoring will focus on:

- Review of regulatory files,
- Accountability records
- eCRFs
- informed consent documentation,
- eligibility criteria,
- medical and laboratory reports,
- protocol and GCP compliance, and
- endpoint ascertainment.

Site monitors will have access to each participating site, study personnel, and all study documentation according to the site monitoring plan. Study monitors will meet with site principal investigators to discuss any problems and actions to be taken, and will document site visit findings and discussions.

15 PUBLICATION POLICY

Following completion of the study, the Principal Investigators are expected to publish the results of this research in a scientific journal. 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 on 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 the date of publication.

As of January 2018, all clinical trials supported by the NIH must be registered on ClinicalTrials.gov, no later than 21 days after the enrollment of the first subject. Results of all clinical trials supported by the NIH, generally, need to be submitted no later than 12 months following the primary completion date. A delay of up to 2 years is available for trials that meet certain criteria and have applied for certification of delayed posting.

As part of the result posting a copy of this protocol (and its amendments) and a copy of the Statistical Analysis Plan will be posted on ClincialTrials.gov.

For this trial the responsible parties are Drs. Nathan Bahr and Alessandro Pasqualotto which will register the trial and post results.

The responsible party does not plan to request certification of delayed posting.

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17 APPENDICES

Schedule of Events

Toxicity Grading Scales

Appendix A. Schedule of Events

		Screen	Enrolment / D1	D2	D4	D 7	D 14	Wk 4	Wk 10	Wk 18[1]	Wk 26	Wk 40[1]	Wk 52	Study End[2]	Sick Visit
Signed Consent Form		●													
Assess Eligibility Criteria			●												
Review Medical History			●												
Aims 1 and 2 Randomization			●												
Aim 3 Randomization											●				
Assess Clinical Status		●	●	●	●	●	●	●	●	●	●	●	●	●	●
Assess Adverse Events			●	●	●	●	●	●	●	●	●	●	●	●	●
Clinical Lab Tests	HIV Test		●												
	CD4 count		●								●[3]				
	Complete blood count		●		●	●	●								●
	ALT, alkaline phosphatase, total bilirubin		●		●	●	●	●	●						●
	Sodium, Creatinine, Potassium, Magnesium		●		●	●	●								●
	PCR		●		●	●	●	●							
	Histoplasma antigen[a]		●				●		●	●[1]	●	●[1,**]	●**		●
	Itraconazole Level (Aim 1, single dose)					●*									●
	Itraconazole Level (Aim 2, daily dose)							●*							●
	Urine Pregnancy Test	●													
	HIV viral load (local standard of care)		●								●***				●
Blood storage for PK and research studies (Aim 1 single dose)			●[b]			●*					●				
Blood storage for PK and research studies (Aim 1 daily dose)			●[b]					●*			●				
ART initiation/change							●								

Hematology – Hemoglobin, hematocrit, WBC and differential count, platelet count.

Biochemistry – Sodium, potassium, magnesium,, urea, creatinine, glucose, bicarbonate, albumin, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase. **Other** – itraconazole serum levels, *Histoplasma* urinary antigen, *Histoplasma* PCR, urine pregnancy test.

Study Intervention – Liposomal amphotericin B is to be administered either once or daily for 14 days (according to study arm). Consolidation therapy (either itraconazole or posaconazole) is to be administered for 26 to 52 weeks (according to study arm).

At baseline, all procedures should be done before study intervention.

[a]Excess urine collected from antigen collection should be stored for future diagnostic test evaluation. At D1, ~30mL of urine should be stored for future research use.

[b]Research only collection at D1 for future studies (e.g., no PK study). Sample storage for other time points for PK studies. [1]If the patient is unable to make an in-person visit, Week 18 and Week 40 may by virtual/phone visits. Thus, *Histoplasma* urine antigen will be considered optional for those visits, if participants are seen in person, an urine antigen collection is expected.

[2]Virtual/phone visit. Week 56 for all those who complete Aim 3, Week 30 for those who only complete Aims 1 and 2.

[3:] Only persons enrolling in Aim 3 with ART adherence concerns

*Drug levels should be done after at least 7 days of azole therapy at the next study visit. Itraconazole levels will be done locally as per standard of care, when available. Blood storage for PK and research studies will be stored and shipped to UMN later.

**Sub-study at select sites in Brazil only

*** Aim 3 only

Study Windows:
D1: no window
D2: +1 day
D4: +2 days
D7: +3 days
W2: -3 days, + 7 days

W4: -6 days, + 7 days

Weeks 10, 18, 26, 40 have a ±7 day window.
Weeks 52 and 56 (and 30 for those who do not enroll in Aim 3) have a ±14 day window.

Appendix B. Toxicity Table Information

Lab abnormalities will be summarized from the statistical database and graded for severity according to the NIAID Division of AIDS (DAIDS) Toxicity Table. All grades 1-5 will be summarized. These laboratory abnormalities will be summarized separately from clinical AEs. As the DAIDS Toxicity Table is a 32 page document, it has not be included herein but is available at the cited website.[30]