Optimizing the Dose of Flucytosine for the Treatment of Cryptococcal Meningitis.

Abbreviation: FLOOR

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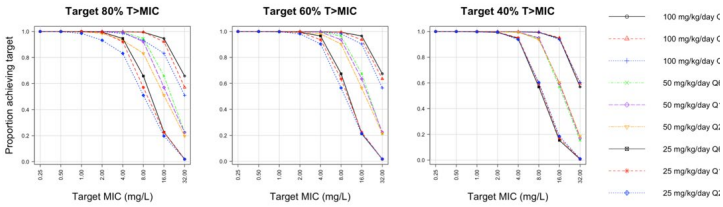
Protocol Summary

Title:	Optimizing the Dose of Flucytosine for the Treatment of Cryptococcal Meningitis.
Abbreviation:	FLOOR
Phase:	**II**
Population:	Phase II: HIV-infected persons in Uganda with cryptococcal meningitis
Rationale:	Cryptococcal meningitis (CM) is a fungal infection that causes a severe syndrome of meningitis that is 100% fatal without antifungal therapy. Even with antifungal therapy, mortality rates remain high, especially in sub-Saharan Africa where the ongoing HIV/AIDS pandemic leads to higher prevalence of cryptococcosis. Combination of amphotericin and flucytosine (5-FC) is the mainstay of therapy for the initial management of CM. Indeed, it has even been shown that effective delivery of these therapies in sub-Saharan Africa can lower mortality rates by 90%. However, even while 5-FC is included on the WHO list of essential medicines, its availability world-wide is limited. Since 5-FC has few clinical indications, it is not manufactured widely, leading to high costs for the medication, and limited distribution in sub-Saharan Africa. While early studies of 5-FC in CM were done in the pre-AIDS era, the dose of 150 mg/kg/day was selected and resulted in significant toxicities, including leukopenia and thrombocytopenia requiring dose reduction or discontinuation of 5-FC. Later retrospective studies of dosing in the AIDS era were inconclusive about the ideal dose to benefit efficacy and toxicity. In 1997, van der Horst performed the first major clinical trial for AIDS related CM demonstrating efficacy of combination of 5-FC with amphotericin B. The selected dose was 100 mg/kg/day, which has been used as standard dosing ever since. Optimizing dose necessary for good clinical outcomes could reduce the daily dose and increase the total number of patients able to be treated at the same cost and supply. In our own studies of *Cryptococcus* in Uganda, the MIC_{50} is approximately 4-8 mg/L. Preliminary models of dosing based on AMBITION pharmacokinetics of 5-FC has shown that lower doses may still be acceptable at targets of 40% or 60% time over MIC.

	Results: *hypothetical* flucytosine probability of target attainment %T>MIC: *TARGET IS NOT KNOWN*  ICCC 2023 Population Pharmacokinetics from AMBITION-cm
Description of Study Intervention:	Standard dosing for 5-FC is 100 mg/kg/day divided in 4 doses over waking hours for 7-14 days during the period of induction therapy. We will deliver doses of: 1. 60mg/kg/day in 3 divided doses for 10 days This most commonly will be 2 500mg tablets three times per day
Prelim Data	Molloy et al. demonstrated in the ACTA trial published in 2018 that 1 week of IV Amphotericin B 1 mg/kg plus oral 5-FC 100 mg/kg is the optimal management for the first week of CM. These are shown to have benefits in mortality and similar rates of fungal clearance from the CSF to other combination regimens.
Phase II Study Design	Prospective, open-label trial to compare the efficacy and safety of lower doses of 5FC during induction therapy to historical controls with standard 5FC dosing. 1. Participants in the trial will receive 60mg/kg/day of 5-FC in 3 divided doses for 10 days, n=36 2. Single-dose liposomal amphotericin (10mg/kg) is preferred, if available. 3. Amphotericin B 0.7-1.0 mg/kg/day may be used if needed. 4. Historical controls drawn from the AMBITION trial will be used as a comparison group, selected weighted by inclusion/exclusion criteria, baseline characteristics and therapies received. Induction therapy for control group participants followed the 2018 WHO cryptococcal guidelines with 7 days of 5-FC 100mg/kg/day and 7 days of IV Amphotericin deoxycholate followed by 1200mg fluconazole/day for 7 days. The intervention group received single-dose liposomal amphotericin plus 5-FC and fluconazole 1200 mg/day.

	All participants will receive fluconazole 1200mg/day during consolidation therapy from day 1 to 14 then 800mg/day from day 15 to 10 weeks, and 200mg/day after 10 weeks. All participants will receive lumbar punctures at diagnosis, day 3, day 5-7, day 10-14, and additionally as required for control of intracranial pressure and documentation of CSF sterilization. Controls from Ambition will be matched for the same LP windows. Therapeutic LPs conducted during the first week have a ~70% relative survival benefit.
Phase II Study Endpoints	**Primary:** 1. Rate of CSF *Cryptococcus* clearance (Early Fungicidal Activity, or EFA), quantified by the change of $\log_{10}$ *Cryptococcus* CFU/mL CSF/day as measured by serial quantitative CSF fungal cultures over ~2 weeks **Secondary:** 1. CSF culture sterility cumulative incidence over 18 weeks 2. 18-week survival time **Key Secondary:** 1. Desirability of Outcome Response (DOOR) as ordinal ranked maximum score tested by Win Ratio. a. 18-week Survival with CSF sterility by 2-weeks b. 18-week survival with CSF culture positivity beyond 2 weeks c. Grade 3 hematological adverse event by 2 weeks d. Grade 4 hematological adverse event by 2 weeks e. Lost to follow up before 18-weeks f. Serious Adverse Event through 18 weeks (e.g. all-cause re-hospitalization, permanent neurologic deficit) g. Death by 18-weeks **Safety & Exploratory:** 1. Incidence of Grade 3-5 Laboratory abnormalities over 2 weeks 2. Incidence of Clinical Grade 3-5 Adverse Events or Serious Adverse Events (SAEs) over 2 weeks 3. Pharmacokinetics of 5FC in blood, CSF, and bodily fluids / tissues.
Phase II Duration:	18 weeks
Phase II Number of Participants	36 CSF culture positive participants with an EFA estimate With adaptive sample size up to 50 CSF CrAg+ participants consented
Phase II Inclusion Criteria	● CSF cryptococcal antigen (CrAg) positive meningitis ● Ability and willingness to provide informed consent ● Willing to receive protocol-specified lumbar punctures

Phase II x Criteria	• Age < 18 years • Inability to take enteral (oral or nasogastric) medicine • Cannot or unlikely to attend regular clinic visits • Receiving chemotherapy or corticosteroids • Suspected Paradoxical immune reconstitution inflammatory syndrome (IRIS) • Pregnancy or breastfeeding • CrCl < 20 mL/minute • Absolute neutrophil count < 500 x10^6 cells/L • Thrombocytopenia < 50,000 x 10^6 cells/L • Patients with prior 5-flucytosine exposure >3 days in the 12 months prior to enrollment • Any condition for which participation would not be in the best interest of the participant or that could limit protocol specified assessments.
Phase II Study Drug and Dosing	● Participants will receive 5-FC 60mg/kg/day for 10 days. ● Participants will receive IV AMB 0.7-1.0 mg/kg/day for 7 days. ● Liposomal Amphotericin B single IV dose 10 mg/kg is preferred, when available. ● All participants will receive fluconazole 1200 mg/day during consolidation therapy from day 1 to 14 then 800 mg/day from day 15 to 10 weeks, and 200mg/day after 10 weeks.
Phase II Monitoring	• All participants will have CSF quantitative fungal cultures performed on Days 1, 2-4, 5-7, and 10-14 of antifungal treatment. • 3x weekly CBC, chemistries, and kidney function tests for duration of induction therapy during hospitalization
Phase II Safety Analysis	The Data Monitoring Committee (DMC) will conduct interim safety analysis after 10 participants are enrolled with >2 weeks of accrued data and calculable EFA. EFA and culture positive Cryptococcus relapse will be monitored by the trial investigators, and if statistical evidence shows that EFA <0.2 log10 CFU/mL/day, then an early DMC will be called.
Phase II Statistical Design Rationale	Early fungicidal activity (EFA) is a quantitative measure of antimicrobial activity for an antifungal regimen in human patients with cryptococcal meningitis. In numerous trials, EFA correlates well with 10-week mortality. Specifically, EFAs worse than 0.20 $\log_{10}$ CFU/mL/day are associated with higher mortality (>50%) and EFA better than 0.3 $\log_{10}$ CFU/mL/day is associated with lower mortality (30-40%). EFA is calculated by measuring the slope of the clearance rate of yeast from CSF, which is calculated from longitudinal quantitative CSF cultures collected over two weeks. By intent to treat analysis, all participants who are enrolled in this study will be included for analysis.
Clinical Trial Sites	1. Mulago Hospital, Kampala, Uganda 2. Kiruddu Hospital, Kampala, Uganda 3. Mbarara Regional Referral Hospital, Mbarara, Uganda

Estimated Time to Complete Enrollment:	Up to 12 months

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Document History

Document	Version Date	Summary of Changes
Original Protocol	30 March 2023	
Original Protocol	14 August 2023	Response to Mulago IRB and UNCST comments. Clarifications of the therapies to be used. Additional evidence added to the background/justification. Minor additions to the protocol listed in the cover letters.
Original Protocol	22 March 2024	Updated the schedule of events and exclusion criteria for better efficiency and to align with the schedule that was used for the comparator group. Response to Mulago REC comments.

Abbreviations

ACTA	Advancing cryptococcal meningitis treatment for Africa
AE	Adverse event
AMB	amphotericin B deoxycholate
ART	antiretroviral therapy
CBC	complete blood count
CDC	Centers for Disease Control and Prevention
CFU	colony forming units
CHAI	Clinton Health Access Initiative
CM	cryptococcal meningitis
COAT	Cryptococcal Optimal ART Timing
CrAg	cryptococcal antigen
CRP	C-reactive protein
CSF	cerebrospinal fluid
CTCAE	Common Terminology Criteria for Adverse Events
DAIDS	Division of AIDS (NIH, NIAID)
DSMB	Data & Safety Monitoring Board
EFA	Early Fungicidal Activity
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GCS	Glasgow Coma Scale
GI	Gastrointestinal
GRAS	generally recognized as safe
ICP	intracranial pressure
IDSA	Infectious Diseases Society of America
IRB	Institutional Review Board
IRIS	immune reconstitution inflammatory syndrome
IQR	interquartile range
IV	intravenous
LFT	liver function tests
LP	lumbar puncture
MOP	manual of operations
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NG	nasogastric
OI	opportunistic infection
PHQ-9	patient health questionnaire – 9
PO	*per os* (by mouth)
SAE	Serious adverse event
SOC	Standard of Care
SoE	Schedule of Events
SOPs	Standard Operating Procedures
SRA	Stringent Regulatory Authority
TEAEs	Treatment emergent adverse events

1. KEY ROLES

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2. BACKGROUND AND SCIENTIFIC RATIONALE

2.1 Background Summary and Study Hypotheses

Cryptococcal meningitis (CM) has emerged as one of the most frequent and deadly opportunistic infections in HIV patients, with a global burden estimated at nearly 1 million cases annually.[1] Early mortality from HIV-associated cryptococcal meningitis remains unacceptably high, in large part due to the high cost, toxicity, and relatively limited repertoire of effective antifungals. The essential role of 5-FC in reducing fungal burden in the first week of therapy has now been firmly established.[2–4] Importantly, access to the important medication 5-FC remains limited in settings where CM incidence and mortality is highest.

The Advancing Cryptococcal Meningitis treatment for Africa trial (ACTA) recently demonstrated in a high quality, randomized controlled trial that Amphotericin B (AMB) in combination with 5-FC had a 38% reduced mortality at 10-week follow-up when compared to fluconazole used in combination with AMB.[2] A cost-effectiveness analysis based on these data showed that the cost of saving 1 life from CM would be only $973.[5]

While there are 3 stringent regulatory authority (SRA) approved manufacturers of 5-FC, for decades these companies have not been registered in Africa.[6,7] Consequently, this low-cost, off-patent, life-saving medication has been widely unavailable in the region of the world where it is needed most. Vitaris, one of the SRA approved manufacturers of 5-FC received WHO pre-qualification to produce 5-FC tablets in 2018.[8] This led to registration in South Africa and development of an access program that provided 5-FC free of charge to tertiary centers throughout the country.[9] In this project, after 5-FC was provided free of charge, these centers saw an 11% mortality reduction compared to a period before 5-FC was freely available.[9]

While funding and production remain limiting factors on distribution of 5-FC in these critical areas, another aspect in need of attention is the optimal dosing of 5-FC. Early studies of 5-FC in CM used doses as high as 150 mg/kg/day.[10,11] In these studies, leukopenia and thrombocytopenia were common, seen in up to 30% of patients receiving 5-FC. Diarrhea and rash were also seen but less frequent.[10,11] Subsequent studies used a lower dose of 5-FC and 100mg/kg/day and found no difference in toxicity compared to groups treated without 5-FC.[12] While these studies show that 5-FC can be used safely and effectively at 100 mg/kg/day, no study has yet shown whether lower doses might yet be effective. This crucial point could greatly affect access to 5-FC by reducing the cost and supplies needed for any given patient with CM.

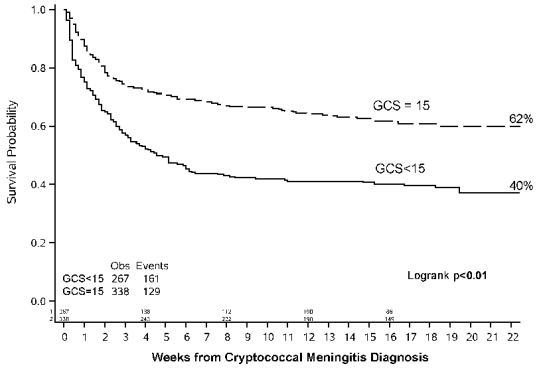
2.2 Burden of Cryptococcal Meningitis

Cryptococcal meningitis has become the most common cause of adult meningitis in many parts of Africa,[13] where cryptococcosis now rivals tuberculosis in all-cause mortality.[14] While long-term survival has improved with widespread use of antiretroviral therapy in high-income countries, early mortality remains high.[15] Furthermore, expanding access to antiretroviral therapy in resource-limited

settings has not yet led to compelling improvements in mortality, with 10-week mortality rates between 24% and 37%, even under optimal research conditions.[16,17] Early mortality rates are often ~70% in routine practice where access to diagnostics or medications is limited or unavailable, intracranial pressure is uncontrolled, or in settings where other barriers to the management of cryptococcal meningitis exist.[18–20] Intravenous administration of amphotericin B deoxycholate is not often possible in resource-limited settings, even when it is available.

Even with amphotericin-based induction therapy, survival is not optimal. Figure 2.2 displays the survival in Uganda from 2013-2017 in the setting of a study using IV amphotericin + fluconazole induction therapy and control of intracranial pressure.[21,22] Approximately, half of the mortality occurs during the first two weeks and the other half during the next 18 weeks, particularly in the first 6 weeks (Figure 2.2). This 18-week time period captures 95% of the 5-year mortality in Uganda.[23]

Figure 2.2: Survival After Cryptococcal Meningitis by Glasgow Coma Scale (GCS) in Uganda



GCS < 15 = altered mental status, GCS = 15 = intact mental status, responsive to voice

The efficacy and safety of induction therapy for cryptococcal meningitis can be evaluated effectively with all-cause mortality as an early clinical outcome. This is because approximately half of the mortality related to CM occurs during the first two weeks.[24] 18-week outcomes will also be assessed for further evaluation of primary and secondary endpoints.

Table 2.2: The 2-week mortality of Ugandans on Amphotericin + Flucytosine with GCS=15

Study	Number of Patients	Number Died	2-week Mortality	95% Confidence Interval
EnACT Stage 1-3	25	1	4.00%	-3.7%, 11.7%

Jarvis et al. NEJM 2022; Uganda GCS=15 data	197	17	8.60%	4.7%, 12.6%
Average	**222**	**18**	**8.10%**	**4.5%, 11.7%**

2.3 Treatment of Cryptococcal Meningitis

Standard cryptococcal meningitis treatment is divided into three phases: a 2 week induction phase, followed by an 8-week consolidation phase, and an extended maintenance phase thereafter for secondary prophylaxis.[24] Table 2.3 outlines the treatment recommendations for cryptococcal meningitis in HIV-infected individuals. Historically, international treatment guidelines have been informed primarily by large multisite clinical trials with endpoints of treatment success and survival at 2 weeks and 10 weeks. Ongoing mortality occurs between 10-18 weeks. After 18 weeks, approximately 90-95% will be alive at 5 years.[23,25]

Table 2.3: 2018, 2022 WHO Antifungal Treatment Recommendations for Cryptococcal Meningitis

Medication and Dose	**Week 1**	**Week 2**	**Week 3-10**	**Week >10**
Amphotericin B (1.0 mg/kg/day) + flucytosine 100mg/kg/day				
Fluconazole 1200mg daily				
Fluconazole 800mg daily				
Fluconazole 200mg daily				Through 12 months
Treatment Phase	**Induction**	**Induction**	**Consolidation**	**Secondary Prophylaxis**

These recommendations are based on a published 2018 phase III randomized trial.[20] This remains the 2022 recommended regimen for locations without access to liposomal amphotericin B (Ambisome®).[21]

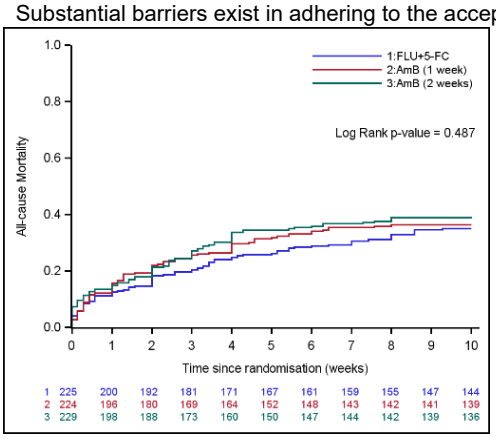
In the published ACTA trial, one week of IV amphotericin B deoxycholate was non-inferior to two weeks of IV AMB (Figure 2.3).[2] The best therapy in the ACTA trial was 1 week of IV AMB + flucytosine with 24% (27/113) 10-week mortality.

Cryptococcal mortality can be reduced by the use of high-dose amphotericin B (0.7-1.0 mg/kg/day), alongside aggressive inpatient management of raised intracranial pressure.[12] In

most resource-limited settings, standard induction therapy for cryptococcosis includes the alternate regimen of IV AMB for 14 days combined with high-dose fluconazole (800-1200mg/day).

The ability of amphotericin to rapidly and consistently sterilize the cerebrospinal fluid (CSF) of cryptococcal patients suggests that amphotericin should be central to any induction strategy.[26]

Figure 2.3: ACTA trial, comparison of induction therapies



Substantial barriers exist in adhering to the accepted guidelines in resource-limited areas, where cryptococcal meningitis is most prevalent. These include costs and availability of drugs, intravenous (IV) drug administration which requires hospitalization, co-administration of IV fluids and supplemental electrolytes, and the severe and potentially life-threatening toxicity of amphotericin which necessitates rapid and reliable laboratory monitoring.[27,28] For these reasons, amphotericin is generally reserved for life-threatening fungal infections, and in the case of cryptococcosis, limited to the induction period. Many centers in low and middle income countries consequently find it difficult to adhere to recommended guidelines for cryptococcal induction therapy. Identifying more effective and less toxic antifungal therapy is necessary to allow for more rapid fungal clearance during the induction phase, which could lead to less dependence on completing long courses of IV medications with increased risk of toxicity.

The combination of amphotericin and flucytosine appears to be rapidly fungicidal and has better survival than alternative 14-day regimens.[2,29–31] Flucytosine can also be associated with severe side effects including hepatotoxicity, bone-marrow depression, and renal toxicity when co-administered with other nephrotoxic drugs such as amphotericin.[32] Furthermore, flucytosine is currently not registered or available in most low and middle income countries. Given the apparent advantages of flucytosine over fluconazole as combination therapy, substantial advocacy efforts are currently underway to secure availability and affordability of flucytosine where *Cryptococcus* is prevalent. In 2019, UNITAID was seeking to make flucytosine available.

In contrast to amphotericin, fluconazole is generally well-tolerated and readily penetrates the central nervous system (CNS).[33] Consolidation and subsequent maintenance therapy therefore relies on extended courses of oral fluconazole (Table 2.3). At typical doses (≤400 mg/day),

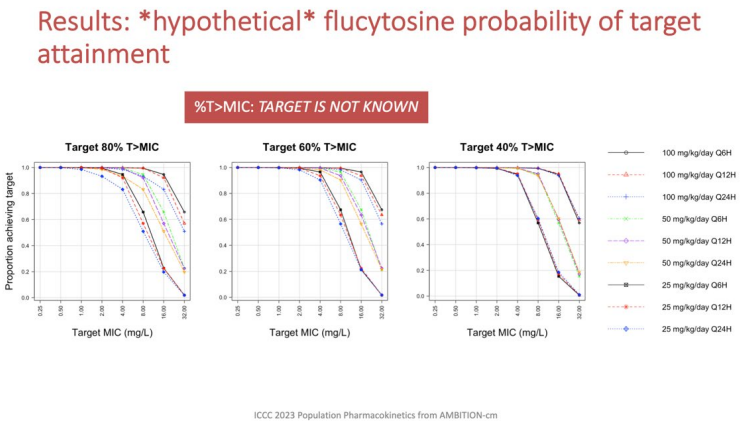
however, fluconazole is fungistatic rather than fungicidal, confirmed in a well-designed clinical trial demonstrating no change in fungal growth by serial quantitative cultures over 2 weeks with 400 mg/day of fluconazole.[26] Even when used in higher, more fungicidal doses (800-1200 mg/day)[19] as is currently being recommended for induction therapy of CM, fluconazole adds a marginal contribution to overall fungal clearance when used together with amphotericin. Fluconazole monotherapy, which is still the standard of care in much of the world where amphotericin use is limited or impossible to administer, leads to suboptimal clearance, resistance and symptomatic relapse.[34,35] More efficacious and less toxic oral antifungals for the treatment of cryptococcosis are urgently needed.

2.4 Rationale for Optimizing 5FC Dose

Dosing for 5-FC was a matter of debate in early trials that established the efficacy of 5-FC in the management of CM. In the era before AIDS, Bennett *et al.* used a lower dose of amphotericin B (0.3 mg/kg/day) in combination with higher dose of 5-FC (150 mg/kg/day) to treat CM in patients receiving immunosuppressive medications [10]. While the addition of 5-FC proved to have superior clinical efficacy, the rate of cytopenias was high (30%).[10] In a subsequent retrospective study of patients with AIDS, average dose of 5-FC was 75-100 mg/kg/day, but was not associated with survival benefit and was associated with 53% of patients having cytopenias necessitating discontinuation of the drug.[36] Finally, van der Horst *et al*. performed a high quality randomized clinical trial using a higher dose of amphotericin (0.7mg/kg/day) with or without 5-FC (100 mg/kg/day)[12]. This trial demonstrated that the addition of 5-FC resulted in higher rates of CSF sterilization and lower mortality rates and no difference in toxicity between groups. However, the dose of 5-FC was not selected based on a systematic analysis, but rather as an attempt to mitigate the toxicity and maximize therapeutic effects.

While there are no studies to date that establish time over MIC as the critical pharmacodynamic property for efficacy of 5-FC, we can extrapolate that time over MIC is an important factor from work on 5-FC and Candida spp.[37] Preliminary modelling based on AMBITION pharmacokinetics by Stott *et al.* demonstrated that 5-FC may reach CSF concentrations above MIC for *Cryptococcus* even at lower and less frequent dosing (**Figure 2.4.1)**. In our own analyses of MIC for 5-FC against *Cryptococcus* the MIC_{50} has been approximately 4 mg/L and MIC_{90} 8 mg/L (**Table 2.4)**. In this hypothetical model displayed in Figure 2.4.1, the proportion of patients receiving 5-FC dosing of 50 mg/kg/day still achieve target time over MIC more than 80% of the time even in the strictest target.

Figure 2.4.1: Pharmacokinetic modelling of flucytosine CSF levels at lower doses and frequencies.



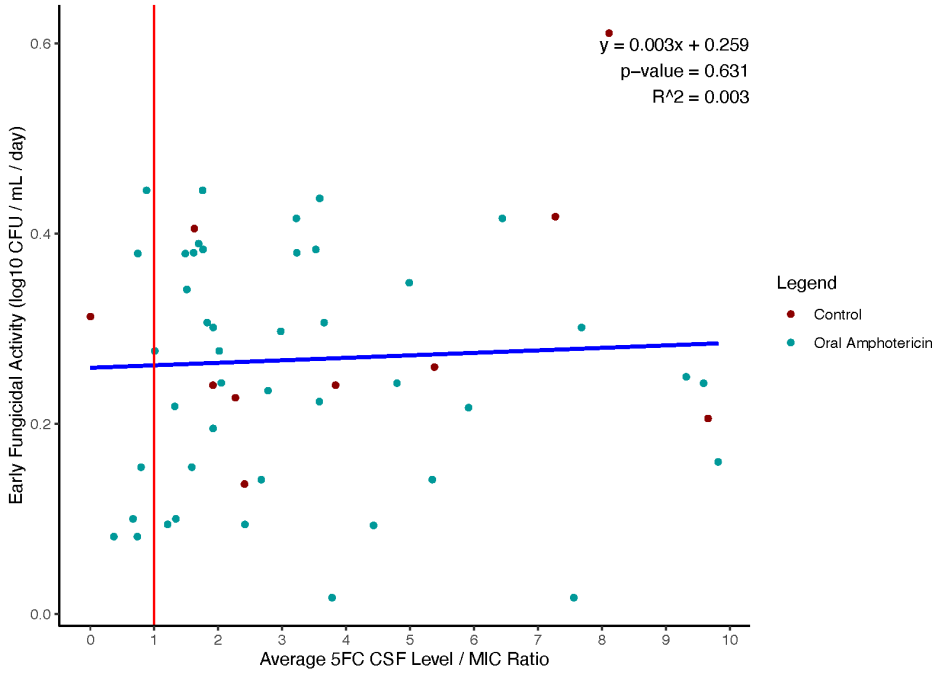
Recently, Jezewski *et al*. endeavored to better understand the mechanisms of how flucytosine functions in the *in vivo* environment.[66] For years, 5-FC has been understood to have a synergistic effect when used in combination with amphotericin or fluconazole.[67-69] However, this synergism has been poorly understood and difficult to replicate *in vitro*. One of the conditions that has been thought to potentiate the flucytosine effects is the elevated level of CO_2 in the body compared to ambient atmospheric levels. In the Jezewski *et al*. study, isolates grown at elevated CO_2 concentrations had a 2 to 64-fold reduction in MIC, depending on the CO_2 concentration. We have also found, in our own studies that the ratio of 5FC CSF concentrations to MIC is quite high, with a median ratio of 2.98 (**Figure 2.4.2**). These data indicate that in addition to the strong evidence by Stott *et al* above that lower doses of flucytosine will still achieve levels above MIC, the target MIC might be even lower than we have identified in our isolates (**Table 2.4**).

During phase 2 of the AMBITION-*cm* trial, which established the effectiveness of single high dose liposomal Amphotericin 10 mg/kg for induction treatment of CM. Amphotericin was paired with high-dose fluconazole without 5-FC.[38] In this phase 2 trial, single dose liposomal Amphotericin B 10 mg/kg with fluconazole 1200 mg/day for 14 days demonstrated an EFA of -0.52 log10 CFU/mL/day. This was found to be non-inferior to the standard regimen of liposomal Amphotericin 3 mg/kg/day for 14 days with the same dose of fluconazole. These data suggest that the dose of 5-FC can be safely adjusted for optimization while maintaining the mortality benefit of CM induction therapy.

Table 2: MIC for flucytosine in *Cryptococcus* isolates from participants in the EnACT clinical trial. The top section are the MIC values for the initial screening CSF, prior to significant exposure to 5-FC. The second section is the last CSF with positive *Cryptococcus* growth during treatment. The χ^2 p-value tests for a statistical difference in MIC between the control and oral amphotericin groups.

MIC (µg/mL)	***Control (n, %)***	***Oral Amphotericin (n, %)***	***Total (n, %)***	***χ^2 p-value***
Screening Lumbar Puncture				
≤2	2 (14.3%)	7 (21.9%)	9 (19.6%)	0.451
4	9 (64.3%)	15 (46.9%)	24 (52.2%)	
8	3 (21.4%)	6 (18.8%)	9 (19.6%)	
16	0 (0%)	4 (12.5%)	4 (8.7%)	
Last Positive Lumbar Puncture				
≤2	0 (0%)	3 (10.0%)	3 (7.3%)	0.302
4	7 (63.6%)	12 (40.0%)	19 (46.3%)	
8	4 (36.4%)	15 (50.0%)	19 (46.3%)	

Figure 2.4.2: Scatterplot of the ratio of average 5-FC CSF level to MIC, with high-end outliers removed. The median 5-FC / MIC ratio was 2.98 and mean was 4.72, indicating the 5-FC levels were markedly higher than MIC in the overall population. Points below the red line indicate 5-FC levels below MIC at any point during treatment (n=6 participants with ratio <1). There is no relationship between EFA and the average 5-FC CSF level to MIC ratio. The blue trendline is based on a linear regression formula that is displayed in the top right of the chart.



2.5 Early Fungicidal Activity

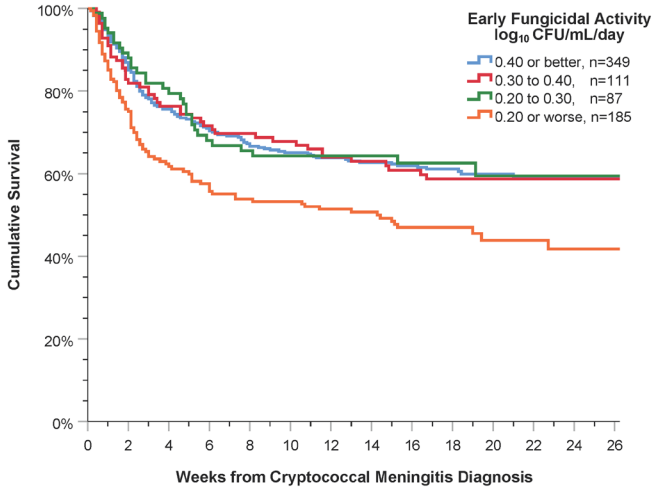
Rate of fungal clearance, or early fungicidal activity (EFA), provides a measure for evaluating the efficacy of induction therapy for CM. Bicanic *et al* have demonstrated in a pooled series of Phase II clinical trials that the rate of cerebrospinal fluid sterilization correlates with clinical outcomes.[40]

Quantitative CSF culture yeast clearance is generally logarithmic over the first 2 weeks; thus, the clearance can be estimated by linear regression of $\log_{10}$ colony-forming units (CFU) per mL

of CSF per day of therapy as a unit of measure (i.e., change in log_{10} CFU/mL CSF/day). This approach has allowed for small innovative phase II clinical trials to estimate the early fungicidal activity of newer induction regimens with dramatically smaller sample sizes, using early fungicidal activity as a surrogate marker.

Similarly in Uganda, the relationship of EFA to 10-week and 26-week survival in patients with CM has been investigated in several clinical trials In a series of three NIH-sponsored clinical trials from 2010 to 2018,[41] an EFA worse than 0.20 log_{10} CFU/ml/day was strongly associated with increased mortality (P=0.001). Survival was comparable among AMB regimens with EFAs greater than 0.20 log_{10} CFU/ml/day.

Figure 2.5: Early Fungicidal Activity versus 26-week Survival with IV AMB + Fluconazole



Below are examples of EFA spaghetti plots (**Figure 2.5.A**), where each line is an individual research participant, and each dot is a CSF quantitative culture value in log_{10} CFU/mL CSF units. In general, CSF yeast clearance is approximately linear (on a log_{10} scale). With IV AMB, the rate of clearance improves. With IV AMB + 5-FC, the rate of clearance improves further over IV AMB + fluconazole alone. Increases in the first 1 day of therapy are common, but after Day 2 the majority have a steadily decreasing CFU/mL count over 14 days of induction therapy. The occasional outlier patient (~5%) might have increased CSF culture values over time (even with IV AMB + 5FC).

Figure 2.5.A: Example of Serial Quantitative CSF Cultures to calculate EFA

Fluconazole 400mg/day in Cape Town [26]	Fluconazole 1200mg/day in Mbarara, Uganda [19]	IV AMB x 5day with fluconazole 1200mg in Mbarara, Uganda [42]	IV AMB + 5FC x 14 days in Cape Town [17]

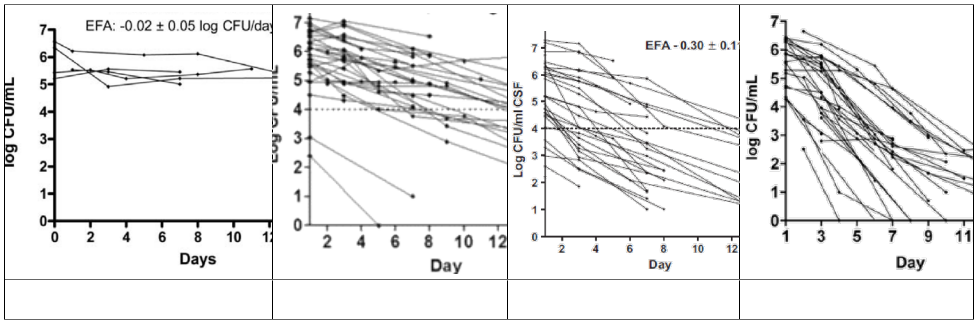
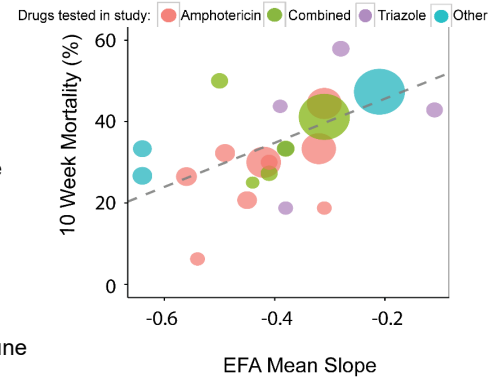


Table 2.5: Trials comparing CSF *Cryptococcus* clearance Early Fungicidal Activity (EFA) by regimen.

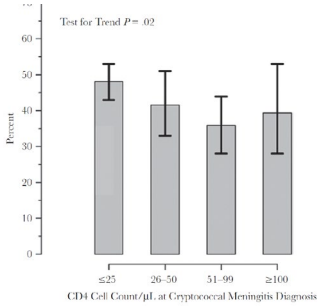
Induction Regimen	EFA	± SD	N	Ref
Single-dose Amphotericin + 5FC + fluconazole (1200 mg/day)	0.40	0.13	407	43
Amphotericin	0.31	0.15	99	
Amphotericin + 5FC	0.42	0.10	100	30
Amphotericin + fluconazole (800 mg/day)	0.32	0.10	99	
Amphotericin + 5FC	0.41	0.22	21	
Amphotericin + fluconazole (800 mg/day)	0.38	0.18	22	44
Amphotericin + fluconazole (1200 mg/day)	0.41	0.35	23	
Amphotericin + voriconazole	0.44	0.20	13	
Amphotericin (5 days) + fluconazole (1200 mg/day)	0.30	0.11	30	42
Amphotericin + 5FC	0.49	NA	30	45
Amphotericin + 5FC + INF-?	0.64	NA	60	
Amphotericin (7 days) + fluconazole (1200 mg/day)	0.38	0.20	19	46
Amphotericin (7 days) + fluconazole (1200 mg/day) + 5FC	0.50	0.15	18	
Fluconazole (1200 mg/day)	0.18	0.11	30	19
Fluconazole (800 mg/day)	0.07	0.17	30	
Amphotericin (1 mg/kg/day)	0.48	0.28	49	26
Fluconazole (400 mg/day)	0.02	0.05	5	
Amphotericin + fluconazole (800 mg/day)	0.36	0.25	223	25
Amphotericin + fluconazole (800 mg/day) + sertraline	0.43	0.39	128	21
Fluconazole (1200mg/day)	0.11	0.09	20	47
Fluconazole (1200mg/day) + flucytosine	0.28	0.17	21	
Amphotericin 1 week (either 5FC or fluconazole)	0.40	0.24	179	
Amphotericin 2 week (either 5FC or fluconazole)	0.42	0.25	182	
Fluconazole (1200mg/day) + flucytosine	0.26	0.18	182	2
Amphotericin + 5FC	0.46	0.25	186	
Amphotericin + fluconazole (1200mg/day)	0.36	0.23	175	

Similarly, a separately published analysis found a correlation between EFA and 10-week mortality (P=0.04) using a larger collection of studies (**Figure 2.5.B**).[48] This analysis used the reported EFA from the publication, which differed slightly across publications and did not have primary data, thus was unable to account for differing demographics across highly diverse HIV-positive and HIV-negative cohorts. The included studies came from: the US, Canada, India, Thailand, Indonesia, Laos, Vietnam, Uganda, South Africa, Malawi, Botswana, Zimbabwe and Netherlands. This analysis only included randomized trials, and excluded two fluconazole monotherapy studies,[19,26] which had poor EFAs <0.20 $\log_{10}$ CFU/ml/day and high mortality on the basis that these are clearly inferior therapies. This exclusion misses the point that EFA is a quantitative marker of microbiologic activity in humans.

Shortcomings in EFA: EFA is not a perfect surrogate marker for all-cause mortality on an individual patient level basis. Thus, EFA has been criticized.[48] There are two problems with EFA: 1) Persons who rapidly die after diagnosis may not have a computed EFA (as an EFA requires at least two time points); 2) Death in persons with advanced AIDS could be from other opportunistic infections and/or AIDS-related cancers; 3) Death after cryptococcal meningitis is influenced also by the immune system. As we have reported, cryptococcal meningitis follows the "damage-response framework for microbial pathogenesis"[49]—whereby those with the worst immune response (e.g. lowest CD4 T cell count) and highest pathogen burden have high mortality—but surprisingly those with highest CD4 counts and lowest fungal burden (i.e. sterile CSF cultures), actually have relatively higher mortality as well (**Figure 2.5.C**).[50] Similarly, over abundant immune responses, e.g. unmasking cryptococcal IRIS occurring early after HIV therapy initiation, is highly detrimental.[51] Excess dysregulated inflammation in the brain is often fatal.



Thus, EFA cannot capture all factors associated with mortality, but EFA can assess the microbiologic activity of an antifungal regimen. At present, there is no immune directed therapy for cryptococcosis, thus the only therapeutics we have (and that are FDA-approved) are focused on antifungal therapy. Thus, while the immune system contributes to mortality on an individual patient basis, the group EFA of an antifungal regimen has been associated with overall mortality as displayed in Figures 2.4.B.



In this trial, the primary endpoint is EFA as the clearance rate of *Cryptococcus* yeast in CSF. EFA is a direct quantitative measure of the antimicrobial activity of 5-FC used in combination with AMB therapy in humans with cryptococcal meningitis.

2.6 Potential Risks and Benefits

2.6.1 Potential Risks

First, the EFA, which as described above, indicates the rate of fungal clearance and correlates with mortality due to CM will be followed by the study team. If EFA is consistently <0.2 $\log_{10}$ CFU/mL/day after 10 enrolled patients, then the trial will be suspended until an interim review

can be completed. In addition, all patients will be receiving IV AMB which is also a gold standard therapy and reduces mortality related to CM, in addition to fluconazole.

As discussed above 5-FC is a critical aspect of the induction phase of treatment for CM, decreasing the fungal burden in the CNS and improving mortality. Given this, there is a risk that decreasing the dose of 5-FC will lead to insufficient CSF concentrations to achieve fungal clearance and reduced mortality. For this reason, we will implement several safety measures.

As discussed above, our trial will use the induction therapy regimen established in the AMBITION-cm trial and now recommended by the updated WHO guidelines.[4] During phase 2 of that trial, high dose liposomal Amphotericin B with fluconazole achieved an EFA of -0.52 $\log_{10}$ CFU/mL/day.[38] These data provide strong evidence that optimizing the dose of 5-FC can be safely studied in the same context without significantly reducing mortality.

The risks associated with the traditional IV AMB 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.

Amphotericin B is an FDA pregnancy category B (no evidence of risk in humans); however, all participants will also receive flucytosine and fluconazole, which are both potential teratogens at high doses. Therefore, subjects must use effective forms of contraception to avoid becoming pregnant. Pregnant women will be excluded.

2.6.2 Potential Benefits

The primary benefit for this study will be to improve access to 5-FC for patients in greatest need of this therapy. It is hoped that lower doses of 5-FC will be equally effective compared to the traditional dosing, which will reduce the cost of therapy for individual patients and eventually HIV care programs. A secondary benefit by participating in the study is that the lower doses may be associated with lower toxicities hence providing data to recommend decreasing costs of safety monitoring in the long term. High doses of 5-FC are associated with leukopenia, thrombocytopenia and acute renal injury, especially when used in conjunction with IV AMB. Lower doses of 5-FC will likely have reduced burden of side effects on individual patients.

Participants will receive scheduled lumbar punctures at baseline, Day 3 ±1, Day 7 ±2, and Day 14 ±2 and additionally as required for control of intracranial pressure and documentation of CSF sterilization. Receipt during the first week of at least one therapeutic lumbar puncture with control of intracranial pressure has a 69% relative reduction in 10 day mortality in Uganda.[52] Thus participants will likely benefit from study participation through receipt of scheduled lumbar punctures with control of intracranial pressure.

There is the potential benefit of access to single high dose liposomal AMB, which has recently been shown to be as effective as the 7-day course of IV AMB deoxycholate. When available to the study team, liposomal AMB will be used which will also reduce incidence of side effects related to AMB deoxycholate.

3. OBJECTIVES

3.1 Study Objectives

3.1.1 Phase II Trial Objective

To demonstrate the reduced dose of 5-FC, when combined with IV AMB for induction therapy of CM is non-inferior compared to traditional dosing of 5-FC in reducing fungal burden of *Cryptococcus*

3.1.2 Phase II Secondary Objective

- To determine if there remains a mortality benefit to 5-FC for induction therapy at reduced dosing.
- To assess the overall safety and tolerability of 5-FC at reduced dosing.

3.2 Study Outcome Measures

Primary Endpoint

1. CSF culture clearance at 2 weeks (Early Fungicidal Activity, or EFA: change of $\log_{10}$ *Cryptococcus* CFU/mL CSF/day as measured by serial quantitative CSF fungal cultures)

Key Secondary Endpoint

1. Desirability of Outcome Response (DOOR) as ordinal ranked maximum score tested by Win Ratio.
   a. 18-week Survival with CSF sterility by 2-weeks
   b. 18-week survival with CSF culture positivity beyond 2 weeks
   c. Grade 3 hematological adverse event by 2 weeks
   d. Grade 4 hematological adverse event by 2 weeks
   e. Lost to follow up before 18-weeks
   f. Serious Adverse Event through 18 weeks (e.g. all-cause re-hospitalization, permanent neurologic deficit)
   g. Death by 18-weeks

Secondary Endpoints

1. CSF culture sterility cumulative incidence over 18 weeks
2. 18-week survival time

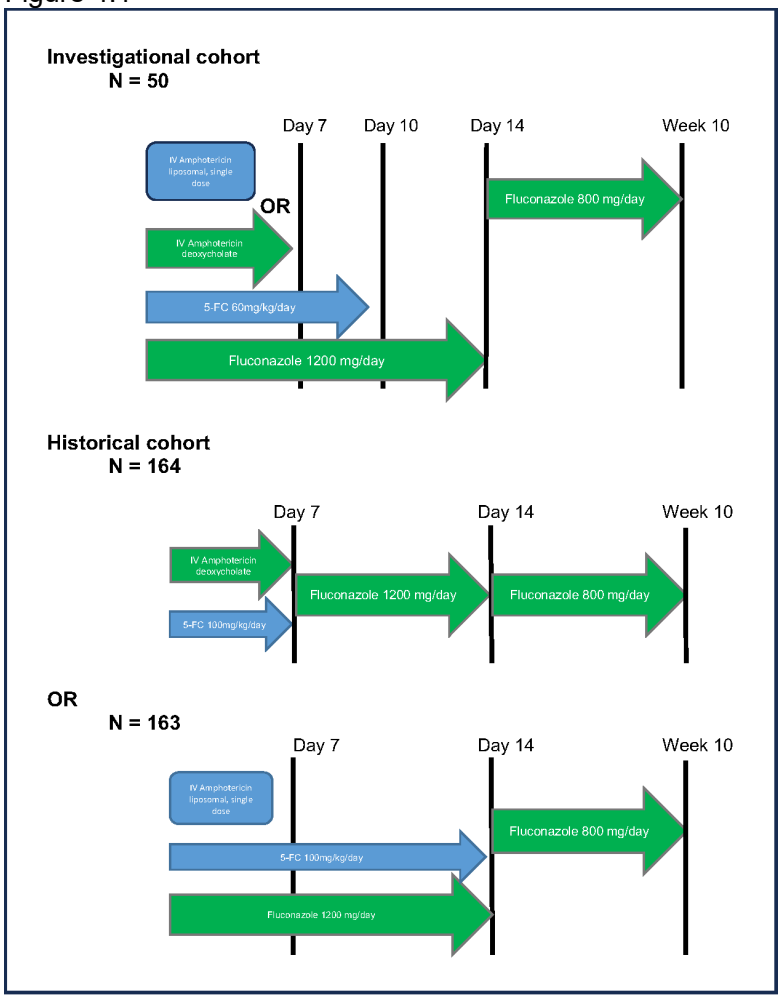
Safety & Exploratory Endpoints

1. Incidence of Grade 3-5 abnormal laboratory test results (as per the NIAID DAIDS toxicity grading scale) through Week 2 of the study
2. Incidence of clinical Adverse Events (Grade 3-5) or SAEs through week 2
3. Pharmacokinetics of 5-FC concentrations in blood, CSF, and other tissues.

4. STUDY DESIGN

4.1 Phase II Study Design

Figure 4.1



This is a single-arm trial with historical controls to compare the efficacy and safety of reduced dosing of 5-FC induction therapy for the treatment of cryptococcal meningitis in combination with IV AMB to standard of care, with EFA as the primary endpoint.

Participants will be enrolled into an investigational cohort at study entry.

1. The investigational cohort will receive 60mg/kg/day of 5-FC divided into 3 doses per day for 10 days, n~50 total enrolled to achieve n=36 CSF culture positive.
2. The control group will consist of historical participants in the AMBITION trial
3. All participants will receive liposomal Amphotericin B 10 mg/kg, single-dose infusion, if available.
4. If liposomal Amphotericin is unavailable, participants will receive IV amphotericin deoxycholate 0.7-1.0 mg/kg/day for 7 days.
5. All participants will receive fluconazole 1200 mg/day during induction therapy from day 1 to 14 then consolidation therapy 800 mg/day from day 15 to 10 weeks, and maintenance therapy of 200 mg/day after 10 weeks.
6. All participants will receive lumbar punctures at diagnosis, day 3, day 5-7, day 10-14, and additionally as required for control of intracranial pressure and documentation of CSF sterilization. Therapeutic LPs conducted during the first week have a ~70% relative survival benefit.

Investigational cohort will receive:

- IV Amphotericin B deoxycholate 1 mg/kg/day for 7 days or IV Liposomal Amphotericin B 10mg/kg single dose.
- Oral flucytosine (5FC) 60mg/kg/day for 10 days in 3 divided doses
- Oral fluconazole 1200mg/day from Day 1 to 14
- Oral fluconazole 800mg/day from Day 15 to Week 10
- Oral fluconazole 200mg/day thereafter

The control cohort will be drawn from the AMBITION trial that was conducted partly in Uganda.[43] This was a randomized clinical trial that evaluated single high-dose amphotericin B (10 mg/kg) with fluconazole (1200 mg/day) and flucytosine (100 mg/kg/day) for 2-week induction therapy of cryptococcal meningitis compared to standard amphotericin deoxycholate (1 mg/kg/day) with flucytosine (100 mg/kg/day). The trial enrolled participants who were HIV positive with a first episode of cryptococcal meningitis diagnosed by cryptococcal antigen or India ink staining. Participants were excluded if they were pregnant, had already taken amphotericin or fluconazole for >48 hours, had a previous serious reaction to the study medications or were taking a medication that would be contraindicated with the study medications. In Uganda, the trial enrolled 163 participants in the control arm and 164 in the intervention arm for a total enrollment of 327 Ugandan participants. The control cohort will be drawn from Ugandan participants. If IV liposomal amphotericin B is used in the investigational cohort then they will be drawn from the intervention arm. If IV deoxycholate amphotericin B is used in the investigational cohort then they will be drawn from the control arm. We will compare the EFA of all the control participants to the interventional participants using multivariate mixed linear regression, adjusting for confounding factors such as CD4+ count, GCS<15 or not, prior exposure to ART or not and other important clinical factors. We will also compare 10-week survival in the

interventional and control cohorts, using multivariate Cox regression, adjusting for the same clinical factors as the mixed regression model.

This 2018 and 2022 WHO recommended regimen is based on a phase III randomized clinical trial demonstrating that:

i) 1 week of IV AMB had equivalent survival as 2 weeks of IV AMB;[2]
ii) 1 week of IV AMB combination therapy was less toxic than 2 weeks of IV AMB;[2] and
iii) Adjunctive flucytosine had superior survival to adjunctive fluconazole.[2]

A DSMB review by the full committee will occur after 10 participants have been enrolled with >2 weeks of accrued data. A local Ugandan community member will be invited to attend open DSMB meetings. The DSMB safety reports will be submitted to the IRBs and regulatory authorities.

Timing of ART Initiation or Switch.

ART will be initiated for ART-naïve participants ~6 weeks after CM diagnosis, per current standard of care.[25] Persons enrolled with a failing ART regimen will be switched to 2nd line HIV therapy at ~6 weeks while persons who have initiated ART in the preceding 2 weeks will have their ART interrupted and re-initiated ~6 weeks after CM diagnosis, in accordance with consensus best practices.[53] ART management decisions for persons on ART longer than 2 weeks will be at investigators' discretion. Outpatient visits will occur every 2-4 weeks at the study site outpatient HIV clinic until the conclusion of the study at 18 weeks. Patients can elect to continue to receive routine HIV care at the site after study completion. If persons have active cryptococcal-related issues (e.g. IRIS or relapse) ongoing at 18 weeks, the participant will continue to be followed by the study team until their condition has stabilized.

Prior published outcome data suggest no overall difference in 18-week survival between those who present ART-naïve vs. ART-experienced on a failing regimen.[51]

4.2 Pharmacokinetic Studies

PK studies will be conducted on CSF from each lumbar puncture performed during the study and on selected plasma specimens. Timing of blood and CSF collections will be recorded. For a subset of participants, we will do a more intensive plasma sampling regimen by collecting multiple peripheral blood samples after the first dose of 5-FC that day.

In Kampala, cell pellets of CSF white cells and peripheral blood mononuclear cells (PBMCs) will be collected for potential immunologic studies.

4.3 Antifungal Susceptibility Testing

Minimum inhibitory concentration (MIC) testing will be performed on all participants in the investigational cohort with *Cryptococcal* growth on CSF culture. Broth microdilution, as recommended by the European Committee on Antimicrobial Susceptibility Testing will be used to determine the MIC.

5. STUDY ENROLLMENT AND WITHDRAWAL

5.1 Inclusion Criteria

- CSF cryptococcal antigen (CrAg) positive meningitis
- Ability and willingness to provide informed consent
- Willing to receive protocol-specified lumbar punctures

Exclusion Criteria

- Age < 18 years
- Inability to take enteral (oral or nasogastric) medicine
- Cannot or unlikely to attend regular clinic visits
- Receiving chemotherapy or corticosteroids
- Suspected paradoxical immune reconstitution inflammatory syndrome (IRIS)
- Pregnancy or breastfeeding (tested on screening)
- CrCl < 20 mL/minute*
- Absolute neutrophil count < 500 x 10^6 cells/L*
- Thrombocytopenia < 50,000 x 10^6 cells/L*
- Patients with prior 5-flucytosine exposure $\geq$3 days in the 12 months prior to enrollment
- Any condition for which participation would not be in the best interest of the participant or that could limit protocol specified assessments.

Rationale for Phase II Trial Criteria

The inclusion and exclusion criteria of the Phase II trial are meant to be broad to enroll a representative sample of persons in resource-limited areas, and thereby be broadly generalizable. Flucytosine and fluconazole are known teratogens in the first trimester. Amphotericin is the preferred drug of choice during pregnancy. Since therapy includes flucytosine or fluconazole, pregnant and breastfeeding women are excluded.

Other exclusion criteria are intended to minimize bias and maximize likelihood of completing the study or need to customize therapy (e.g., pregnant women).

Note on subjects with positive CSF CrAg but subsequent negative CSF cultures:

Since CSF CrAg is more sensitive than CSF culture in very early disease with a low fungal burden,[18,54] CSF culture-negative patients with a positive CSF CrAg will be assumed to have early CM and be eligible for the study and included in the analyses. Approximately 10-15% of participants are estimated to be culture negative. CSF cultures take 7-14 days for results, thus are not a realistic criterion for enrollment as therapy is initiated based on CSF CrAg results. CSF

CrAg LFA is markedly more sensitive than India ink microscopy.[54] Although these participants will not contribute to the quantitative CSF clearance data, they will contribute to all other endpoints for survival, safety, tolerability, and toxicity – all of which are important outcomes.

This trial will use adaptive sample size, requiring 36 CSF culture positive participants and enrolling up to 50 total participants with cryptococcal meningitis.

Note on subjects with previous history of cryptococcal meningitis
Subjects with a previous history of prior, known cryptococcal meningitis will be included in the trial as long as they did not receive >2 days of 5-FC during their previous treatment. The rationale for this is that most patients in Uganda would have previously been treated with fluconazole during induction therapy and relapse is highly associated with fluconazole resistance.[34] These patients may still benefit from an induction regimen that includes 5-FC and would be ideal candidates for this therapy when it becomes more widely available.

****Note on laboratory exclusion criteria***
Subjects can be enrolled into the study before the laboratory data is available. Labs should be sent on enrollment and should be available within 72 hours. Subjects meeting laboratory exclusion criteria will be withdrawn from the study (See section 5.6).

5.2 Treatment Procedures

5.2.1 Selection of Study Population

For the phase II trial, potential research participants with active cryptococcal meningitis will be identified by active surveillance during hospitalization by the on-site study teams and consented for screening. Secondary surveillance will be via the site microbiology laboratories with study team notification of new positive culture or CrAg results.

5.2.2 Timing of Study Enrollment

For the phase II trial, potential participants would be determined as eligible after lumbar puncture results. The diagnostic lumbar puncture is considered a routine medical procedure; with meningitis considered a medical emergency. Participants can be enrolled anytime in the first 2 days of 5-FC therapy. This allows time for informed consent without any undue, coercive time pressure. Receipt of >2 days of 5-FC therapy is an exclusion.

5.3 Informed Consent Process

5.3.1 Study Screening and Storage Consent

Since serum and CSF will be stored, consent for specimen storage and data collection will be obtained at the time of the initial diagnostic lumbar puncture (when the cryptococcal meningitis diagnosis is unconfirmed) prior to obtaining consent for the main overall trial. A screening and storage consent (or surrogate consent from caregiver/next of kin) will be required for study personnel to perform a lumbar puncture (LP) screening for CM diagnosis to verify an inclusion criterion. Persons with altered mental status are eligible for screening diagnostic lumbar

puncture for meningitis diagnosis with surrogate consent, even if they are not eligible for the overall study (as it would be unethical to withhold appropriate diagnostics from critically ill patients). This screening and storage consent requests to "record information about their current illness," including presenting symptoms (assessed prior to LP), final diagnosis (based on CSF results and physician diagnosis), and vital status at hospital discharge.

This screening /storage consent may be via this or another research study (e.g., EnACT) in order to make the diagnosis of cryptococcal meningitis and assess for this trial's eligibility criteria. If persons are referred from outside health care facilities with documentation of CM per study inclusion criteria, they will be eligible; however, these persons must have an LP with a full diagnostic CSF analysis performed after study screening consent and prior to entry into the study to confirm the diagnosis and exclude alternative diagnoses.

As meningitis is a medical emergency, after the diagnosis of cryptococcal meningitis is confirmed, persons start on standard IV AMB and 5-FC therapy. The first dose of 5-FC is study day 1.

5.3.2 Informed Consent

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continuing throughout the individual's study participation. After CM diagnosis and eligibility criteria are confirmed, informed consent will be obtained by a study investigator who will provide potential subjects with an IRB approved consent form in English or Luganda (Kampala) or Runyankole (Mbarara). Informed consent must occur prior to participants receiving 12 or more doses of 5-FC. There is a window of time to allow for informed consent process without any undue, coercive time pressure or unethical withholding of antifungal therapy until consent is provided. Participants may have received fluconazole and remain eligible. However, potential participants will be given appropriate time to consider the study. At the time of informed consent, a complete history and physical examination will be performed to verify inclusion/exclusion criteria.

During the Informed Consent process, the investigator will describe the purpose, risks, and benefits related to the study. Each aspect of the forms will be explained in detail with the potential subject, and the potential subject will have the opportunity to ask any questions regarding the study. The investigator obtaining informed consent will ask questions to assess the subject's understanding. The investigator will state that participation is voluntary and that subjects may refuse participation or withdraw at any time without prejudice to their clinical care. Persons who decline participation will continue the same CM induction therapy according to current standards of care.

Since this study may require additional blood draws beyond the standard of care, additional consent will be required from subjects receiving blood draws for stored samples for future study purposes. In this case, samples will be stored indefinitely, unless the subject has a change of mind and asks for them to be destroyed.

Specimens do need to be collected as pharmacokinetics is a key component of the study.

If, in the opinion of the investigator, potential participants who do not have appropriate comprehension, the investigator must re-explain the study or determine whether the participant's current mental capacity is diminished due to the CM and pursue surrogate consent.

Consent for Lumbar Punctures: Lumbar punctures will be explained in detail in the meningitis screening sample storage consent and main trial informed consent form. All follow up therapeutic LPs are deemed standard of care to control intracranial pressure and document clearance of infection. Participants retain the right to refuse a follow up LP, although this is not recommended as therapeutic LPs have a strong survival benefit. One follow up therapeutic LP is associated with a 69% relative survival benefit in the first ~10 days.[52] A second therapeutic LP in the second week has a 50% reduction in 30-day mortality (33% vs 22%) in Uganda.[55] Refer to Section 8.2 for the recommended LP schedule.

Storage Consent: Informed consent for long-term storage of samples for future research testing is necessary by Ugandan regulations. This will be completed according to the COAST protocol which is a parallel study into which all FLOOR participants will be enrolled. Refer to Section 12.6 for further details.

5.3.3 Capacity to Consent

Assessment of the capacity to consent is required for all participants in the study that have a GCS of 15. The UCSD Brief Assessment of Capacity to Consent (UBACC) will be administered to all subjects. The UBACC is an adapted, validated tool consisting of 10 questions worth 3 points each for assessing the capacity to consent in clinical trials.[56] If GCS is 15, then UBACC will be administered to assess capacity to consent. If the subject scores 15 or better on the assessment, then they can self-consent. **A GCS of <15 or UBACC assessment score of <15 means that the individual does not have the capacity to consent**. Documentation that the assessment was administered and completed will be included in the study file.

5.3.4 Surrogate/Proxy Consent

Subjects who are unable to give informed consent due the lack of capacity to consent or other barrier may enroll in the study with surrogate consent.

5.3.5 Literacy

A person who speaks and understands the language of the informed consent document, but does not read and write, can be enrolled in a study by "making their mark" via a thumbprint on the informed consent document.

In this event, an impartial, literate third party must witness the entire consent process and sign the informed consent document. The witness's name, signature, and relationship must be recorded on the informed consent document. A member of the study team is not an impartial third party.

5.3.6 Waiver of Informed Consent

CrAg LFA screening by fingerstick, serum, and/or plasma is to be performed as routine diagnostic care (without written informed consent) due to the high prevalence of CrAg+ individuals hospitalized. At Mulago Hospital in Kampala, Uganda in 2010, the prevalence of

CrAg+ individuals with CD4<100 was 21% on the infectious disease ward.[57] In Uganda, this point-of-care CrAg testing has been standard of care to rapidly identify persons with disseminated cryptococcal infection.

5.4 Blinding

This study will be a single arm phase II trial. Study medications will be dispensed by a study pharmacist of record.

5.5 Premature Discontinuation of the Intervention

A study subject can discontinue the study intervention (low-dose 5-FC) in the event of any of the following:

- Any clinical adverse event (AE), laboratory abnormality, concurrent illness, or other medical condition or situation occurs such that continued participation with an intervention would not be in the best interest of the subject
- Decompensation of the cryptococcal meningitis
- Subject wishes to voluntarily discontinue therapy

Subjects are free to discontinue an intervention at any time upon request or in the opinion of the investigator in the event of decompensation and disease progression. Examples of progression would be development of altered mental status, increasing CSF quantitative culture CFU counts by >1 $\log_{10}$ CFU/mL, other medical events leading to an inability to take oral medicines, etc. Subjects discontinuing low-dose 5-FC would then be given standard dose 5-FC, as clinically necessary.

Every effort will be made to undertake protocol-specific study procedures. If voluntary discontinuation occurs, the subject will be asked to continue scheduled evaluations, complete an end-of-study evaluation, and be given appropriate care under medical supervision until the symptoms of any AE resolve or the subject's condition becomes stable. The patient will be offered ongoing follow-up care at the site outpatient HIV clinic or referred to a clinic of their choice if withdrawn from the study.

Alterations in dose will be considered a protocol deviation, since the goal of the trial is to assess the effect of dose-reduction. Patients may remain in the study but dose-adjustments will be documented and reported to the IRB.

Subjects discontinuing the intervention will receive standard of care therapy through 18 weeks.

5.6 Study Withdrawal

By consenting to the trial, participants are consenting to receiving the study treatment, study follow-up and study data collection. However, a study subject may discontinue from further ongoing study participation at any time if:

- Subject wishes to voluntarily withdraw.
- Fulfillment of early withdrawal criterion: absolute neutrophil count < 500 x 10^6 cells/L, thrombocytopenia < 50,000 x10^6 cells/L, or renal creatinine clearance < 20 mL/minute

- Any unacceptable toxicity or adverse event (ie. Development of severe renal dysfunction or leukopenia on treatment).

Subjects are free to withdraw from participating in the study at any time upon request. If voluntary withdrawal occurs, the subject will be asked to complete an end-of-study evaluation and be given appropriate care under medical supervision until the symptoms of any AE resolve or the subject's condition becomes stable. The patient will be offered ongoing follow-up care at the site outpatient HIV clinic or referred to a clinic of their choice if withdrawn from the study. Participants who withdraw from Mulago hospital will not be contacted after withdrawal of consent.

Subjects may discontinue the study intervention (as per Section 5.5) and remain in the research study for safety monitoring and assessment of endpoints, receiving standard of care therapy.

Subjects withdrawing from the study will be recommended to receive standard of care therapy.

6. MEDICATIONS TO BE USED IN THE TRIAL

With the exception of the intervention of low-dose 5-FC, all medications received in this study are considered standard of care and will be provided by the study. HIV antiretroviral medicines will not be provided by the study but are freely available, regardless of study population, via the public sector HIV clinics.

6.1 Cryptococcal Meningitis Management

Medication management is summarized in Table 6.2, as per 2018 and 2022 cryptococcal WHO guidelines,[4,58] which exceed the local standard of care.

Table 6.2: 2018 and 2022 WHO Treatment Regimen for Cryptococcal Meningitis

Medication and Dose	**Week 1**	**Week 2**	**Week 3-10**	**Week >10**
Amphotericin B (1.0 mg/kg/day) + flucytosine 100mg/kg/day [a]				
Fluconazole 1200mg daily				
Fluconazole 800mg daily				
Fluconazole 200mg daily				Through 12 months
Treatment Phase	**Induction**	**Induction**	**Consolidation**	**Secondary Prophylaxis**

Notes: [a] Flucytosine (5FC) 100 mg/kg/day preferred where available, otherwise fluconazole at 800–1200mg/day in divided doses with two weeks of amphotericin B. KCl 40–60 mEq/day should be given with amphotericin.

Individual patient factors often alter patient care and fluconazole dosage on an individual basis at physician discretion, such as acute renal failure, duration of cryptococcal culture positivity, CM relapse, medication intolerance, and hepatic P450 drug-drug interactions (e.g. rifamycin).

As well, some individuals will remain CSF culture positive after 14 days of amphotericin, thus higher dose fluconazole with fungicidal activity (1200 mg/day) may be used until outpatient clinic enrollment at Study Week 4. For persons with persistently positive cultures, refer to Section 7.4 on outpatient clinical management of CM. Induction therapy using a combination of amphotericin and either high-dose fluconazole or flucytosine is recommended as the microbiologic activity is greater than that of amphotericin alone. Subjects who remain CSF culture positive should continue on at least induction-dose fluconazole until culture negative.

6.2 Other Antifungals Used in Cryptococcal Treatment

6.2.1 Amphotericin B

Amphotericin B is an antifungal polyene. Amphotericin is well known for its frequent and potentially severe side effects. For this reason, it is used only for the treatment of life-threatening fungal disease, such as CM, where it may be the only effective treatment available. In all cases, possible life-saving benefits of amphotericin must be balanced against its untoward and dangerous side effects.

6.2.1.1 Amphotericin B Deoxycholate

Amphotericin B deoxycholate is available in single vials. Each vial contains a sterile, non-pyrogenic, lyophilized cake providing 50 mg amphotericin B and 41 mg sodium deoxycholate buffered with sodium phosphates. Amphotericin B is solubilized by the addition of sodium deoxycholate to form a mixture, which provides a colloidal dispersion for intravenous infusion following reconstitution.

Typical AMB deoxycholate dosing for CM is 0.7-1 mg/kg daily. Amphotericin should be administered by slow intravenous infusion over a period of approximately 4 hours. The recommended concentration for intravenous infusion is 0.1 mg/mL (1 mg/10 mL).

Acquisition: Any nationally registered, GMP-manufactured, and/or FDA-approved formulation is acceptable.

Product Storage and Stability: Prior to reconstitution, AMB deoxycholate should be stored under refrigeration 2° to 8°C (36° to 46°F) and protected against exposure to light.

Study administration for all treatment arms:

Subjects will receive the WHO standard 7-day course of IV amphotericin B deoxycholate 0.7-1.0mg/kg/day.

Amphotericin is well known for its frequent and often severe side effects, as outlined in Appendix A. Significant infusion reactions must be anticipated with a tendency toward IV-associated phlebitis. To help prevent this, IV lines will be flushed after amphotericin is administered and peripheral IV sites generally rotated at least every 3 days. With IV AMB, provide 1–2 L saline/day, along with careful monitoring for renal dysfunction and particularly electrolyte abnormalities (K and Mg). Electrolyte management with amphotericin is essential, and will follow WHO treatment guidelines including supplemental potassium and magnesium.[53]

Guidelines for use of IV AMB and management of common toxicities associated with amphotericin B are included in the MOP and may be consulted, at the site investigator's discretion. Participants who permanently and prematurely discontinue IV AMB because of an AE will be followed closely until resolution of the AE can be documented. Those who require additional IV AMB in any arm will be tracked, as additional IV AMB is a secondary endpoint.

When not receiving IV AMB, additional IV fluids will be given as clinically indicated.

6.2.1.2 Liposomal Amphotericin B

Liposomal amphotericin B (AmBisome®), Gilead, Inc. is dosed at 10 mg/kg/dose in a single dose.

Acquisition: Liposomal preparations of amphotericin B are rarely available in Africa, but may be more so in the future. AmBisome® may be substituted for AMB deoxycholate depending on availability.

Product Storage and Stability:

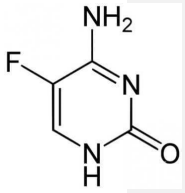
Liposomal amphotericin B may be stored at temperatures up to 25°C.

Study Administration:

Subjects will receive the WHO revised standard single high dose liposomal Amphotericin B 10 mg/kg/dose.[4] Liposomal amphotericin will be the preferred regimen when available to the study team. If liposomal Amphotericin is not available then Amphotericin deoxycholate will be used.

The same guidelines for use of IV AMB and management of its toxicities as described above in Section 6.2.1.1. will be followed.

6.2.2 Flucytosine



Flucytosine (5-fluorocytosine) is a fluorinated pyrimidine that is available as 250mg and 500mg oral capsules. Flucytosine is a white crystalline powder and the following inactive ingredients: cornstarch, lactose, and talc.

Typical dosing is 100mg/kg/day divided in 6-hour intervals. Flucytosine is rapidly and virtually completely absorbed following oral administration.

Flucytosine is taken up by fungal organisms via the enzyme cytosine permease. Inside the fungal cell, flucytosine is rapidly converted to fluorouracil (5-FU) by the enzyme cytosine deaminase. Fluorouracil exerts its antifungal activity through the subsequent conversion into several active metabolites, which inhibit protein synthesis by being falsely incorporated into fungal RNA or interfere with the biosynthesis of fungal DNA through the inhibition of the enzyme thymidylate synthetase.

Acquisition: Any GMP-manufactured Flucytosine is acceptable.

Product Storage: Flucytosine should be stored at room temperature between 15-30ºC.

Study administration:

Investigational cohort: Subjects enrolled will receive 10 days of flucytosine at 60mg/kg/day in 3 divided doses. Three divided doses are a general recommendation. Based on the number of tablets or GI tolerability, the number of doses can be altered. Table 6.2.2 provides an example approximate dosing schedule.

Table 6.2.2 Example Daily Dosing Regimen by Weight

Weight	Total 500mg Tablets Per Day	Pills per Dose	Mg/Kg Dose Range
<30	Weight based dosing	Respective pills every 8 hours	
30-38	4	2, 1, 1	54.1 - 66.7
38-44	5	2, 2, 1	56.8-65.8
45-53	6	2, 2, 2	55.6-66.7
54-62	7	3, 2, 2	56.2 - 64.8
63-70	8	3, 3, 2	57.5 - 63.5
71-79	9	3, 3, 3	57.0 - 63.4
80-87	10	4, 3, 3	57.5 - 62.5

88-94	11	4, 4, 3	58.5 - 62.5
95-103	12	4, 4, 4	58.3 - 63.2
>104	Weight based dosing		

Estimated weight is acceptable to use (e.g. in comatose or confused persons unable to stand on a scale). Dose should be adjusted once a measured weight is available. There is a general preference to round up when between weight bands or when in doubt.

Cautions. Flucytosine must be given with extreme caution to patients with impaired renal function or bone marrow depression. Since flucytosine is excreted primarily by the kidneys, renal impairment may lead to accumulation of the drug. Flucytosine serum concentrations should be monitored to determine the adequacy of renal excretion in such patients. Dosage adjustments should be made in patients with renal insufficiency to prevent progressive accumulation of active drug. Patients may be more prone to depression of bone marrow function if they: 1) have a hematologic disease, 2) are being treated with radiation or drugs which depress bone marrow, or 3) have a history of treatment with such drugs or radiation. Bone marrow toxicity can be irreversible and can lead to death. Frequent monitoring of hepatic function and of hematopoietic system is indicated during therapy.

Side Effects. Other common side effects include headache, rash, nausea, vomiting, diarrhea, and abdominal pain. The potential side effects will be discussed with patients at study entry, with close monitoring for adverse events.

6.2.3 Fluconazole

Fluconazole is a synthetic triazole antifungal agent that is available in 200 mg oral tablets. Fluconazole is a white crystalline solid which is slightly soluble in water and saline. Diflucan tablets contain 200mg of fluconazole and the following inactive ingredients: microcrystalline cellulose, dibasic calcium phosphate anhydrous, povidone, croscarmellose sodium, FD&C Red No. 40 aluminum lake dye, and magnesium stearate.

Typical dosing is 200-1200 mg daily. Duration of dosage depends on severity of infection. The oral bioavailability of fluconazole is >90%.

Fluconazole is a highly selective inhibitor of fungal cytochrome P450 dependent enzymes. It is a potent CYP2C9 inhibitor and moderate CYP3A4 inhibitor. Thus, patients who are on fluconazole and other drugs metabolized through CYP2C9 and CYP3A4 should be monitored.

Acquisition: When available, fluconazole will be obtained from the Ministry of Health via the Pfizer Diflucan Donation Programme (as per standard clinical practice). Any GMP-manufactured fluconazole is acceptable.

Product Storage and Stability: Fluconazole needs to be stored below 86°F (30°C).

Study administration: Fluconazole is an oral medication, used for the treatment of many fungal infections including CM. After induction therapy, 800mg/day will be given until sterilization of CSF can be documented and effective ART is initiated, as outlined in Table 6.2. If there is fluconazole toxicity, the dose can be reduced to 400 mg/day for consolidation therapy after negative cultures for Cryptococcus have been documented. It can be further reduced to 200 mg/day after 10 weeks for secondary prophylaxis, assuming there is no reason to continue high dose fluconazole (history of CM relapse, CM-IRIS, concurrent fungal infection, or other reasons per medical officer clinical judgment).

The justification for the increased dose is based on recent studies that have shown that fluconazole is more potent and safely tolerated at higher doses.[19,42,44,46] In a study from Mbarara, for example, Longley et al showed that fluconazole was more rapidly fungicidal when administered at a dosage of 1200 mg per day versus 800 mg per day.[19] The current 2018 and 2022 WHO cryptococcal guidelines recommend 800 mg/day for 8 weeks during consolidation therapy (Week 3 to Week 10).[4,58]

Possible side effects of the medication are rare, but include headache, rash, nausea, vomiting, diarrhea, elevated alkaline phosphatase, and abdominal pain. Patients will be warned of potential side effects at study entry, and closely monitored for adverse events. Fluconazole doses of 800 mg or above will be divided into at least twice daily to avoid side effects.

6.3 Accountability Procedures for the Study Intervention

Clinical personnel involved in the dispensation and administration of study drugs to be used in the study will adhere to GCP practices. Pharmacies utilized in the study will maintain the supply and record keeping of all study drug and antifungal dispensing.

6.4 Assessment of Subject Adherence to Study Interventions

While subjects are hospitalized, study personnel (nurse or medical officer) will directly oversee administration of study medication to ensure compliance and record time of administration. Administration of antifungals will be by directly observed therapy while subjects are hospitalized during the first 1-2 weeks. Upon hospital discharge during outpatient management, self-reported adherence collected via a patient diary and pharmacy records will be used to assess study drug adherence. Patient diaries will include at minimum, the date the dose was taken, amount of dose administered, and number of doses taken in one day.

7. PHASE II TRIAL STUDY SCHEDULE

7.1 Schedule of Events

Procedures		Screening	Enrollment	Day 3	Day 5-7	Day 10-14	Week 4	Week 6	Week 8	Week 10	Week 12	Week 18	Premature Discontinuation
Signed Consent Form			X										
Assess Eligibility Criteria		X	X										
Review of Medical History		X	X										
Enrollment			X										
Assess Clinical Status			X	X	X	X	X	X	X	X	X	X	X
Assess Adverse Event			X	X	X	X	X	X					X
Clinical Labs	Na, K, creatinine	X	X		X	X		X					
	CBC	X	X		X	X		X					
	CD4		X										
	ALT, T-bilirubin		X					X					
	CSF Collection & Storage	X		X	X	±[1]	±[2]						
	Urine Pregnancy Test	X											
Research Labs	Storage of Blood for PK and research studies		X[3]										
	ART Initiation/Change							X					
	Quantitative Neurocognitive										X		

[1] Day 14 CSF can be collected between days 10-14. If CSF cultures are sterile from ≥7 days prior (i.e. culture obtained on day 3 is sterile on day 10), lumbar punctures may be deferred at Day 14. Participants may be discharged from the hospital after 7 days, if ambulatory and clinically stable.
[2] If CSF culture is positive at Day 14 then an LP must be performed at Week 4. For those who remain culture positive thereafter, follow up LPs should be conducted as per best practices to document sterility.
[3]Optional: Peripheral blood mononuclear cells (PBMCs) – Kampala only (optional)

All study visits have an allowable visit window of ±1 day for Day 3-7 visit, ±2 days from Day 7-14 visits, and 4 Weeks and beyond have ±1 week visit window.

7.2 Enrollment

At the time of initial hospital presentation, participants presenting with suspected CM will be evaluated by the study team. An LP will be performed by the study team per standard of care for evaluation of a patient with signs of meningitis (Section 8.2). For women of childbearing potential, a urine pregnancy test will be administered.

Study staff will verbally screen for inclusion/exclusion criteria when introducing the patient information sheet at the start of the informed consent process. Diagnostic CSF analysis includes:

- CSF white cell count
- CSF protein
- Quantitative fungal culture[59]
- Cryptococcal antigen (CrAg) by lateral flow assay[54]
- CSF storage for possible PK studies or future immunology assays

Documented cryptococcal meningitis will be defined as either CSF fungal culture positive for *Cryptococcus* species and/or CSF CrAg positive. As trial enrollment will be within the first 48 hours after diagnosis, CSF CrAg results will be used as the inclusion criteria, as the CSF fungal culture takes >48 hours. CSF analysis performed at the local approved microbiology laboratory. The purpose of the first lumbar puncture is to make the etiologic diagnosis of meningitis (an inclusion criterion) and collect baseline CSF parameters and CSF specimens prior to antifungal therapy. As with every LP in this trial, CSF will be sent for quantitative cryptococcal cultures.

If the participant meets all inclusion/exclusion criteria and completes the informed consent process by signing the informed consent document, they will be considered eligible for enrollment into the study.

A physician assessment at this time will include:

- Complete medical history
- Vital Signs
- Complete physical examination

At enrollment, current signs/symptoms occurring must be recorded. All signs/symptoms must be recorded on CRFs at time of enrollment, so that new AEs may be correctly assessed and documented on an ongoing basis. Pre-existing Grade 3-4 events are commonly expected among participants at study entry and will likely include: fatigue, weight loss, anorexia, nausea, vomiting and neurologic (behavior/altered mental status/cognitive/headache) abnormalities.

Within ± 72 hours of enrollment, baseline laboratories will be collected for:

- Complete blood count (CBC)
- Chemistry panel (e.g., Na, K, creatinine at minimum, with calculated creatinine clearance)
- CD4 T cell count, as possible
- HIV RNA (viral load)
- Liver function tests (ALT, T-bilirubin)
- Specimen Storage
- Peripheral blood mononuclear cells (PBMCs) – Kampala only (optional)

We expect that most patients that undergo screening will already have an HIV diagnosis. In the event of unknown HIV-status, HIV screening will be the rapid test per the current hospital protocol of universal HIV testing of all hospitalized patients (i.e. opt-out). Additional

screening for AIDS-related OIs may occur as per standard medical practice, particularly for tuberculosis.

Study days are numbered starting from the first day of diagnosis (Day 1 = the day the subject receives first dose of IV AMB therapy). The format of the visit code will be 'xx.y', where 'xx' is the study week, and 'y' is the study day of the corresponding week. For example, day 1 of the study is the day the subject received the first dose of IV AMB and will be denoted '00.1". Day 10 of the study (1 week and 3 days) would therefore be denoted '01.3'.

Participants may receive up to 2 days of standard dose 5-FC and remain eligible, so as to allow participants adequate time for their decision on whether to participate in the research project as well as to allow for weekends or holidays. The number of 5-FC doses should start with the first investigational dose received, and the schedule of events adjusted accordingly.

7.3 Hospital Follow-up Visits

Hospital follow up visits have a visit window, as defined below, to accommodate weekends and/or holidays when the local laboratories are closed. Participants will generally be seen by a study investigator daily during hospitalization recording administration of study medicine, antifungal medicines, and additional IV fluids.

Follow up Visits

Day ~3

A study medical officer will assess between day 2-4:

- Interval history occurring since the prior study visit
- Vital signs
- Focused physical and neurologic examination, directed by symptoms
- Medications and adherence (as appropriate)
- Adverse events (AEs)

Laboratory monitoring will include, at a minimum:

- Serum creatinine
- Serum chemistry (Na, K)
- Lumbar puncture with quantitative fungal culture
- Storage of plasma, serum, and CSF.

Day 5-7

A study medical officer will assess during day 5-7:

- Interval history
- Vital signs
- Focused physical and neurologic examination, directed by symptoms
- Medications and adherence (as appropriate)

- Adverse events (AEs)

Laboratory monitoring will include, at a minimum:

- CBC with autodiff
- Lumbar puncture with CSF quantitative culture
- Storage of plasma, serum, and CSF.
- Optional: Peripheral blood mononuclear cells (PBMCs) – Kampala only (optional)
- Optional: Intensive PK sampling of peripheral blood with samples taken 1, 2, 4, 7, 12, and 23 hours after the first dose of 5-FC that day

Participants can be discharged from the hospital after day 7 if they are fully ambulatory and their clinical condition has been deemed stable by the study medical officer, but return for a Day 14 outpatient study visit.

Day 10 - 14

A study medical officer will assess between day 8-14:

- Interval history
- Vital signs
- Focused physical and neurologic examination, directed by symptoms
- Medications and adherence (as appropriate)
- Adverse events (AEs)

Laboratory monitoring will include, at a minimum:

- Serum creatinine
- Serum chemistry (Na, K)
- CBC with autodiff
- ± Lumbar puncture with CSF quantitative culture (at physician discretion, see below*)
- Storage of plasma, serum, and CSF.

* A lumbar puncture at Day 10-14 is at physician discretion, based on earlier quantitative culture results. If participants have sterile cultures from ≥7 days prior (i.e. Day 3 or Day 5-7 CSF culture, respectively) AND have an absence of ongoing symptoms of elevated intracranial pressure (e.g. headache), then physicians may opt to not perform the lumbar puncture.

If life-threatening (Grade 4) lab abnormalities are present or develop during the course of the study, laboratory monitoring shall occur with greater frequency for subject safety. Example:

- Creatinine: if values > 3 mg/dL (>265 mmol/L), Creatinine will be repeated at least daily until stable
- Potassium: if values ≤ 2.5 mEq/L or ≥ 6.5 mEq/L potassium will be repeated STAT and at least daily until improving

In the event of a prolonged hospitalization, the study team will continue to follow the patient daily in the hospital, with assessments, evaluation, and monitoring undertaken according to expected routine standards of care. If any subject is hospitalized at the time of their expected clinic follow-up visit (Week 4, 6, 8, or 10), the study visit will occur while hospitalized.

7.4 Clinic Follow-up Outpatient Visits

7.4.1 Clinic Registration, Orientation, and ART counseling

All subjects will be scheduled to return for outpatient clinic registration in ~1-2 weeks after hospital discharge for clinic orientation and ART counseling, as necessary. After discharge from the hospital, patients will be connected with their local HIV clinic for consideration of ART initiation. ART counseling and initiation will occur per standard of care at 4-6 weeks per WHO guidelines.[4,25]A study nurse will call the patient to verify plans for attending clinic. If subjects are physically unable to attend clinic by ~ 4 weeks, a home visit will occur by study personnel, or a driver will be sent to retrieve the subject if necessary. If there are health concerns (and specifically if the subject needs to be retrieved due to poor functional status and/or inability to ambulate), a study physician visit will occur as clinically needed for a 'sick visit', in addition to the scheduled events, as described in detail below.

At the first outpatient visit, the subject's general health, hydration status, and need for re-hospitalization will be assessed. Locator information including a street geographical mapping to the home or latitude/longitude coordinates of the home and up to 3 telephone contacts will be obtained to minimize lost to follow up.

Outpatient visits have a ±1 week visit window, but substantial deviation with scheduling is discouraged. Visits beyond week 6 can be done by phone.

Week 4 Outpatient Study Visit

The Week 4 visit is optional unless the day 10-14 CSF was not sterile. A medical officer will assess:

- Interval history
- Review of medications and adherence
- Document any adverse events
- Focused physical and neurologic exam, directed by symptoms
- Laboratory evaluation, for all participants:
  - Lumbar puncture if Day 14 is not sterile
- Additional Research labs
  - ± Whole blood for PBMC isolation - immunology studies in Kampala
  - Storage of blood for pharmacokinetic / immunology studies
- Consider ART initiation (or switch to second line ART) if clinically stable

In the event of a prolonged hospitalization or re-hospitalization, the schedule of events will continue per schedule with lab monitoring and procedures conducted in the hospital.

A lumbar puncture will be repeated only if the most recent CSF analysis is culture positive at time of visit.

Pharmacy will reconcile previous study medicines and dispense another 2-week supply.

Week 6 Outpatient Study Visit

At the Week 6 visit, a medical officer will assess:

- Interval history
- Review of medications and adherence
- Focused physical and neurologic exam, directed by symptoms
- Laboratory evaluation:
  - Complete blood count (CBC)
  - Serum sodium, potassium, creatinine
  - ALT, AST, T-bilirubin, alkaline phosphatase
  - Lumbar puncture if Week 4 is not sterile
  - Storage of leftover plasma and serum for pharmacokinetic / immunology studies
- Consider ART initiation (or switch to second line ART) if clinically stable
- Week 6 is the end of the collection period for expedited SAE reporting as all care afterward is the observational standard of care therapy.
- A lumbar puncture will be repeated only if the most recent CSF analysis is culture positive at time of visit.

Week 8 Outpatient Study Visit

Routine HIV care is provided.

- Any clinically significant (i.e. symptomatic) laboratory abnormality should be considered for follow up.
- A lumbar puncture will be repeated only if the most recent CSF analysis is culture positive at time of visit.

Week 10 Outpatient Study Visit

At the Week 10 visit, a medical officer will assess:

- Interval history
- Review of medications and adherence
- Focused physical and neurologic exam, directed by symptoms
- Laboratory evaluation follow up of unresolved AEs
- Lumbar puncture will be repeated if no prior sterile culture has occurred.
- Change to Fluconazole 200mg/day secondary prophylaxis; unless there is a reason to continue higher dose fluconazole (history of CM relapse, CM-IRIS, concurrent fungal infection, or other reasons per medical officer clinical judgment), according to current guidelines for long-term secondary prophylaxis.[4]

Week 12 Outpatient Study Visit

Commented [1]: Should move to week 12 to align with coast

At the Week 12 visit, a medical officer will assess:

- Interval history
- Review of medications and adherence
- Focused physical and neurologic exam, directed by symptoms
- Evaluation of quantitative neurocognitive function at 12 weeks.[60]

Week 18 Termination Visit

Week 18 is the final scheduled visit of the study. This visit can be conducted by phone or in-person. The patient will be offered ongoing HIV follow-up care at the site HIV clinic or referred to a clinic of their choice.

18 weeks signifies completion of the study though subjects may require additional evaluation and follow-up in circumstances of ongoing acute complications. Fluconazole will be continued at maintenance dose (200 mg/daily) according to current guidelines for long-term secondary prophylaxis.

7.4.2 Sick Visits

In all subjects with suspected culture-positive relapse, paradoxical IRIS, or other health concerns, an urgent physician's visit will be made. Patients will be given the mobile phone number of the site study team to make urgent visits on their appointment card. At sick visits, study physicians will perform:

- Interval History, specifically emphasizing review of systems and medication adherence
- Vital Signs
- Complete Physical Exam
- LP (if CNS symptoms)
- Radiologic investigation (as clinically indicated)
- Blood draw, as clinically directed

If there is clinical suspicion for meningitis (e.g., therapeutic failure, culture-positive relapse, or paradoxical IRIS), an LP must be performed (with subject verbal consent). The clinical presentation of a culture positive meningitis relapse and IRIS is clinically indistinguishable, and an LP with complete CSF analysis is necessary to differentiate IRIS from culture-positive relapse. MIC will be performed on all relapse meningitis with culture positive CSF. Clinically, paradoxical IRIS has a classic phenotype with the basic triad of:

1. Treated cryptococcal meningitis with a clinical response,
2. Recently started effective ART, and
3. Recurrent, aseptic (culture negative) meningitis.

The primary diagnostic consideration is in excluding cryptococcal relapse in which the CSF culture would be positive.

After a prior response to CM treatment with resolution of symptoms, evidence suggesting clinical worsening or new signs/symptoms would commonly include increased headache with or

without fever or neurological deficits associated with increased intracranial pressure, increased lymphocytic CSF pleocytosis and/or development of cerebral cryptococcal abscess by contrast CT scan.

For persons with seizures, loss of consciousness, or focal neurologic deficit (other than cranial nerve VI palsy), a head CT will be performed to exclude other intracranial pathology or contra-indication to lumbar puncture (if available). If a CT is not available, LP should be performed at physician discretion. Cranial nerve VI palsy is common (20%) with increased intracranial pressure >200 mm H_2O. The purpose of repeat LP will be to differentiate CM relapse as well as to therapeutically relieve elevated intracranial pressure when present. Diagnosis of non-CNS cryptococcal IRIS will be based on clinical signs (e.g., lymphadenopathy), imaging, and/or biopsy of the relevant site with histopathology and/or culture to exclude alternative etiologies (e.g., AFB).

All other non-IRIS or non-relapse clinical events will be in accordance with current guidelines and existing clinic practices. Refer to Section 10 on AEs and for additional information.

Any CSF obtained during sick visits will have complete CSF analysis (Section 8.2.1). Remaining CSF will be stored for PK studies and future immunology studies.

7.5 Termination of Study

At study termination, the reason for study termination will be documented. Reasons for study termination are study completion (after 18 weeks), withdrawal of consent, death, transfer of care, or loss-to-follow-up. Analysis is by intention to treat. At the study termination visit, the following will be documented:

- Interval history
- Vital status
- AEs, updates / final outcomes

7.5.1 Early Termination

In case of early termination, a reason for study termination will be documented. Reasons for early termination include withdrawal of consent, death, transfer of care, or loss-to-follow-up. An attempt will be made to schedule a termination visit. Assessment and laboratory evaluations for an early termination visit will be the same as the Week 18 visit.

If subjects withdraw from the study, they remain eligible for ART per national guidelines. Persons withdrawing will be encouraged to continue to receive ART from the outpatient clinic or referred to a clinic of their choice for ongoing HIV care and management of CM.

Subjects enrolled in the study but choosing to leave the hospital early against medical advice will continue to participate in the study if they wish. Additional phone calls by study personnel will encourage the subject to seek follow up HIV 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 participation must have a specific request made to them of whether continued collection of vital status (dead / alive) via an 18-week visit or a phone call at a minimum is allowable.

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 HIV-care will be arranged and a request made for a Week 18 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 outpatient to assess interval history and vital status.

8. STUDY PROCEDURES/EVALUATIONS

8.1 Procedures

8.1.1 Clinical History

8.1.1.1 Complete History

A complete medical history will review prior HIV history, TB history, other past medical history, review of systems, medications, and allergies.

8.1.1.2 Interval History

At each subject contact, investigators will seek information on adverse events by open ended questioning and, as appropriate, by examination. This will include structured assessment of the: interval history of the present illness, review of systems, adverse events, new medications, medication adherence.

All clearly related signs, symptoms, and abnormal diagnostic results will be recorded and grouped under one diagnosis. After entry, all signs/symptoms consistent Grade 1-5 clinical adverse events must be recorded at each study visit (refer to Section 9.1). Laboratory abnormalities do not need to be individually documented on an AE form unless accompanied by a clinical event.

8.1.2 Physical Exam

8.1.2.1 Complete Physical Exam

A complete physical exam includes: HEENT (head, eyes, ears, nose, and throat), neck, chest, cardiovascular, abdomen, extremities, skin, and neurologic exam, as well as GU exam as culturally appropriate.

8.1.2.2 Focused Physical and Neurologic Exam

A focused physical and neurologic exam will be directed at current symptoms and complaints with a targeted physical exam by study physicians at a minimum.

8.1.2.3 Quantitative Neurocognitive Performance Score (QNPZ-8)

Neurocognitive function testing may occur at week 12 visit (±2 weeks) using a quantitative neurocognitive performance (QNPZ-8) score in Kampala.

QNPZ-8 is derived from a test battery which includes:

- Grooved Pegboard test
- Color Trails 1 test
- Color Trails 2 test
- WAIS-III Digit Symbol test
- Finger Tapping test
- Hopkins Verbal Learning test – revised (HVLT-R), Learning and Delayed Recall
- Semantic Verbal Fluency test (category fluency)

8.1.2.4 Depression Screening

Prevalence of Depression as per the Patient Health Questionnaire (PHQ-9) will be collected at 12 weeks. PHQ-9 Total Score Depression Severity Scoring is:

1-4 Minimal depression
5-9 Mild depression
10-14 Moderate depression
15-19 Moderate severe depression
20-27 Severe depression

8.2 Lumbar Puncture and CSF Analysis

8.2.1 Diagnostic Lumbar Puncture (LP) with Complete CSF Analysis

All patients with suspected meningitis will be screened for cryptococcosis by CrAg lateral flow assay (LFA). A CrAg LFA will initially be evaluated on whole blood obtained via fingerstick, with the results (return time = 10 minutes) directing subsequent work-up of CSF obtained by diagnostic LP. The screening informed consent process will be started after the fingerstick CrAg is collected. If the fingerstick CrAg is positive, a diagnosis of cryptococcal meningitis will be entertained and CSF studies will be directed to confirm/exclude this diagnosis. To evaluate for CM, a CrAg LFA will be performed on CSF obtained at the bedside during the LP procedure.

In cases of CM confirmed by bedside CrAg LFA, additional CSF studies will be obtained. Refer to the most current CSF Lab SOP on meningitis diagnostics. All LPs will be performed with manometers and have opening intracranial pressure (ICP) and closing pressure recorded. Quantitative fungal cultures will be performed. Remaining CSF samples will be stored at the site laboratory for future pharmacokinetic and pathophysiology studies.

If the screening fingerstick CrAg is negative, a diagnostic LP will be performed and non-specific CSF studies will be obtained to ascertain alternative diagnoses, refer to CSF Lab SOP for current details.

8.2.2 Therapeutic LPs with Quantitative Cultures

After cryptococcal diagnosis is known, follow up LPs will be performed to reduce ICP and document quantitative fungal culture to determine if the CSF is sterile. When an elevated opening CSF pressure is present (>20 cm H2O), the therapeutic reduction of pressure is clinically indicated.[62,63] We have previously demonstrated that a therapeutic LP conducted during the first week of therapy has a 70% relative reduction in mortality.[46]

All follow up 'therapeutic LPs' will be analyzed for quantitative fungal culture. Should a persistent headache recur anytime during the study, a therapeutic LP must be recommended to decrease the intracranial pressure. These follow up therapeutic LPs require verbal consent of the participant per clinical practice.

8.2.3 Quantitative Fungal Cultures

Fungal cultures will be performed per the SOP: "Protocol for quantitative CSF microbiology cultures for *Cryptococcus neoformans*" as provided by Drs. Tom Harrison and Tihana Bicanic.[59,61] The quantitative culture SOP includes an undiluted, standard CSF fungal culture, the result of which will be used for CM management. Cryptococcal isolates will be frozen at ≤ -20ºC in cryovials with 1mL of 25% glycerol. The purpose of storing isolates is to enable external quality assurance and enable future susceptibility testing as needed. Cryptococcus isolates will be stored indefinitely.

8.2.4 Frequency of LPs

An initial LP is required for CM diagnosis at the time of enrollment with written informed consent. Therapeutic LPs will be performed at Day 3 ±1 and Day 7 ±2, and whenever clinically indicated for the reduction of elevated ICP as recommended by IDSA and DHHS guidelines.[62,63] Subjects with a positive CSF culture at Day 14 (estimated ~30%) will be recommended to have a follow up LP by the Week 4 outpatient visit to document whether ongoing culture positive cryptococcosis is continuing. Additionally, subjects with a positive CSF culture at Week 4 (estimated ~10%) will be recommended to have a follow up LP by the week 6 outpatient visit to document whether ongoing culture positive cryptococcosis is continuing.

The majority of individuals being treated for CM are expected to have sterile CSF cultures by Week 4. Those with ongoing CSF culture positivity should have follow up LPs to document clearance of the cryptococcal infection, prior to decreasing the fluconazole maintenance dosing. Until CSF is known to be culture negative, fluconazole should remain dosed at 800 mg/day.

Serial LPs are recommended as the standard of care for controlling elevated ICP in CM. According to the current standard of care, if a subject has elevated intracranial pressure

(>20 cm H2O), an LP should be performed therapeutically to reduce elevated intracranial pressure.[18,64] Therapeutic LPs conducted within the first one week of therapy have a ~70% relative reduction in mortality.[52]

All follow-up LPs must have verbal consent from the subject prior to performing the LP per clinical practice. As LPs are invasive procedures, subjects will need to give consent prior to performing follow up LPs. The initial diagnostic LP at screening visit requires written informed consent. Follow up LPs require verbal consent. Subjects may decline follow-up LPs at their own risk, but at minimum will be offered therapeutic LPs at Day 3 ±1 and Day 7 ±2.

Once subjects are CSF culture negative (given at least 7 days of culture growth) and have a normal CSF opening pressure (< 20 cm H2O), they should not have a routine follow up LP, unless new symptoms develop. If new symptoms develop concerning for CM-IRIS or CM relapse, an LP will be recommended for the subject for diagnosis to guide clinical management.

8.3 Laboratory Evaluations

All routine clinical tests as specified on the schedule of events will be performed at the local site lab. Print out of lab results will be placed into research charts.

8.3.1 Hematology

CBC monitoring will be encouraged as part of routine care at the start of induction antifungal therapy, initial evaluation for ART, and whenever clinically indicated. Scheduled draws will occur at enrollment, Day 5-7, Day 14, and Week 6.

8.3.2 Chemistry

Serum creatinine, sodium, potassium, and ALT, total-bilirubin will be monitored at the local approved site laboratory as part of routine care at the start of CM therapy, initial evaluation for ART, and whenever clinically indicated. Serum aliquots of 0.5-1mL will be frozen at -80ºC. An estimated creatinine clearance can be obtained using the Modification of Diet in Renal Disease (MDRD) Study equation:[65]

GFR (mL/min/1.73 m2) = 175 x (Scr)-1.154 x (Age)-0.203 x (0.742 if female) x (1.212 for African)

8.3.3 CD4 Profile

CD4 profiles will be encouraged on initial HIV diagnosis as part of routine care and at 10 weeks.

8.3.4 Stored Specimens

Any extra serum and plasma collected for clinical testing (e.g., creatinine and potassium) will be stored for later potential cytokine analysis (Bio-Rad, Hercules, CA) via a Luminex Magpix instrument to investigate biomarkers which predict risk of mortality and/or IRIS pathogenesis. The volume of stored blood should always be < 30mL for all pathogenesis studies.

The CSF supernatant will be frozen for pharmacokinetic and pathogenesis studies.

If a subject later withdraws storage consent (after initial consent), their study chart should be marked for 'No Storage,' and the PIs notified in order to destroy any stored specimens.

8.4 Specimen Storage

Specimens processed at the study site will be per the laboratory protocols for the site lab for clinical tests. Extra serum, plasma, and CSF must be stored at -80ºC.

8.5 Biohazard Containment

Appropriate blood and secretion precautions will be employed by all personnel during blood draws, lumbar punctures, and shipping and handling of all specimens for this study, as currently recommended by the CDC, the NIH, and national guidelines.

9. ASSESSMENT OF SAFETY

This is a clinical trial investigating whether a lower dose of flucytosine is safe and effective compared to standard dosing. There is evidence that lower doses reach adequate levels above MIC for *Cryptococcus*.

Participants will also receive fluconazole and/or Amphotericin B as well as HIV therapy and cotrimoxazole. HIV therapy will be provided via the public health system. This study will provide antifungal therapy while hospitalized, if otherwise unavailable. Outpatient standard of care fluconazole will be provided by this research study.

9.1 Adverse Event (AE) Definitions

The intervention during this trial is the reduced dose of flucytosine for induction therapy.

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. An AE can therefore be any unfavorable or unintended sign (including abnormal laboratory findings), symptom, or disease temporally associated with the use of a medicinal product. Any symptom, sign, illness or experience that develops or worsens in severity during the course of the study are considered AEs.

Any medical condition already present at the time of screening should not be reported as an adverse event unless the medical condition or signs or symptoms present at baseline changes in severity or seriousness at any time during the study. In this case, it should be reported as an adverse event.

As a diagnosis may have multiple signs or symptoms associated with the AE (e.g., sepsis presenting with fever, hypotension, confusion), a single diagnosis for the AE is preferred over reporting each individual symptom.

Clinically significant abnormal laboratory or other examination (e.g., ECG) findings that are detected during the study or are present at the time of screening and significantly worsen during the study are defined and should be reported as adverse events. The Investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding, or other abnormal assessment is clinically significant. Clinically significant abnormal laboratory values occurring during the clinical study will be followed until repeat tests return to normal, stabilize, or are no longer clinically significant. Any abnormal test that is determined to be an error does not require reporting as an adverse event. Laboratory abnormalities will be separately categorized from clinical AEs.

Worsening of the index indication (cryptococcal meningitis) due to insufficient therapeutic effect of study drug is captured as an efficacy measure (i.e., death, poor early fungicidal activity) and in general will not be considered an adverse event but are captured in other individual trial endpoints. As participants enter the trial with Grade 4 cryptococcal meningitis, worsening is not possible, unless death occurs.

9.2 Assessment of Adverse Events by the Investigator

The Investigator will assess the severity (intensity) of each adverse event as mild, moderate, severe, or life threatening.

9.2.1 Severity of Adverse Events

The term severity is defined as the intensity grade or level for a specific event, i.e., mild, moderate, severe, or life-threatening. Importantly, severity is *not* the same as seriousness, which is based on participant/event *outcome or action* criteria usually associated with events that pose a threat to a subject's life or functioning (ICH E2A). The *Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events (v2.1, July 2017)* will be used. Laboratory AEs will be defined using the statistical database by DAIDS Table grading thresholds.

The Investigator will assess the severity (intensity) of each adverse event as mild, moderate, severe, or life threatening and will also categorize each adverse event as to its potential relationship to study drug using the categories of yes or no.

Assessment of severity:

The severity of all adverse events should be graded as per The *Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events (v2.1, July 2017 or*

most current). For those adverse events not listed, the following grading system should be used:

- Mild (Grade 1): Transient symptoms, awareness of sign/symptom, but easily tolerated and no interference with patient's daily activities.
- Moderate (Grade 2): Marked signs/symptoms that interfere with a patient's usual activities, but still acceptable.
- Severe (Grade 3): Incapacitating signs/symptoms which cause considerable interference with the patient's daily activities, unacceptable.
- Life-threatening (Grade 4): Life-threatening or disabling adverse event.
- Death (Grade 5): Death-related adverse event.
    - Death from pre-existing cryptococcal infection is part of the survival endpoint, which is expected to be up to 20% of enrolled subjects. Cryptococcal meningitis deaths without other AEs should not be additionally reported as a Grade 5 AE. The objective is to discern Grade 5 deaths due to adverse events versus deaths from the progression of the pre-existing cryptococcal meningitis. Overall survival will be separately analyzed as the secondary endpoint.

9.2.2 Causality of Adverse Events

The probable causality of the investigational study drug being related to the AE will be collected.

Definitely related	There is a certainty that the AE is related to the study drug.
Probably related	There is a high likelihood that the AE is related to the study drug.
Possibly related	There is a likelihood that the study drug is the cause of the AE, but other causes cannot be ruled out.
Unlikely to be related	It is not likely that the AE is related to the study drug, and other more likely causes are present.
Unrelated	Evidence exists that the AE is related to something other than the study drug.

AEs will use pre-defined causality for the common, expected medical conditions associated with HIV/AID (e.g., TB, new opportunistic infections), other infections (e.g., malaria, pneumonia) and cryptococcal meningitis complications. Refer to Manual of Operations for details.

Therapeutic failure of the antifungal therapy alone is not deemed as causal for an AE (i.e. it is unrelated).

The most common disease-related complications and/or standard of care treatment-related side effects include:

- Death due to pre-existing cryptococcosis (up to 20% of enrolled subjects);
- Meningitis complications: e.g., seizures, cranial nerve palsies, increased intracranial pressure
- Transient renal insufficiency in the first 14 days after study entry due to IV amphotericin (20%);
- Cryptococcal IRIS events (~20%);
- AIDS-related opportunistic infections;
- Hospitalization or death due to immune reconstitution inflammatory syndrome (IRIS), cryptococcal culture-positive relapse, or AIDS opportunistic infection (20-25%);[18,19,26]
- Common ART or flucytosine side effects (e.g., anemia, leukopenia)
- Common IV amphotericin side effects (e.g., anemia, hypokalemia, renal failure)
- Hospital nosocomial infections and other complications (e.g., aspiration pneumonia)

9.3 Adverse Events of Special Interests

AEs of special interest will be queried of participants while hospitalized on a daily basis.

9.4 Serious Adverse Event

AEs are classified as serious or non-serious. A S*erious adverse event* is:

- fatal
- life-threatening
- requires re-hospitalization (after hospital discharge for the initial, pre-existing meningitis)
- prolonged hospitalization (due to a new AE; not due to pre-existing meningitis or HIV/AIDS)
- 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 (e.g., anaphylaxis)

All AEs that do not meet any of the criteria for serious will be classified as *non-serious adverse events*. A standard medical problem, e.g., malaria, which in theory if untreated could result in a fatal or life-threatening event, is not by itself a SAE. If the AE is of sufficient severity to result in hospitalization, then the AE is a SAE.

Routine outpatient medical therapy for a common, expected illness in the study population (e.g., malaria, TB) is not a SAE.

All SAEs will be:

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

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

Death due to pre-existing cryptococcal meningitis is not a SAE. Death due to a complication of meningitis (e.g., new seizure) would be a SAE.
Prolongation of hospitalization must be due to a new AE, not the pre-existing cryptococcal meningitis or pre-existing HIV/AIDS and related conditions. A *new* AE must cause the prolonged hospitalization for this to be considered a SAE. Hospitalizations due to cryptococcal meningitis commonly may last 2-4 weeks.

9.5 Serious Adverse Event Reporting – Procedures for Investigators

Initial reports

All SAEs occurring from the time of informed consent through 2 weeks within 72 hours of the knowledge of the occurrence (this refers to any adverse event that meets any of the aforementioned serious criteria).

Follow-up reports

The Investigator must continue to follow the patient until the SAE has subsided or until the condition becomes chronic in nature, stabilizes (in the case of persistent impairment), or the patient dies.

Within 24 hours of receipt of follow-up information, the investigator must update the SAE form for the study and submit any supporting documentation (***e.g.***, patient discharge summary or autopsy reports) to the IRB via e-mail. If it is not possible to access the CRF system, refer to the procedures outlined above for initial reporting of SAEs.

9.6 Recording of Adverse Events

Clinical AEs (Grades 3-5) occurring during the first 2 weeks of the research study will be recorded in the CRF, and the Investigator will give his or her opinion as to the relationship of the AE to the study drug treatment (i.e., whether the event is related or unrelated to study drug administration).

All AEs will be documented. A description of the event, including its date of onset and resolution, whether it constitutes an SAE or not, any action taken (e.g., changes to study treatment), and its outcome should be provided, along with the Investigator's assessment

of causality (i.e., the relationship to the study treatment). For an AE to be a suspected treatment-related event, there should be at least a reasonable possibility of a causal relationship between the protocol treatment and the AE. Adverse events will be graded according to The *Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events (v2.1, July 2017)*.
If the AE is serious, it should be reported immediately to the IRB. Other untoward events occurring in the framework of a clinical study after enrollment are to be recorded as AEs. Laboratory test results do not require individual additional reporting on CRFs as these will be summarized from the statistical database for Grade 1 lab AEs and above.

9.7 Reporting Period for Adverse Events

All AEs regardless of seriousness or relationship to study treatment, spanning from the signing of the informed consent form until 10 weeks are to be recorded on the CRF. The AEs occurring from the signing of the ICF until the first dosing of the study drug will be the pre-treatment AEs. The AEs occurring at or after the first dosing until completion of treatment will be the treatment-emergent AEs (TEAEs). The study will continue with a follow-up period after ~30 calendar days post completion of study drug through 10 weeks.

All AEs resulting in discontinuation from the study should be followed until resolution or stabilization. All new AEs occurring during this period must be reported and followed until resolution unless, in the opinion of the Investigator, the AE or laboratory abnormality/ies is/are not likely to improve because of the underlying disease. In this case, the Investigator must record his or her reasoning for this decision in the patient's medical record.

9.7.1 Laboratory AEs

As laboratory anomalies are common in this population with advanced HIV/AIDS and causality difficult to discern, all laboratory values will be graded but not assigned causality individually.

All lab results shall be reported on 'Blood Results CRF.' Lab abnormalities will be summarized from the statistical database and graded for severity according the DAIDS Toxicity Table. All grades 1-5 will be summarized. These laboratory abnormalities will be summarized separately from clinical AEs.

9.8 Study Medication Discontinuation

This study medicine can be halted or interrupted if the investigator of record has concern of harm by the study drug. If the participant wishes to dose reduce due to intolerance of GI side effects (e.g. nausea), this is allowable. This is not a protocol deviation, but tolerability is an exploratory endpoint. The participant may discontinue the study medicine and remain in the study. Any dose reduction or discontinuation should be recorded on the 'Med CRF' for the study medicine. Before discontinuing the medication, the investigator should consider dividing the scheduled daily dose into smaller doses or dose reduction.

9.9 Pre-existing Conditions

A pre-existing condition is one that is present at study entry. All persons in the trial have life-threatening (Grade 4) cryptococcosis and HIV/AIDS. At study entry, any clinically significant abnormality should be recorded on the study CRF as a pre-existing condition and graded 1-4. Cryptococcosis has a variety of symptoms associated with this disease state, and these symptoms will be expected to wax and wane. Each symptom of cryptococcal meningitis (and the disseminated cryptococcosis present thereof) is not an AE; however, new complications of cryptococcosis would be a new AE. Examples of complications would include: new seizures, new aspiration pneumonia, new obstructive hydrocephalus, etc.

All pre-existing conditions must be clearly documented at study enrollment as new Grade 3-5 AEs diagnosis are a study endpoint. As a diagnostic evaluation may be underway at time of enrollment (e.g. tuberculosis), the investigator should document if the condition was present prior to enrollment, even if the final diagnosis was made after enrollment. If a participant enters the study with a diagnosis and then the frequency, intensity, or the character of the condition worsens by Grade +1 level during the 2-week reporting period, the event should be defined as a new AE.

At the end of 2 weeks, any new clinically significant findings/abnormalities that meet the definition of an AE in retrospective comparison to the pre-existing conditions must also be recorded and documented as an AE(s).

9.10 Pregnancy Reporting

If the patient or partner of a patient participating in the study becomes pregnant during the 18-week study, the Investigator should report the pregnancy to the IRB within 24 hours of being notified. The IRB will then forward the exposure in-utero form to the investigator for completion.

If a patient becomes pregnant while in the study, their medical care will be customized in discussion with the trial PIs. Safety and monitoring procedures will continue to be performed. The major concern is the known teratogenic effect of high-dose fluconazole in the first trimester.

The patient should be followed by the investigator until completion of the pregnancy. If the pregnancy ends for any reason before the anticipated date, the investigator should notify the IRB. At the completion of the pregnancy, the investigator will document the outcome of the pregnancy. If the outcome of the pregnancy meets the criteria for immediate classification as an SAE (i.e., postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly), the investigator should follow the procedures for reporting an SAE.

9.11 Expedited Reporting

During the AE reporting period of 2 weeks to comply with national regulations, reports of all suspected unexpected serious adverse reactions (SUSARs) will be submitted to the IRB with oversight within 7 calendar days of the study site awareness of the AE. Relevant follow-up information will subsequently be communicated within 8 days.

Reporting will be via the ‘Adverse Event Reporting CRF.’ Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator’s binder.

All other suspected unexpected serious adverse reactions will be reported to the FDA, applicable competent authorities and Ethics Committees as required as soon as possible but within a maximum of 15 days of first knowledge by the Sponsor. Relevant follow-up information will subsequently be communicated within 15 days.

The Sponsor will also inform all investigators as required.

Listings of SAEs related to 5FC will be included in the Development Safety Update Report.

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

- Study identifier
- Study Site
- Subject number
- Date of event
- A description of the event
- Medical treatments given/discontinued
- Working diagnosis
- Current vital status

Within the 7 calendar days following the event, the investigator will provide further information in the form of a written narrative. This will be documented along with any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing SAEs will be provided promptly to the study sponsor and local IRB of record.
Any health authority safety reports will also be submitted to the Uganda National Drug Authority, both expedited and on an annual basis.

10. CLINICAL MONITORING

10.1 Site Monitoring Plan

Site monitoring will be conducted to ensure that the human subject protection, study procedures, laboratory, study intervention administration, and data collection processes are of high quality and meet the sponsor, ICH E6 and regulatory guidelines. The objectives of a monitoring visit are to:

- Verify the existence of signed informed consent documents and documentation of the Informed Consent Form process for each monitored subject;
- Verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs;
- Compare data abstracts with individual participants' records and source documents (laboratory analyses and test results, and any other relevant original subject information);
- Help ensure investigators are in compliance with the protocol.

10.2 Medical Monitor

The medical monitor will review the overall clinical progress of the study and provide protocol guidance and clarifications as needed. The medical monitor will review AE CRFs for the appropriateness of: working/final diagnoses, SAE designation, AE severity grade,

expectedness, and relatedness to study medication. The medical monitor will provide feedback to the study investigators, requesting further documentation, as needed.

10.3 Data and Safety Monitoring Board (DSMB) Safety Reviews

Refer to the DSMB Charter for full details.

- DSMB safety reviews will occur after 10 participants have been enrolled and have accrued approximately 2 weeks of follow-up data.

The DSMB committee will consist of Dr. Peter Williamson as the chairperson, Dr. Agnes Kiragga, Dr. Felix Bongomin, Dr.Emilio Letang, and Dr. Kenneth Ssebambulidde. The trial PIs and statistician will present to the DSMB in an open session before the DSMB enters into a closed, private session. A quorum of three members will be required to meet, including the DSMB statistician. A local Ugandan community member will be invited to attend open DSMB meetings. The PhD study biostatistician will prepare summary progress reports on tolerability, safety, and efficacy within approximately 30 days of 10 participants being enrolled and accruing 2 weeks of follow up data. At time of the safety reviews, all available data will be utilized, particularly with respect to control subjects.

Data are unblinded, thus the PI, on-site investigators, and other team members will additionally monitor for any unexpected concern. If concerns are raised, this will prompt an unscheduled DSMB safety evaluation.
If there are unexpected safety concerns of serious and potentially related AEs, these AEs will be reviewed by the DSMB, and reported to the relevant regulatory authorities with oversight. Unexpected safety concerns may result in suspension of further trial interventions or closure to further enrollment. The study PIs, DSMB chair, and sponsor each retain the authority to suspend additional enrollment and study interventions for the entire study; however, consensus agreement will be sought. Examples of findings that might trigger a safety review are the number of SAEs overall, the number of occurrences of a particular type of SAE, severe AEs/reactions, or increased frequency of events. In particular, the investigators will be monitoring for culture positive Cryptococcal relapse. This has been rare in previous trials and a small number of cases would be cause for an early safety review. The biostatistician will perform a formal analysis as per the statistical plan.

Reporting to the IRB of record will occur with annual reporting, and after each DSMB report. We will report all deaths, including cryptococcal related deaths to the IRB.

11. STATISTICAL CONSIDERATIONS

11.1 Statistical Analysis Plan

Descriptive statistical methods will be used to summarize the data from this study, with hypothesis testing performed for the primary efficacy endpoint. Unless stated otherwise, the term “descriptive statistics” refers to number of subjects (N), mean, median, standard deviation,

standard error, minimum, and maximum for continuous data and frequencies and percentages for categorical data. All data collected during the study will be included in data listings. Unless otherwise noted, the data will be sorted first by treatment assignment, subject number, and then by date. Unless specified otherwise, all statistical testing will be two-sided and will be performed using a significance (alpha) level of 0.05. The statistical analyses will be conducted with the SAS® software package version 9.4 or higher or R Studio® 2022.07.1 or higher. All analyses will be subject to formal verification procedures. Analysis will be by intent-to-treat principle.

11.2 Timing of Analyses

The primary analysis of the primary and key secondary endpoints will be conducted once the last patient completes 2 weeks of the study or discontinues prematurely, and the resulting database is cleaned and locked.

A follow-up analysis will be conducted including all data through Week 18 once the last patient completes 18 weeks on study or discontinues prematurely.

11.3 Statistical Hypothesis

We hypothesize that a regimen with lower-dose 5-FC will be well tolerated, have reduced toxicity compared to a regimen with standard dose 5-FC, will have a superior rate of CSF fungal clearance compared to historical fluconazole monotherapy, and will have a statistically non-inferior rate of CSF fungal clearance compared to the historical standard-dose 5-FC. Full details of statistical considerations for making comparisons with historical controls will be provided in Statistical Analysis Plan.

11.4 Primary Endpoint:

The primary endpoint is CSF early fungicidal activity (EFA) during induction treatment: as measured in the change in $\log_{10}$ CFU/mL of CSF/day and as assessed with general linear models. EFA from the low-dose 5-FC group will be compared to historical controls of standard-dose IV amphotericin + standard dose flucytosine and fluconazole.

11.4.1 Sample Size & Statistical Power

Similar to phase II TB trials, rate of CSF yeast clearance has been used in moderate size phase II cryptococcal trials to demonstrate microbiologic efficacy as proof of concept. When the EFA is better than 0.20 CFU/mL/day, there is reasonably good survival after cryptococcal meningitis (Figure 2.5). Once the EFA is better than approximately 0.30 $\log_{10}$ CFU/mL/day, there is less obvious incremental gains in survival beyond this threshold.[61]

Overall, the EFA is a quantitative measure of microbiologic activity in humans of an antifungal regimen.

For the EFA on a lower-dose 5-FC, we will have 36 participants with adequate data to calculate the EFA (i.e. non-sterile CSF culture at baseline and at least 1 follow up LP) and a standard deviation of approximately 0.20.

The CSF EFA will be interpreted in the context of other antifungal regimens published EFAs and the concurrent controls (Table 2.5). For example, if the lower-dose 5-FC arm has an EFA >0.30 log_{10} CFU/mL/day and standard deviation of $\pm$0.20, then the low dose 5-FC regimen will establish superiority compared to fluconazole 1200 mg regimen and non-inferiority with the all oral 5FC + fluconazole regimen. For a low-dose 5-FC arm EFA of 0.35, non-inferiority compared to the concurrent controls will be established with a margin of 0.17. For an EFA better than 0.30 log_{10} CFU/mL/day, survival is relatively similar across antifungal regimens, as per section 2.5 and Figure 2.5.

A subsequent statistical analysis of the ASTRO clinical trial has demonstrated that 30 to 40 patients with EFA is sufficient to determine a significant difference in EFA. This was based on a bootstrap analysis of the participants of the ASTRO trial that is documented in table 11.4.1 below.

Table 11.4.1: EFA Calculated with the ASTRO Study (n=378)

Replicate Size	500 Bootstrap EFAs from randomly selected replicates
N	Mean (95%CI)
10	-0.454 (-0.768, -0.219)
15	-0.450 (-0.688, -0.257)
20	-0.449 (-0.664, -0.269)
30	-0.445 (-0.608, -0.292)
40	-0.445 (-0.593, -0.308)
50	-0.446 (-0.569, -0.316)
378 Actual	-0.444 (-0.489, -0.399)

11.5 Secondary Endpoints

Key Secondary Endpoint

DOOR as ordinal ranked maximum score tested by Win Ratio.

a. 18-week Survival with CSF sterility by 2-weeks
b. 18-week survival with CSF culture positivity beyond 2 weeks
c. Grade 3 hematological adverse event by 2 weeks
d. Grade 4 hematological adverse event by 2 weeks
e. Lost to follow up before 18-weeks
f. Serious Adverse Event through 18 weeks (e.g. all-cause re-hospitalization, permanent neurologic deficit)
g. Death by 18-weeks

Secondary Endpoints

1) CSF culture sterility cumulative incidence over 18 weeks using Gray's method. All persons who are non-sterile at baseline will be included with all CSF culture data considered.
    a) CSF culture sterility at 2 weeks, 6 weeks, 10, and 18 weeks will be summarized. All persons who are non-sterile at baseline will be included with all CSF culture data considered through the relevant study visit window.
2) 18-week survival time

11.6 Safety and Exploratory Endpoints

1) Laboratory anomalies (Grade 3-5 AEs) as per the NIAID DAIDS toxicity grading table will be summarized for the incidence over 2 weeks of having at least one event and the mean number of events with cumulative incidence and linear regression models, respectively. If the cumulative incidence of a laboratory AE for any given anomaly (e.g. anemia, acute kidney injury) is >10%, the lab measurement will be individually summarized as a continuous variable. This is the key safety endpoint.
    a) Grade 1-2 AEs will also be collected and summarized but are not the protocol scientific endpoint.
2) Clinical adverse events (Grade 3-5 AEs or SAEs) will be summarized via cumulative incidence function.
    a) Note: Clinical AEs are listed separately from laboratory anomalies as lab AEs occur in the majority of this critically ill population with advanced AIDS and receiving IV AMB.
3) Tolerability will be assessed as the percent of received and tolerated doses versus total daily doses as a continuous variable for the experimental low-dose 5-FC and control standard dose 5-FC groups over the first two weeks.
    a) Dropouts and study withdrawals will be summarized.
4) Descriptive analyses for the lower-dose 5-FC on pharmacokinetics of 5-FC concentrations in blood, CSF, and other bodily fluids/tissues; both antemortem and possibly postmortem.

11.7 Statistical Power for Secondary Outcomes

Secondary endpoints are primarily descriptive in nature without statistical power for individual dose comparison unless the effect size is large.

12. ETHICS/PROTECTION OF HUMAN SUBJECTS

12.1 Ethical Standard

This study is to be conducted according to US and international standards of Good Clinical Practice (International Conference on Harmonization guidelines), Declaration of Helsinki, and

International Ethical Guidelines for Biomedical Research Involving Human Subjects, applicable government regulations for Uganda, U.S., international and Institutional research policies and procedures. All investigators must have received human subject protection and Good Clinical Practice training prior to human subject involvement.

12.2 Institutional Review Board

Prior to the initiation of the study at the clinical research site, the protocol, all informed consent forms and the participant Information materials will be submitted to and approved by the local IRB of record. Likewise, any future amendments to the study protocol will be approved by each site's IRB.

This protocol and any amendments will undergo review and approval by the local site IRB in Kampala, Uganda and the Uganda National Council of Science and Technology (UNCST) under FWA00001293, University of Minnesota (FWA00000312), and all other relevant local/national IRBs for any clinical site.

12.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the research study and continuing throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the subjects and their families. Consent forms describing in detail the study interventions/products, study procedures, and risks are given to the subject and written documentation of informed consent is required prior to enrollment and starting the study product. Consent forms will be IRB-approved, and the subject will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the subject and answer any questions that may arise. The subjects will sign the informed consent document prior to any procedures being done specifically for the study. Procedures being performed for standard medical care (e.g., lumbar puncture, CSF diagnostic testing) may be performed prior to informed consent. Meningitis is a medical emergency, and routine medical care should not be withheld, endangering potential participants.

The subjects should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

12.4 Subject Confidentiality

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party without prior written approval of the sponsor. For research uses, all data will be de-identified and coded with the clinic medical record number.

Protected health information will only be shared through a secure network and will not be used or sent through WhatsApp, Zoom, or email.

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

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study, or the data will be released to any unauthorized third party without prior written approval of the sponsor. For research uses, all data will be de-identified and coded with the clinic medical record number.

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

12.5 Study Discontinuation

In the event that the study is discontinued, subjects remain eligible for ART according to national guidelines and as provided by PEPFAR, Global Fund, or Ministry of Health sources. Participants will be encouraged to continue to receive ART from the outpatient clinic or referred to a clinic of their choice for ongoing HIV care and management of CM.

12.6 Future Use of Stored Specimens

According to Ugandan regulations, a separate informed consent for long term storage of samples for future research testing is necessary. The “SCREENING STORAGE CONSENT FORM FOR MENINGITIS” asks for consent for long term storage of samples. Subjects have the option to consent for long term storage of samples for this study only and/or for future research. If a subject declines storage consent, this will be noted on their lab order entry form and in their study chart, and specimens will be discarded after appropriate clinical testing is completed. The purpose of the long-term storage is to enable: 1) future diagnostic testing for assays which are not currently commercially available (e.g., multi-band semi-quantitative CrAg LFA; new TB tests); 2) pathogenesis studies to better understand immunology and microbiology of cryptococcal meningitis in relation to clinical outcome; 3) pharmacokinetic studies of antifungal medications; 4) antifungal susceptibility testing of *Cryptococcus* isolates.

There will not be a separate storage informed consent form for the FLOOR study. However, all FLOOR participants will be co-enrolled in the ongoing COAST observational study. This study includes a separate storage informed consent form.

13. DATA HANDLING AND RECORD KEEPING

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

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

13.1 Source Documents

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, research charts, laboratory notes, memoranda, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial.

The research team are the primary healthcare providers for participants after they enter into the trial and will manage all routine care for the participants. The research team will work together with the hospital team for complete hospital management and the hospital clinical team will be involved for emergent and urgent needs of the participant when the research team is not available or actively providing care on the wards.

The Daily Clinical Review form will serve as the source for clinical observations obtained by the study team, as this is the first location where data are recorded. During outpatient visits, CRFs are completed first with information then transcribed into the electronic medical record.

Study data forms will be digitally scanned for permanent record keeping and for enabling rapid resolution of any discrepancies.

The site will maintain appropriate medical and research records for this trial, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects.

Information Type	Source Document	Rationale / Record Location
Lab Values	Paper Lab Report	In Research chart
Microbiology Values	Paper Lab Report	In Research chart
CSF CrAg Bedside Test	Screening CRF	CrAg testing is performed at bedside by trained study staff and the results are recorded on a CRF
Pregnancy Test	CRF	Point-of-care pregnancy test is performed by study staff in the hospital, recorded on the CRF
Medical History	Screening CRF and Meningitis CRF	Past medical history is obtained directly from the patient and recorded on the Screening and Meningitis CRFs. Hospital Records are not used.
Physical Exam, or Clinical Observations	Daily Clinical Review Form	Clinical observations are recorded directly in the Daily Clinical Review Form. Hospital records are not used and are not the source document.
Study Medicine Dispensing	Pharmacy Dispensing Log	Research Pharmacy in locked file cabinet
Study Medication Administration	Daily Clinical Review Form and Nurses Notes; Participant Adherence Diary	Research staff record study medication administration on daily clinical review forms and nurses notes while participant is in hospital; Participants self-report study medication administration after hospital discharge in a patient adherence diary.
Concomitant Medications	Med CRF	Research team prescribes all medications. Medications are recorded on Med CRF by the prescribing provider.
Clinical Adverse Events	AE Log	Research staff record AEs on the AE Log. Participants contact research study team for all their health care needs. (Given 24/7 contact phone numbers and reimbursed for transport costs for scheduled or sick visits). All healthcare once enrolled is provided without cost (within reasonable relatedness to HIV, meningitis, or any AE).
Lab Adverse Events	Paper Lab Report	In Research chart

13.2 Data Management Responsibilities

All source documents and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse events must be graded, assessed for severity and causality, and reviewed by the site PI or designee.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. During the study, the investigator must maintain complete and accurate documentation for the study.

13.3 Data Capture Methods

Data entry will occur via computer database using DataFax system.

13.4 Types of Data

Data for this study will include safety, laboratory (clinical, immunologic and microbiological), and outcome measures (e.g., survival, hospital duration, performance status).

13.5 Study Records Retention

The investigator will retain study essential source documents for 15 years after the completion of the study or at least 2 years after market authorization or formal discontinuation of the product whichever is longest. Digital images of the source documents will be retained for an indefinite period.

13.6 Protocol Violations and Deviations

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

Protocol Deviation: Any change, divergence, or departure from the IRB approved study procedures in a research protocol that does not have a major impact on the subject's rights, safety or well-being, or the completeness, accuracy and reliability of the study data. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions will be developed 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 to use continuous vigilance to identify and report protocol deviations within 5 working days of identification, or within 5 working days of the scheduled protocol-required activity.

13.7 Quality Control and Quality Assurance

A QC/QA Quality Management plan will be implemented centrally via the DataFax system which incorporates quality control for complete record keeping.

This study may be subject to a quality assurance audit by Matinas or its designee, as well as inspection by appropriate regulatory authorities.

14. ADMINISTRATIVE

14.1 Publication of Research Findings

The Principal Investigators are expected to publish the results of this clinical trial in a scientific journal. Publication of the results of this trial will be governed by the co-PIs: Drs Meya and Boulware in accordance with standard academic practices with the freedom to publish without restriction. The investigators agree to submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide optional comments.

The clinical study plan and the results of the study will be published on www.ClinicalTrials.gov in accordance with 21 CFR 50.25(c).

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. The NIH Public Access Policy ensures the public has access to the published results of NIH funded research.

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Appendix A: Adverse Events observed with IV amphotericin + fluconazole therapy

Cryptococcal Optimal ART Timing (COAT) Trial in Uganda used amphotericin and fluconazole with a randomized timing of ART initiation. Data below are as published in the manuscript's supplemental appendix.[18] In total, 436 Grade 3-5 adverse events occurred in 177 subjects with cryptococcal meningitis. The majority were asymptomatic lab abnormalities, related to AIDS, cryptococcosis, or amphotericin B deoxycholate therapy.

Figure S3a. Cumulative Incidence of Grade 3−5 Adverse Events

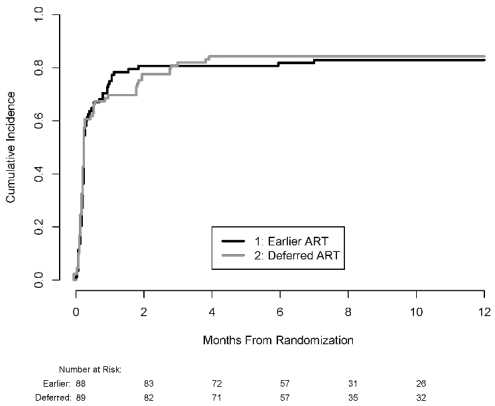

Figure S3b. Cumulative Incidence of Grade 4 or 5 Adverse Events

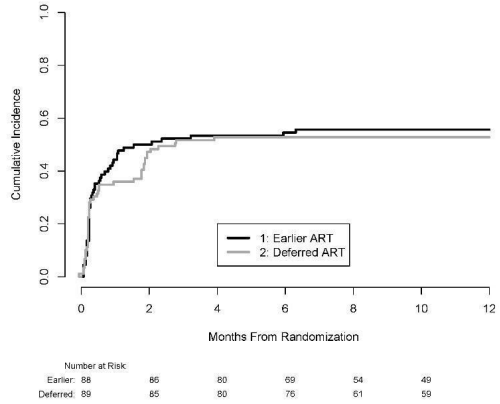

Figure S3a displays the cumulative incidence of time to a first Grade 3-5 adverse event by trial arm as defined by the NIAID DAIDS Toxicity Grading Scale, 2009. The AE distribution by body system was:

Distribution by Body System	**Earlier ART**	**Deferred ART**
Hematology	99 (47.8%)	128 (55.9%)
Chemistries	65 (31.4%)	53 (23.1%)
Infection	13 (6.3%)	26 (11.4%)
Neurological	11 (5.3%)	5 (2.2%)
Gastrointestinal	6 (2.9%)	8 (3.5%)
Cardiovascular	6 (2.9%)	5 (2.2%)
Systemic	4 (1.9%)	2 (0.9%)
Skin, dermatological	2 (1.0%)	1 (0.4%)
Respiratory	1 (0.5%)	1 (0.4%)
Overall (Number of events)	**207 (100%)**	**229 (100%)**

The most commons adverse events included anemia (n=88), neutropenia (n=54), leukopenia (n=24), hyponatremia (n=21), hypokalemia (n=23), and elevated creatinine (n=18).

Grade 5 events occurred in 14 (15.9%) persons randomized to earlier ART and 11 (12.3%) randomized to deferred ART. Grade 5 events did not include deaths from *Cryptococcus*.

The line listing of AEs was:

COAT Trial: Adverse Event Line Listing		**Grade 3-5 Adverse Events**			**Grade 4 and 5 Adverse Events**	
Body System	**MedDRA Term**	**Earlier ART**	**Deferred ART**		**Earlier ART**	**Deferred ART**
Cardiovascular	Cardiac arrhythmias	1	0		1	0
	DVT	4	1		1	0
	Pulmonary embolus	0	1		0	1
	Tachycardia	0	1		0	0
Chemistries	Alanine aminotransferase (ALT) increased	2	1		0	0
	Aspartate aminotransferase (AST) increased	5	4		2	1
	Blood alkaline phosphatase increased	2	0		1	0
	Blood bicarbonate decreased	6	5		1	2
	Blood bilirubin increased	1	0		0	0
	Blood sodium decreased	14	7		8	3

	Blood sodium increased	0	2		0	1
	Hyperkalemia	2	0		2	0
	Potassium serum decreased	15	8		4	1
	Serum creatinine increased	8	10		2	2
Gastrointestinal	Decreased appetite	1	1		0	0
	Diarrhea	3	2		1	1
	Nausea and vomiting	2	2		1	1
Hematology	Anemia	41	47		23	31
	Leukopenia	11	13		6	3
	Neutropenia	23	31		12	12
	Thrombocytopenia	4	13		1	3
Infection	Abdominal pain localized	0	1		0	0
	Acinetobacter bacteremia	0	1		0	1
	Acute pneumonia	1	0		0	0
	Bronchopneumonia	1	0		0	0
	Burkholderia cepacia complex	0	1		0	0
	Cystitis, Klebsiella	1	0		1	0
	Disseminated tuberculosis	0	2		0	2
	Klebsiella sepsis	1	1		1	1
	Pulmonary tuberculosis	0	1		0	0
	Salmonella bacteremia	0	1		0	0
	Salmonella sepsis	0	1		0	0
	Sepsis	1	4		1	3
	Septicemia due to pseudomonas	1	1		1	0
	Staphylococcus aureus septicemia	1	0		1	0
	Tuberculosis	0	1		0	1
Neurological	Acute mental status changes	5	3		4	0
	Intracranial pressure increased	3	0		0	0
	Muscle weakness right-sided	2	0		2	0
	Sensory neuropathy	1	0		0	0

Respiratory	Dyspnea	1	1		1	
Dermatological	Erythroderma	1	0		0	
	Rash - morbilliform	0	1		0	
Systemic	HIV wasting syndrome	0	1		0	
	Pyrexia	1	0		0	