Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

ACTA Protocol and Statistical Analysis Plan

This supplement contains the following items:

1. Original protocol, final protocol, summary of changes.
2. Original statistical analysis plans (main study and adjunct), final statistical analysis plan, summary of changes.

A RANDOMISED CONTROLLED TRIAL OF ORAL FLUCONAZOLE PLUS FLUCYTOSINE VERSUS AMPHOTERICIN B-BASED THERAPY, FOR ONE OR TWO WEEKS, FOR INITIAL TREATMENT OF HIV-ASSOCIATED CRYPTOCOCCAL MENINGITIS.

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Authorised by:

Name: Thomas S Harrison   Role:

                                                On behalf study consortium

Signature: Date: 29th December 2011

GENERAL INFORMATION

This document describes the AMPHOTERICIN B VS OPTIMISED ORAL THERAPY trial and provides information about procedures for entering patients into it. The protocol should not be used as an aide-memoire or guide for the treatment of other patients; every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to the registered investigators in the trial. Clinical problems relating to this trial should be referred to the Investigators.

- **Compliance**

The trial will be conducted in compliance with the protocol, ICH GCP Guidelines, and other regulatory requirements applying in the countries in which the trial will be conducted.

- **Sponsor**

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- **Trial Manager/Monitor**

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CONTENTS

Abbreviations and Glossary

AE	Adverse event
Ag	Antigen
AIDS	Acquired Immune Deficiency Syndrome
ALT	Alanine transaminase
AmB	Amphotericin B
AR	Adverse reaction
ART/ARV	Anti-retroviral therapy/ Anti-retroviral
CF	Consent form
CRF	Case Report Form
CSF	Cerebrospinous fluid
EFA	Early Fungicidal Activity
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
IDMC	Independent Data Monitoring Committee
Int Inv	International Investigator(s)
PBMC	Peripheral Blood Mononuclear Cell
PI	Principal Investigator
PIS	Patient information Sheet
Plts	Platelets
PMN	polymorphonuclear leukocyte
SAE	Serious adverse event
SAR	Serious adverse reaction
SOP	Standard operating procedures
SUSAR	Suspected unexpected serious adverse reaction
TB	Tuberculosis
TSC	Trial Steering Committee
UAR	Unexpected adverse reaction
5FC	Flucytosine

1. SUMMARY

1.1 Lay summary

Cryptococcal meningitis is a leading cause of death in HIV-infected patients in Africa. With current treatments the mortality rates are very high, varying between 30% to over 50% at 10 weeks in different series. A study of the global burden of cryptococcal disease estimated 3-month mortality to be 70% and that cryptococcal disease was associated with 500,000 deaths per year in Sub-Saharan Africa.

The current standard for initial treatment is with 2 weeks of amphotericin B-based therapy in developed country settings. However in many settings in Africa, amphotericin B is not available or not used due to the requirements with amphotericin B therapy for in-hospital care with intravenous administration, close blood monitoring, and fluid and electrolyte supplements, and the associated extra medical, nursing, and laboratory costs. Instead, many centres have relied on fluconazole, a safe and well-tolerated oral treatment, available free of charge through a donation programme. However, fluconazole monotherapy is much less rapidly active in controlling infection, and is associated with high mortality and also the development of secondary resistance.

The combination of fluconazole with a second oral drug, flucytosine, has been shown to lead to much more rapid control of infection, and to be associated with fewer deaths than fluconazole alone in a small study. In addition, shorter 5-7 day courses of amphotericin B have been shown to be much better tolerated than 2 weeks amphotericin B, reducing the need for intensive monitoring of treatment, and the duration of hospitalization. Such short course amphotericin B would be much more easily implemented in the many centres in Africa and Asia currently using fluconazole, and may not be associated with any loss in efficacy compared with 2-week courses.

Therefore, this study will compare the best oral treatment, a combination of fluconazole and flucytosine, with a one week amphotericin B-based strategy, and with the standard of 2 weeks amphotericin B, in resource-limited settings where implementation of 2 weeks of amphotericin B would be difficult to sustain, and therefore would not be used unless shown to be superior to more readily implementable alternatives.

Additionally, fluconazole and flucytosine will be compared as additional drugs to be given with amphotericin B, in the 2 amphotericin B treatment strategies. Amphotericin B plus flucytosine is the standard combination used in developed country settings, but flucytosine is not currently available in much of the rest of the world. Fluconazole is freely available in Africa through a donation programme and is safer and requires less monitoring, and is also an effective second drug based on smaller, phase II studies. A recent trial from Vietnam showed no significant differences between fluconazole and flucytosine as second drugs in the main analysis although results after longer follow-up favoured flucytosine. Thus, whether fluconazole is an acceptable alternative to flucytosine remains to be determined. The study will address this question with greater numbers than in the Vietnam study, and in the African setting with timely initiation of antiretroviral therapy.

1.2 Abstract and summary of trial design

Type of design

An open label, phase III randomised 3-arm non-inferiority trial to compare alternative strategies for the initial treatment of HIV-associated cryptococcal meningitis.

Disease/patients studied

Patients diagnosed with a first episode of cryptococcal meningitis on basis cerebrospinal fluid (CSF) India ink stain or cryptococcal antigen testing and confirmed by CSF culture.
If not known, human immunodeficiency virus (HIV) seropositivity to be confirmed.

Trial interventions

Study Regimen 1: Fluconazole 600 mg bd plus

flucytosine 25 mg/kg qds for 2 weeks.

Study Regimen 2: Amphotericin B (AmB) 1 mg/kg/d plus EITHER

2A: fluconazole 1200 mg/d, OR 2B: flucytosine 25 mg/kg qds, for **7** days

Study Regimen 3: Amphotericin B (AmB) 1 mg/kg/d plus EITHER

3A: fluconazole 1200 mg/d, OR 3B: flucytosine 25 mg/kg qds, for **14** days

In regimen 2, patients will receive fluconazole 600 mg bd during the second week. In all arms, after 2 weeks, patients will receive fluconazole 800 mg/d until antiretroviral therapy (ART) started (at 2-4 weeks after start antifungal therapy), then fluconazole 400 mg/d to complete 10 weeks treatment, and fluconazole 200mg/d thereafter.

1.3 Outcome measures

Primary outcome measure

- Mortality at 2 weeks by treatment group (regimen 1 and regimen 2 vs regimen 3)

Secondary outcome measures

- Mortality at 10 weeks by treatment group, as above
- Mortality at 2, 10 weeks by treatment group (regimens [2A + 3A] vs regimens [2B + 3B]; and 2A vs 2B, 3A vs 3B)
- Mortality at 2, 4, and 10 weeks by treatment group, as above, adjusted for site and other possible confounders.
- The proportion of patients in each arm suffering clinical and laboratory-defined adverse events.
- Rate of clearance of infection by treatment group based on CSF quantitative cultures at baseline and day 7
- The proportion of patients in each arm suffering pre-defined IRIS reactions to 10 weeks

Duration

Patients will be admitted and clinical response monitored daily for the first 1-2 weeks. Outpatient follow up will be at 2 (for any patient discharged before 2 weeks), 4, 6, 8 and 10 weeks after starting therapy.

Data recording

Data will be recorded on paper case record forms (CRFs), kept at the local centre, then entered into a central database using a Datafax-type system, or double-entered into an Access-based database.

Sample Size and Funding

Using a non-inferiority design with a 10% non-inferiority margin and 5% type 1 error; and assuming 85% 2-week survival in the 2-week AmB arm (regimen 3), would require 157 patients per arm at 80% power, 184 per arm at 85% power, and 219 per arm at 90% power. At least 570 patients total (190 per arm) will be studied in order to achieve minimum 85% power, allowing for 2% losses to follow-up at this early endpoint, based on prior experience, including at trial sites.

MRC (UK) have agreed to support the Malawi and Zambia sites (570 patients) and ANRS have been asked to support the Cameroon site (110 patients), which would allow the power to be increased to 90%.

The numbers of patients treated with fluconazole, or flucytosine in the amphotericin B arms will be a minimum of 190, and 226 with ANRS support. In all scenarios, these numbers are well in excess of the 100 per arm in the Vietnam trial. 190 would give powers of 0.74, 0.81, and 0.87, to detect an increase in 2 week survival to 90% with the most effective adjunctive treatment from 80%, 79%, or 78%, respectively, with the least effective adjunctive treatment. The trial would provide sufficient data to base policy with regard which drug should be used with amphotericin B in Africa.

1.4 Ancillary studies/sub-studies

- A PK/PD sub-study will be developed

- A cost-effectiveness analysis will be developed

- Cryptococcal isolates will be saved and substudies developed

- CSF samples will be saved and used in immunological and proteomic analyses

- PBMC will be saved for later host genetic studies of susceptibility to cryptococcal infection.

All isolates and samples will be fully anonymised. Isolates and samples will also be shared with members of the cryptococcal research community.

1.5 Flow diagram

Trial entry, randomisation, treatment and analysis

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                        │    ELIGIBLE PATIENTS     │
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STUDY REGIMEN 1	STUDY REGIMEN 2	STUDY REGIMEN 3
Fluconazole 1200 mg/d + 5-FC 100 mg/kg/d	AmB 1 mg/kg/day + Fluconazole 1200 mg/d *or* 5-FC 100 mg/kg/d **7 day** Fluc 1200 mg/d **7-14d**	AmB 1 mg/kg/day + Fluconazole 1200 mg/d *or* 5-FC 100 mg/kg/d
14 days		**14 days**

FOLLOW UP TREATMENT
Fluconazole 800 mg/d until ART (at 2-4 weeks), then 400 mg/d to 10 weeks, then 200 mg/d

PATIENT FOLLOW UP
Lumbar punctures day 1, 7.
Outpatient follow-up to 10 weeks

ANALYSIS OF OUTCOME MEASURES

2. BACKGROUND

2.1 Background and Relevant studies/trials

Cryptococcosis is a very common opportunistic infection in patients with late stage HIV infection, especially in Southern and East Africa and South and South East Asia [1-3]: for example, cryptococcosis accounts for 20% and 24% of AIDS-defining diagnoses in the North and Northeast of Thailand, respectively (corresponding figures for tuberculosis were 22% and 27%) [4], and cryptococcosis is the commonest cause of adult meningitis in many areas of Africa with high HIV prevalence [5,6]. Despite expansion of ART programmes, cases of cryptococcal meningitis have not decreased in many Africa centres, and are unlikely to in the near future, since as many HIV-infected individuals continue to enter stage IV disease, and therefore become vulnerable to development of cryptococcal disease, as are accessing ART [7].

Furthermore, treatment is unsatisfactory: 10 week mortality in SE Asia and Latin America has ranged between 19% and 43% at 10 weeks [8-11]. In Africa where resources are most restricted, mortality has ranged from 24% at 10 weeks, in a trial in South Africa using amphotericin B, in which the patients were relatively selected, to 95% at 12 weeks in an unselected cohort treated with low dose fluconazole [12-18].

The combination of high incidence and difficulties with treatment mean cryptococcosis is a very common cause of death in AIDS patients in Africa and other parts of the developing world. For example, cryptococcosis accounted for 17% and 13% of all deaths in two cohorts of HIV-infected patients from Uganda [19,20] and 44% of all deaths in a series from South Africa [21]. For comparison, TB caused 6%, 5% and 13% of deaths, respectively, in these studies. A recent CDC analysis estimated that cryptococcal disease is associated with over 500,000 deaths per year in Sub-Saharan Africa alone [3].

A number of other factors combine to further increase the urgent need for improvement in the acute treatment of cryptococcal infection:
(1) Expansion of antitretroviral programmes in many areas raises the prospect of transforming the long-term prognosis of patients presenting with cryptococcal meningitis, *provided* they survive the acute phase of the illness. Unfortunately around 50% of patients do not currently survive long enough to benefit from antiretroviral treatment [15,16].
(2) Fluconazole is now widely available and affordable. It is effective and safe as maintenance treatment and its availability means gains in survival from improved initial management of cryptococcal meningitis will not be lost through lack of effective maintenance therapy.
(3) A large proportion of cases of cryptococcal disease represent the initial presentation of HIV infection and the AIDS-defining illness [10] and therefore without much more widespread and early testing for HIV infection only a proportion of cases are potentially preventable through early antiretroviral treatment (ART) or prophylactic strategies.

Fluconazole monotherapy

Many centers in Africa continue to rely on fluconazole, available free through the Pfizer donation program [22] and also in relatively inexpensive generic form. However, results with fluconazole monotherapy, especially at conventional dosages of up to 800 mg/d have been poor in Africa. In a series from Zambia the median survival with fluconazole 200 mg/d monotherapy was 19 days compared to 10 days in untreated patients [12]. Mortality with fluconazole 200 mg/d in a Ugandan trial was 40% in the first two weeks and 64% at two months [13]. In Cape Town, a retrospective study with incomplete follow up, with a median time of follow up for discharged patients of just 36 days, and patients lost to follow up censored, nevertheless reported around 50% 10 week mortality with 200 or 400 mg/d [14]. This represents a minimum estimate, and the 10-week mortality in unselected patients treated with fluconazole at up to 800 mg/d is probably significantly more than

50%. In a small cohort from Uganda with complete follow up fluconazole at 800 mg/d was associated with a 60% 10 week mortality [18]. It was in the light of these data that investigators in a recent global burden study estimated the 3-month mortality associated with cryptococcal disease in Sub-Saharan Africa to be 70% [3].

In Cape Town, in a small group of patients, using serial quantitative cultures, fluconazole at 400 mg/d was shown to be essentially fungistatic, at least over the first 2 weeks of treatment [Figure 1B, 15]. While there is evidence for a dose-response effect with fluconazole [18, 23-27], and plasma concentrations of fluconazole in patients with fungal infection are known to increase linearly with doses up to 2 g/d [28], even with a dose of 1200 mg/d, the rate of clearance of infection is markedly less rapid than with amphotericin B-based treatment, even when this is for one week only (figure 1A and D, [15,18]):

Figure 1: Rate of clearance data for amphotericin B at 1 mg/kg/d for 7 days (A), and for fluconazole at 400 mg/d (B), 800 mg/d (C), and 1200 mg/d (D), from [15] and [18].

Supporting the clinical relevance of such studies, rate of clearance of infection was recently shown to be independently associated with 2- and 10-week mortality in a combined cohort that now totals over 300 patients (Figure 2, [29]):

Figure 2: Kaplan-Meier survival curves by rate of clearance of infection divided into quartiles (from [29])

In addition to inadequate antifungal activity, there are other reasons to avoid fluconazole monotherapy:

1. The prolonged period with a high viable organism load associated with fluconazole monotherapy may predispose to the development of secondary fluconazole resistance. Such resistance was a significant problem in Cape Town when initial therapy was with fluconazole [30], but disappeared following a switch to more-rapidly active amphotericin-B based therapy.

2. Prolonged active infection could also increase the risk of immune reconstitution reactions following introduction of ART. Although data on this point are lacking, of relevance, the risk of developing culture negative symptomatic relapse after ART was shown to be associated with the burden of infection, as assessed by serum cryptococcal antigen titer, prior to starting ART [31]. Consistent with an increase in the incidence and/or severity of IRIS when fluconazole monotherapy is used, in a recent study from Harare, Zimbabwe, fluconazole 800 mg/d with rapid initiation of ART was associated with very high mortality [32]. Given the high early mortality in patients waiting to access ART, delaying ART to avoid this problem, if fluconazole is used, is also not an attractive option.

In the light of these data, the Southern African HIV Clinicians Society concluded that amphotericin B-based treatment, even if shortened to one week, should be given, if at all possible, in preference to fluconazole [33]. Thus, the weight of evidence suggests fluconazole monotherapy is inadequate as induction treatment; and the question for the many centres still currently using fluconazole at up to 800 mg/d, is not whether this treatment is an appropriate option for the future; but which of the alternative regimens offers the best combination of efficacy and sustainability in a particular setting?

Standard 2-week amphotericin B induction

A 2 week initial induction phase with amphotericin B is widely accepted as the gold standard therapy for HIV-associated cryptococcal meningitis if resources and facilities allow [34]; largely based on a landmark study by van der Horst and colleagues, the rationale of which was to try to get early control of infection with rapidly fungicidal amphotericin B, with or without flucytosine, but then to avoid the toxicities associated with these drugs by switching to consolidation and then maintenance treatment with the better tolerated azoles [35]. The results, published over a decade ago, remain the best to date from a phase III trial. Overall 10-week mortality was between 10 and 23%.

Unfortunately in many centres in Africa, 2 weeks' induction therapy with amphotericin B may be difficult to sustain. In addition to the costs of the drug, which may be substantial in local terms [36], are the requirements for hospitalization, intravenous drug administration, including nursing time and expertise in siting and maintaining i/v access for a drug which causes considerable phlebitis, saline fluids, and electrolyte replacement, and regular, rapid and reliable laboratory monitoring for renal function, electrolytes, and hemoglobin.

However, while acknowledging the challenges of amphotericin B therapy, if it were shown that 2 weeks amphotericin B induction is superior to more readily implemented alternatives (see below), then the extra resources required could be justified. We have considerable experience in introducing amphotericin B therapy in centres previously using oral fluconazole therapy. It is vital that amphotericin B therapy is monitored, and potassium replaced, as required, and that saline fluid supplementation is given, provided no contraindication, to reduce nephrotoxicity [37,38]. To reduce the need for oral potassium supplements that can exacerbate nausea, it has been our practice to add a small amount (20 mmol) of potassium to a daily 1 L of saline pre-hydration fluid. After starting collaborative work at Jooste Hospital, Cape Town, one week [15] and then 2 weeks amphotericin B [17] was introduced as the standard of care, in place of fluconazole. And in South Africa as a whole,

following a reduction in the cost of amphotericin B [36], the proportion of patients treated with amphotericin B induction has progressively increased [Nelesh Govender, personal communication]. Of note, major donors have provided amphotericin B to countries with more resource limitation than South Africa including Uganda (PEPFAR), where 2 weeks amphotericin B is given in Mulago hospital [16], and Malawi, where sites for this study are located.

Short course amphotericin B induction
A one-week induction with amphotericin B would be much more easily implemented than a full, standard 2 weeks course, in resource limited settings, and may not be associated with a significant reduction in efficacy. A very significant reduction in the burden of infection in terms of the CSF CFU counts of between 3 and 4 logs can be achieved with 7 days of amphotericin B at 1 mg/kg/d [Figure 1A, 15]. Perhaps related to the long half life of amphotericin B, the rate of clearance of infection with 7 days amphotericin B, at 1 mg/kg/d, even if measured over 14 days, was not noticeably less rapid than when 14 days amphotericin B was given to patients at the same hospital: -0.48 log CFU/d with 7 days compared -0.45 and -0.56 log CFU/d with 0.7 and 1 mg/kg/d respectively, for 14 days [15,17].

In addition, renal impairment and anemia, which are dose-related, and usually manifest during the second week of induction [17], may be reduced, and iv access is much easier to maintain, and line infections may be reduced, with 7 rather than 14 days. In a recent cohort from Uganda, 5 days amphotericin B was extremely well tolerated and associated with a 26% 10 week mortality, less than that observed for prior cohorts at the same centre treated with high dose fluconazole (Muzoora, Taseera, Harrison et al, unpublished data). Again, rate of clearance, measured over 14 days, was impressive despite the short duration of amphotericin B (-0.30 log CFU/d). Of note, although during this study amphotericin B was fully monitored with 5 days, none of 30 patients required early discontinuation, and adjustments in potassium supplementation and fluids were very rare (Muzoora et al in preparation). This would suggest strongly that even if, in wider implementation, monitoring was not to a study standard, short-course amphotericin B could still be introduced and given safely.

Although small and underpowered, a randomized study from Thailand found induction with one week of amphotericin B not to be substantially inferior to two weeks [39].

Combination therapy with amphotericin B
The combination of amphotericin B plus flucytosine is recommended in international guidelines, based on consistent evidence for synergy in vitro, in animal models, and in clinical studies [34]. However, flucytosine is not currently available in much of the developing world. Fluconazole is freely available in Africa through a donation programme and is generally safer and requires less monitoring. Despite the antagonism between amphotericin B and azoles that has been observed in some systems [40], most animal model data suggest amphotericin B plus fluconazole is also a very effective combination against C. neoformans [41,42]. In Thailand, amphotericin B and fluconazole at 400 mg/d was more rapidly fungicidal than amphotericin B alone, although the difference did not reach statistical significance [10]. Amphotericin B plus fluconazole at 800 mg/d is more promising. In a recent phase II study by Pappas and colleagues, amphotericin B plus fluconazole at 800 mg/d was associated with the highest proportion of successful outcomes (combined endpoint of sterile CSF, survival, and neurological stability or improvement) at 2, 6, and 10 weeks, in comparison with amphotericin B alone and amphotericin B plus fluconazole 400 mg/d [43]. A Phase III study comparing amphotericin B alone with amphotericin B plus flucytosine and with amphotericin B plus fluconazole 800 mg/d has recently been completed in Vietnam [ISRCTN95123928, www.controlled-trials.com, Day J et al, ICAAC 2011]. The data remain inconclusive with regard the comparison of flucytosine and fluconazole as second drugs: there were no significant mortality differences between fluconazole and flucytosine as second drugs in the main, 2 and 10 week analyses (HR for death at 10 wks, fluconazole vs flucytosine: 1.15 (0.7,1.9), p=0.6; adjusted HR 1.5 (0.9, 2.6) p=0.1), although results after longer 6 month follow-up favoured flucytosine (HR 1.4 (0.9,2.2), p=0.1, adjusted HR 1.8 (1.1, 2.9) p=0.01), and rates of clearance of infection were more rapid with flucytosine. Thus, whether fluconazole is an acceptable alternative to flucytosine remains to be determined. The study

will address this question of the optimal drug to give with amphotericin B with greater numbers than in the Vietnam study and in the African setting with timely initiation of antiretroviral therapy: Patients randomized to each of the 2 amphotericin B arms will be assigned to receive either flucytosine or fluconazole as second agents.

An optimised oral regimen

In the recently published Infectious Diseases Society of America (IDSA) guideline [34], fluconazole 800mg/d is not recommended, even in resource-limited settings. Fluconazole monotherapy is not recommended; if amphotericin B is not available, the recommended oral regimen is fluconazole 1200 mg/d with flucytosine; and in absence flucytosine, fluconazole 1200 mg/d. This is based on:

1. Data showing a dose response effect for fluconazole going up at least to 1200 mg/d in 2 separate Phase II studies:

Longley et al [18], in Mbarara, Uganda found significantly more rapid rate of clearance of infection with fluconazole at 1200 compared with 800 mg/d, and also fewer deaths in high dose arm, although this was not statistically significant (Figure 1). Of note, rate of clearance of infection has been shown to be associated with survival at 2 and 10 weeks [29]. The 10 week mortality with 800 mg/d was 60%, emphasizing the need for more effective therapies. Mifelchik et al. [27] in an earlier US study, using a combined clinical and culture conversion endpoint, and fluconazole dose escalation from 800 mg to 2 g/d, found the percentage of patients alive with a negative CSF culture at 10 weeks increased up to 1600 mg/d. These data build on earlier evidence for a dose response between 200 and 800 mg/d. Accepting the caveats of comparing across trials and the small numbers of patients in the higher dose cohorts, median time to CSF sterilization was 64 days with 200-400 mg/day [23], mean time to sterilization 41 days with 400mg/day [24], and median times 21 and 33 days with 800mg/day [25,26].

In terms of safety, 800 mg/d has been shown to be a safe in large randomized trials in this and other indications [43,44]; 1200 mg/d is given routinely by many experts in coccidioidal meningitis [45], and has been given to between 100 and 200 patients with cryptococcal meningitis in phase II studies [18, 46, Muzoora et al, Jackson et al, unpublished data]; and fluconazole doses up to 2 g have been given to small numbers of patients with cryptococcal meningitis and other fungal infections [27, 28]. The numbers treated at the highest doses are very small, but serious side effects seem not to be very frequent, at least up to 1600 mg. In Uganda and Lilongwe, there was no suggestion of increased toxicity, in particular, no liver function disturbance, at the 1200 mg/d dose, although the numbers involved (about 100 patients) means continued vigilance is needed.

2. Consistent evidence for the benefit of adding flucytosine to fluconazole in a combined regimen

Based on encouraging murine data [47], a cohort of patients was treated with fluconazole 400 mg/day plus flucytosine 150 mg/kg/day for 10 weeks [48]. The median time to CSF sterilization was relatively short at 23 days. By 10 weeks at this dose, 28% of patients had side effects requiring discontinuation of flucytosine. However, over 95% of participants tolerated 2 weeks therapy. In Uganda, flucytosine for 2 weeks was well tolerated and additive with low dose fluconazole (200 mg/day) [13]. In a study by Larsen and colleagues, flucytosine 100 mg/kg/d was given for 4 weeks with increasing doses of fluconazole and again had a benefit (higher percent of patients alive with a negative CSF culture at 10 weeks) that was most pronounced with fluconazole at 800 – 1200 mg/day [27]. In that study, grade IV neutropenia (<500 x 10^9/L) occurred in 18% of patients given flucytosine for 4 weeks, without evidence of increased infection.

In Malawi, subsequent to the fluconazole dosage study in Uganda, the fungicidal activity of fluconazole 1200 mg/d was compared with and without the addition of oral flucytosine 100 mg/kg/d for 2 weeks (Figure 3, [46]). Flucytosine led to a very significant further increase in EFA, to -0.28 log

CFU/d (compared -0.11 log CFU/d with fluconazole monotherapy), to a level similar to amphotericin B alone at 0.7 mg/kg/d in Thailand (-0.31 log CFU/d, [10]). Of note this rate of clearance with oral combination therapy also approaches the level above which there is less evidence that a further increase in the rapidity of kill is associated with a further improvement in clinical outcome (please see Figure 2 [29]), further supporting the equipoise of comparing this oral therapy with amphotericin B–based induction treatment.

Although not powered for clinical endpoints, there were fewer deaths in the combination arm at 2 weeks that reached borderline significance (Figure 3, p=0.05 [46]). There were more episodes of neutropenia in the combination arm, although no increase in infection related serious adverse events.

Figure 3. Rate of clearance with fluconazole 1200 mg/d plus flucytosine 100 mg/kg/d in Lilongwe; and survival with this combination in comparison with fluconazole at 1200 mg/d alone, from [46]

This recent controlled trial is especially relevant – having been conducted in Malawi against a standard of fluconazole monotherapy. The rationale for using the rate of clearance endpoint is that these studies can be used to make some choices about drug dosages and regimens – as to which are the most promising to take forward to clinical endpoint trials.

In all the earlier studies above, flucytosine was given in higher dose and for longer than in recent studies and in this proposal. In the large MSG trial and in Thailand, flucytosine at 100 mg/kg/d for 2 weeks was vey well tolerated with full blood count monitoring only, without real time flucytosine levels [10, 35]. The reasons for the lack of toxicity observed may be found in a PK substudy from Thailand in which levels of flucytosine in patients on oral flucytosine formulation at this dosage were well below those usually associated with bone marrow depression, although enough to stay above MIC for the whole dosing interval, ensuring maximum boosting of fungicidal activity [49]. In ongoing studies in South Africa and Malawi using 2 weeks of flucytosine with amphotericin B and/or fluconazole, 5% of 123 patients developed grade IV neutropenia in the first 2 weeks (authors' unpublished data). Thus, flucytosine at the historically low doses used now (100 mg/kg/d), is associated with a risk of neutropenia that requires monitoring – but is reversible and rarely requires drug discontinuation.

Flucytosine is not generally available at present in Sub Saharan Africa BUT there is no reason it should not be produced at low cost by generic manufacturers if data showed it to be an essential component of optimal therapy. Flucytosine is a simple molecule that is off patent, and used to be

registered and marketed in South Africa [36]. Advocacy for increased access is already underway based on the Malawi combination oral therapy trial.

Thus, in this trial, amphotericin B-based therapy, of two weeks duration, will be compared with amphotericin B for one week and with an optimal oral regimen of fluconazole 1200 mg/d plus flucytosine. If either an optimal oral regimen or one week of amphotericin B prove to be non inferior to 2 week amphotericin B treatment, these treatment options could be introduced widely in other resource limited settings. If not, efforts will need to be made to improve medical and laboratory facilities and staffing so that 2 weeks of amphotericin B can be given safely in a wider variety of settings. Of note, a cost effectiveness sub-study will be developed to help in these policy decisions.

2.5 Risks and benefits

Phase II studies based on rate of clearance of infection have identified regimens (oral combination fluconazole plus flucytosine, and short course amphotericin B) that are much more rapidly fungicidal than oral fluconazole monotherapy [15,46], and of similar rapidity of action while being less toxic and easier to implement than 2 weeks induction with amphotericin B [10,17]. These alternatives would also potentially allow earlier discharge from hospital, and would be implementable in a much wider number of centres and than 2 weeks' induction with amphotericin B.

Rate of clearance of infection has been shown to be associated with clinical outcome at 2 and 10 weeks [29]. Thus, patients in the trial will benefit from more effective therapies than fluconazole, still currently used in many centres in Africa, including one of the study sites, Blantyre in Malawi.

The important side effects of amphotericin B are predictable, dose-dependent, and reversible. A one week course should be much better tolerated than 2 weeks. While 2 weeks of amphotercin B is the standard induction therapy in HIV-associated cryptococcal meningitis in settings without resource restriction, whether the side effects associated with this duration (anaemia and renal impairment), although reversible, may result in the alternative regimens being studied being as effective in terms of clinical endpoints is the question being addressed by the study. A small and underpowered study suggested no loss of efficacy with a shorter induction with one week of amphotericin B [39].

As discussed above, flucytosine carries a risk of neutropenia that requires monitoring, but at the dose and duration to be used is well tolerated. Data from phase II studies suggest any increase in side effects due flucytosine is more than balanced by superior antifungal efficacy, with a strong trend in favour of its addition to fluconazole, in terms of clinical endpoints [46].

Fluconazole has been used in small numbers of patients up to 2 g/d [27,28]. At 1200 mg/d it has been used in several phase II studies of cryptococcal meningitis [18,46], and has also been used in other serious systemic fungal infections, notably coccidioidal meningitis [45]. 1200 mg/d has not been found to be associated with any increase in serious side effects, in particular no increase in liver function abnormalities, compared to lower doses [18,46]. Azole antifungal drugs have been associated with QT prolongation. In a prior trial using 800 mg/d no significant prolongation of QT interval was reported [50]. Paired ECG at baseline and day 7 will be recorded for the first 60 patients (includes 40 on fluconazole at 1200 mg/d). These will be analysed to decide whether further ECG monitoring is required. Of note, 1200 mg/d has recently been adopted as the recommended dose for cryptococcal meningitis in Malawi.

Therefore, serious toxicity is not expected with the short course and dose of flucytosine and the short duration of relatively high dose fluconazole to be used in this study. Patients in the trial will be closely monitored for adverse events and the Independent Data Monitoring Committee (IDMC) will review safety and efficacy data regularly.

All patients who enroll in the study will benefit indirectly as it is well-established that the outcome for patients enrolled in clinical trials is almost always better than patients in routine care. Because cryptococcal meningitis is often the initial presentation of HIV infection, without improvements in

antifungal therapy, cryptococcosis will remain a leading cause of death of HIV-infected patients in Africa and Asia even as antiretroviral therapy becomes more widely available.

2.2 Population

The study population will be HIV-seropositive patients with cryptococcal meningitis, at the participating centre, who fulfil the inclusion/exclusion criteria outlined below.

See **section 3** for full details.

2.3 Rationale and objectives

The principal aim of the trial is:-

To determine if alternative induction regimens of
1. Fluconazole 600 mg bd plus flucytosine 25 mg/kg qds for 2 weeks; or
2. An amphotericin B-based strategy for 7 days [Amphotericin B (AmB) 1 mg/kg/d plus fluconazole 1200 mg/d, or flucytosine 25 mg/kg qds, for 7 days],
are non-inferior to

3. An amphotericin B-based strategy for 14 days [Amphotericin B (AmB) 1 mg/kg/d plus fluconazole 1200 mg/d, or flucytosine 25 mg/kg qds, for 14 days].

for initial treatment of HIV-associated cryptococcal meningitis.

Secondary Aims.

- To compare flucytosine and fluconazole as additional drugs given with amphotericin B
- To determine the relative tolerability of these regimes.
- To determine the rate of clearance of infection with these regimens, based quantitative cultures at baseline and day 7 and day 14.
- To determine the proportion of patients in each arm suffering pre-defined IRIS reactions to 10 weeks.

2.4 Selection of Centres

The study will be conducted at Kamuzu Central Hospital, Lilongwe, and Queen Elizabeth Hospital, Blantyre, Malawi; at University Teaching Hospital Lusaka, Zambia; and at Central Hospital, Yaounde, Cameroon.

In Lilongwe, 40 patients were recently enrolled in a study with very similar inclusion/exclusion criteria in 10 months (4 per month) [46]. Since then patient numbers have increased. Queen Elizabeth Hospital, Blantyre serves a significantly larger population (approx. 1 million) than Lilongwe, sees an average of 14 culture positive cases per month and enrolled 60 patients in 8 months in a recent observational cohort (7.5 per month), and is expected to enrolled 6 patients per month. At University Teaching Hospital Lusaka, recruitment is conservatively estimated at 4 per month. Thus, with very conservative estimates, a minimum of 168 patients (42 months x 4) could be enrolled at Lilongwe and Lusaka, and 252 at Blantyre in 3.5 years (total 588 patients; giving a total of over 570 patients, 190 patients in each arm, in order to achieve 85% power, allowing for 2% losses to follow-up at the 2 week endpoint, based on prior experience, including at trial sites. Central Hospital in Yaounde has a long-standing collaboration in ANRS studies. It is conservatively estimated that 110 patients could be enrolled there in 2-3 years. Involvement of this additional site with ANRS funding would increase power to 90%.

The trial will be conducted according to GCP with an expert independent external data safety monitoring committee. Ethical and, if appropriate, regulatory approval will be obtained in countries where the study sites are located and in the UK and France.

3. SELECTION OF PATIENTS

3.1 Patient inclusion criteria

1. Consecutive patients $\geq$ 18 yrs with a first episode of cryptococcal meningitis on basis CSF India ink and/or CSF cryptococcal antigen.
2. Willing to agree to HIV testing
3. Willing to consent to participate in the study.

3.2 Patient exclusion criteria

A patient will not be eligible for entry to the study if he/she:

1. ALT>5 times upper limit of normal.
2. PMNs<500×10^6/L
3. Plts<$50,000 \times 10^6$/L.
4. Creat >2.5 mg/dl (225 mmol/L)
5. Pregnancy or lactation.
6. Previous serious reaction to study drugs
7. Concomitant medication that is contraindicated with any study drugs.
8. Already on ART

3.3 Number and source of patients

For selection of centres refer to **section 2.45**
At least 570 patients will be enrolled (190 per arm) to give 85% power. The intake will take place over a 42 month period, and follow up will be for 10 weeks after the start of treatment. The total duration of the study will be 54 months.

4. ENROLMENT & RANDOMISATION PROCEDURE

Patients over 18 years of age with a first episode of cryptococcal meningitis diagnosed on the basis of CSF India ink or cryptococcal antigen test at the local laboratory will be assessed for eligibility for inclusion in the trial. Their details will be recorded in a Screening Register. Patients will be enrolled into the study based on the criteria of eligibility outlined in **sections 3.1 and 3.2**. For those found to be ineligible the reason for non-inclusion should be recorded on the Screening Register.

Patients found to be eligible on assessment of clinical and routine laboratory data will be invited to complete screening (liver function, full blood count and pregnancy test for women of childbearing age) and enter the trial, if eligible. They will be given a Patient Information Sheet (PIS) about the trial and asked if they are willing to agree to participate in the study. Any information entered into the database for the trial, or sent to the laboratory will be identified by a number and their initials but not by name. They will be free to withdraw from the study at any time and if they do so this will not jeopardise their future care. If the staff are satisfied that the patient understands the above

information, and is willing to continue, they will be asked to indicate their consent either by signature or by thumbprint (if the volunteer is illiterate) on the consent form. Illiterate volunteers will be asked to have a witness present (friend, family or another member of staff independent of the study team) to witness the discussion and thumbprint consent. If the volunteer is illiterate and declines to have a witness present, this will be recorded on the informed consent form. Participants will be given a copy of the signed/thumb-printed consent form and an information sheet to take away if they wish. Patients with altered mental status who are unable to consent will be enrolled into the study if their next of kin gives informed consent on their behalf. As soon as the patient's mental status improves they will be given a PIS and consent obtained as above, with care taken to ensure they understand that they are free to withdraw from the study and if they do so this will not jeopardise their future care.

Patients will be randomised individually using a computer-generated programme. Patients with altered mental status on admission will be separately randomised to ensure equal numbers of severely ill patients in each treatment group.

5. TREATMENT OF PATIENTS

5.1 Baseline assessment
i) Baseline data – clinical:

Age, Sex, Significant past medical history, Drug history, Smoking, Residence, Occupation.
Symptoms and duration
Full Examination
Visual acuity (if and once able to co-operate with examination)

ii) Laboratory:

- Haematology and biochemistry
- HIV serology – if status not already known
- CD4 count
- Viral load
- Urine: - pregnancy test if indicated. Analysis for proteinuria
- Chest X-ray, if clinically indicated
- CSF: opening pressure, cell count and differential, protein, glucose, India ink, cryptococcal antigen titre, quantitative fungal culture, organism counts by haemocytometer (at baseline, optional by site), (note CSF will be frozen for later immune parameter analysis).
- ECG for first 60 patients (20 per regimen).

C. neoformans isolates will be stored in TSB and 15% glycerol at minus 80°C.

5.2 Antifungal drugs

Antifungal drugs:
Study Regimen 1: Fluconazole 600 mg bd plus

flucytosine 25 mg/kg qds for 2 weeks.

Study Regimen 2: Amphotericin B (AmB) 1 mg/kg/d plus

EITHER 2A: fluconazole 1200 mg/d, OR 2B: flucytosine 25 mg/kg qds, for 7 days

Study Regimen 3: Amphotericin B (AmB) 1 mg/kg/d plus

EITHER 3A: fluconazole 1200 mg/d OR 3B: flucytosine 25 mg/kg qds, for 14 days

In regimen 2, patients will receive fluconazole 600 mg bd during the second week. In all arms, after 2 weeks, patients will receive fluconazole 800 mg/d until ART started (at 2-4 weeks after start antifungal therapy), then fluconazole 400 mg/d to complete 10 weeks treatment, and fluconazole 200mg/d thereafter.

Management of raised CSF opening pressure:
Will be repeat daily lumbar punctures, consistent with guidelines. See **Appendix 4.**

Antiretroviral Treatment:
Patients will start ART between 2 and 4 weeks after initiation of antifungal therapy, consistent with current guidelines [33,34]. ART will conform to the treatment protocols in use in the ongoing programme of ART at study sites. Currently, this consists nevirapine or efavirenz plus either stavudine and lamivudine or combivir or tenofovir.

Source of drugs

Amphotericin B (Fungizone, BMS) and fluconazole (Diflucan, Pfizer) will be obtained locally. Flucytosine (Meda Pharmaceuticals) will be imported for the study.

5.3 Treatment schedules

Details of Drug Dosages
The doses of drugs to be given to each patient are shown below and are based on the weight of the patient at the time of starting treatment.

Initial treatment (to be given for the first 2 weeks)

Amphotericin B:
1mg/kg/day. Patients will be given 1 L of Normal Saline (plus 20 mmol KCl) per day to help reduce amphotericin B nephrotoxicity (provided no contraindication – such as cardiac failure) [33]. Once reconstituted, amphotericin B will be shielded from UV degradation (however, solution is stable for 24 hours in indoor light).

Flucytosine:

Weight of patient	PO	(tablets)
30-39 kg	500-1000-500-1000	(1-2-1-2)
40-49 kg	1000 mg q 6 hours	(2-2-2-2)
50-59 kg	1000-1500-1000-1500	(2-3-2-3)
60-69 kg	1500 q 6 hours	(3-3-3-3)
70-79 kg	1500-2000-1500-2000	(3-4-3-4)

Fluconazole:

1200 mg/d for the first 2 weeks, given as 1200 mg od or 600 mg bd po, or by NG tube if patient unable to swallow.

Continuation (maintenance) phase treatment

In all arms, after initial induction therapy, patients will receive fluconazole 800 mg/d until ART started (at 2-4 weeks after start antifungal therapy), then fluconazole 400 mg/d to complete 10 weeks treatment, and fluconazole 200mg/d thereafter.

See **Appendix 3** for dose adjustments in renal/hepatic insufficiency and management of expected adverse events.

5.4 Treatment Procedures

Initial intensive phase
Patients will be admitted to hospital for the initial intensive phase (first 7-14 days) of treatment, meaning drug administration can be directly observed and facilitating close clinical and laboratory monitoring.

Continuation phase
Patients will be followed in the outpatient clinic and given medication to take at home.

5.5 Drug Accountability.

Drug stocks will be regularly monitored and the remaining stocks checked against the amounts dispensed.

5.6 Measures of compliance and adherence

The initial induction treatment will be given in a directly observed hospital setting. Adherence to outpatient continuation treatment will be assessed by means of patient interview and tablet counts at follow up visits.

5.7 Non-trial treatment
Drugs not known to be contraindicated with the trial drugs will be permitted.

Medications not permitted/ precautions
Patients receiving ART at diagnosis will not be included in the trial.
Rifampicin – Increase dose of fluconazole by 50% (except for 1200 mg dose in first 2 weeks)
Warfarin – Check INR
Sulfonylurea derivatives – increased risk of hypoglycaemia, check glucose.
Fluconazole is contraindicated in combination with cisapride and the class of antihistamines including terfenadine and astemizole.

Data on concomitant medication
All non-trial treatment taken by the patient will be recorded at enrolment and follow up and in the event of an SAE occurring.

5.8 Dispensing
The drugs will be stored in the pharmacy. The trial staff will collect the amounts needed from the pharmacy. Detailed records of drugs dispensed and received will be maintained by both the pharmacist and the trial staff.

6. ASSESSMENTS AND PROCEDURES

6.1 Follow-Up Schedule

Patients will be admitted and clinical response monitored daily for the first 2 weeks. Outpatient follow up will be at 2 (if discharged before 2 weeks), 4, 6, 8 and 10 weeks after starting therapy.

6.2 Summary of Investigations during Treatment and Follow-Up

CLINICAL
Clinical response will be monitored daily for the first 2 weeks or until discharge; 2, 4, 6 and 8 weeks after discharge. Every effort will be made (for example with mobile telephone calls and financial help with travelling expenses) to obtain accurate and complete follow-up data for 10 weeks after the start of treatment. Particular attention will be paid to the possibility of immune reconstitution reactions after the patients have started ART. Follow up will be greatly helped by the fact that HIV clinics are already well-established at the sites, and all sites have trial experience.

Visual acuity by handheld Snellen - will be done before discharge and 8 weeks after discharge

LABORATORY

Day 5, 9, 13:	FBC and Urea, creatinine, electrolytes
Day 3, 7, 11:	Urea, creatinine, electrolytes
Days 7, 13:	ALT

At the time of monitoring blood tests, an extra 2.5 ml will be taken on up to 3 occasions in the first 2 weeks for drug levels.

Day 7: ECG for first 60 patients.
These paired ECGs at baseline and day 7 will then be examined for any evidence of QT prolongation with fluconazole at 1200 mg/d, and a decision made as to whether continued ECG monitoring is required.

Day 7: Follow up CSF examination
opening pressure, cell count and differential, protein, glucose
quantitative fungal culture

6.3 Procedures for assessing efficacy

Efficacy.
Survival will be recorded at 2, 4 and 10 weeks, as well as date of death or loss to follow up. Cox regression will be used to analyse mortality data. Analyses will be done on both an intention to treat and per protocol basis, and both unadjusted (primary endpoint) and adjusted for possible confounding factors.

Cryptococcal clearance rates will be calculated using a summary statistic for each patient, the rate of decrease in log CFU per ml CSF per day derived from the slope of the linear regression of log CFU against time for each patient, based on quantitative CSF cultures at baseline and on day 7 and 14. A

linear regression model will be used to compare mean rates of decline or early fungicidal activity (EFA) for each treatment arm, giving summary differences with 95%CI and significance levels [14].

A full analytical plan will be drawn up prior to beginning analysis.

6.4 Procedures for assessing safety

Safety. Proportions of patients in each of the treatment arms suffering clinical and laboratory-defined side effects will be compared as well as the mean percent change from baseline of laboratory values in the treatment groups.

The frequency and severity of any immune reconstitution reactions, the factors associated with occurrence of reactions and the frequency of further AIDS-related illnesses will be determined. Logistic regression will be used to compare the proportion of patients developing pre-defined IRIS reactions in each of the arms.

Throughout this study patients will be closely monitored for signs and symptoms of drug toxicity. All toxicities leading to the study therapy being temporarily or permanently discontinued and all Grade 3 or 4 toxicity effects will require thorough investigation with relevant clinical and laboratory tests, as clinically indicated. These should be repeated as needed until final resolution or stabilization of the toxicity. All symptoms and laboratory findings will be graded according to severity using the modified Division of AIDS toxicity criteria. At the time of enrolment, if the patient already has a medical diagnosis whose signs or symptoms worsen during the study to a Grade 3 or 4, this is an adverse event that must be reported.

SAEs will be reported to International investigators as they occur.

For details of safety reporting, expected adverse events and flow chart for assessing and notifying adverse events see section 10, Appendix 3 and Appendix 5.

6.5 Loss to follow-up

Every effort will be made (for example with mobile telephone calls and financial help with travelling expenses) to obtain accurate and complete follow-up data for 10 weeks after the start of treatment.

If a patient fails to attend, the research nurse will visit the home address and make every effort to persuade the patient to attend and continue antifungal and antiretroviral treatment.

6.6 Trial closure

The trial will be considered closed when the last patient has completed 10 weeks in the study and all follow-up and laboratory reports have been received. Early termination could occur if there is an unacceptable level of adverse events, occurring in any of the test arms.

7. WITHDRAWAL OF PATIENTS

In consenting to the trial, patients are consenting to trial treatment, trial follow-up and data collection. If a patient wishes to withdraw from trial treatment, investigators should nevertheless

explain the importance of remaining on trial follow-up, or failing this of allowing routine follow-up data to be used for trial purposes.

Patients may be withdrawn from a trial intervention for severe and intolerable adverse events, or if the patient withdraws consent. Follow-up should be continued unless the patient explicitly withdraws consent for follow-up. For patients moving within or outside the study area, every effort should be made for the patient to be followed up if at all possible.

8. STATISTICAL CONSIDERATIONS

8.1 Method of Randomisation

Patients will be randomised individually using a computer-generated programme. Patients with altered mental status on admission will be separately randomised to ensure equal numbers of severely ill patients in each treatment group.
Details of treatment allocation are shown in **Section 5**.

8.2 Outcome Measures

Primary outcome measure

- Mortality at 2 weeks by treatment group (regimen 1 and regimen 2 vs regimen 3)

Secondary outcome measures

- Mortality at 10 weeks by treatment group, as above
- Mortality at 2, 10 weeks by treatment group (regimens [2A + 3A] vs regimens [2B + 3B]; and 2A vs 2B, 3A vs 3B)
- Mortality at 2, 4, and 10 weeks by treatment group, as above, adjusted for site and other possible confounders.
- The proportions of patients in different arms suffering clinical and laboratory-defined adverse events (grades III and IV)
- Rate of clearance of infection based on quantitative CSF cultures.

8.3 Sample Size

Using a non-inferiority design with a 10% non-inferiority margin and 5% type 1 error; and assuming 85% 2-week survival in the 2-week AmB arm (regimen 3), would require 157 patients per arm at 80% power, 184 per arm at 85% power, and 219 per arm at 90% power. At least 570 patients total (190 per arm) will be studied in order to achieve minimum 85% power, allowing for 2% losses to follow-up at this early endpoint, based on prior experience, including at trial sites (Lilongwe [46])).

MRC (UK) have agreed to support the Malawi and Zambia sites (570 patients) and ANRS have been asked to support the Cameroon site (110 patients), which would allow the power to be increased to 90%.

The numbers of patients treated with fluconazole or flucytosine in the amphotericin B arms will be a minimum of 190, and 226 with ANRS support. In all scenarios, these numbers are well in excess of the 100 per arm in the Vietnam trial. 190 would give powers of 0.74, 0.81, and 0.87, to detect an

increase in 2 week survival to 90% with the most effective adjunctive treatment from 80%, 79%, or 78%, respectively, with the least effective adjunctive treatment. The trial would provide sufficient data to base policy with regard which drugs should be used with amphotericin B in Africa.

Interim Monitoring and Analyses

For the fluconazole vs flucytosine sub-study in strategies 2 and 3, an interim review will be done after 100 patients have been enrolled in each of these two arms. The purpose will be to assess whether there is i) sufficient evidence which indicates superiority of one regimen to the other (in which case from this point on, all patients will receive the more superior drug with amphotericin B) or clear indication of futility (in which case all patients will revert to fluconazole combined with amphotericin B in these two arms), or that the evidence is inconclusive in which case no change will be made to the study design. Adapting the design in this way in scenarios i and ii will enable us to provide the best treatment options for patients and to generate the maximum knowledge of the efficacy of that regimen.

Doing the interim review at a low significance level will not affect the power for the final comparison (between fluconazole and flucytosine as second drugs) at the end of the trial.

8.4 Preliminary Analysis Plan (see also 6.3 and 6.4 above)

After data cleaning, analysis will proceed according to a pre-designed analysis plan. The primary analysis will be by both intention-to-treat and per protocol.

After the crude primary analysis has been performed, an adjusted analysis will be performed including covariates which may influence outcome.

A full analysis plan will be developed before the final analysis is conducted.

8.5 Analysis of adverse events:

The primary endpoint for adverse events is the occurrence of an SAE or a Grade 4 adverse event.

9. TRIAL MONITORING

The purposes of trial monitoring are to verify that :
- The rights and well-being of human subjects are protected.
- The reported trial data are accurate, complete, and verifiable from source documents.
- The conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with the applicable regulatory requirement

9.1 Extent and nature of monitoring

The sites will be visited at regular intervals in order to monitor the conduct of the trial. These visits will be made by the Trial Monitor/Manager. Regular visits will also be made by the International Investigators and the Trial Statistician. The frequency of monitoring visits will be according to need but will be no less than every 6 months in each centre.

9.2 Site monitoring

At monitoring visits the data entered in the CRFs and database will be checked against available source data according to the procedures described in the trial monitoring plan filed in the Trial Master File. Data stored will be checked for missing or unusual values (range checks) and checked for consistency within participants over time. If any such problems are identified any data which are changed should be crossed through with a single line and initialled. Particular attention will be given to:

(a) Verifying, for the study drugs:

   (i) That storage times and conditions are acceptable, and that supplies are sufficient throughout the trial.

   (ii) That the study drugs are supplied only to subjects who are eligible to receive it and at the protocol specified dose(s).

   (iii) That subjects are provided with necessary instruction on properly taking study medication.

   (iv) That the receipt and use of study drugs at the trial sites are controlled and documented adequately.

(b) Verifying that the local investigator follows the approved protocol and all approved amendment(s), if any.

(c) Verifying that written informed consent was obtained before each subject's participation in the trial.

(d) Ensuring that the Principal Investigator has received the current Investigator's Brochure, all documents, and all trial supplies needed to conduct the trial properly and to comply with the applicable regulatory requirement(s).

(e) Ensuring that the local investigator and the investigator's trial staff are adequately informed about the trial.

(f) Verifying that the local investigator and the investigator's trial staff are performing the specified trial functions, in accordance with the protocol and any other written agreement between the sponsor and the local investigator/institution, and have not delegated these functions to unauthorized individuals.

(g) Verifying that the local investigator is enrolling only eligible subjects.

(h) Reporting the subject recruitment rate.

(i) Verifying that source documents and other trial records are accurate, complete, kept up-to-date and maintained.

(j) Verifying that the local investigator provides all the required reports, notifications, applications, and submissions, and that these documents are accurate, complete, timely, legible, dated, and identify the trial.

(k) Checking the accuracy and completeness of the CRF entries, source documents and other trial-related records against each other. In particular:

(l) The data required by the protocol are reported accurately on the CRFs and are consistent with the source documents.

(m) Any dose and/or therapy modifications are well documented for each of the trial subjects.

(n) Adverse events, concomitant medications and intercurrent illnesses are reported in accordance with the protocol on the CRFs.

(o) Visits that the subjects fail to make, tests that are not conducted, and examinations that are not performed are clearly reported as such on the CRFs.

(p) All withdrawals and dropouts of enrolled subjects from the trial are reported and explained on the CRFs.

(q) Informing the local investigator of any CRF entry error, omission, or illegibility. Any corrections, additions, or deletions made, are dated, explained (if necessary), and initialled by the local investigator or by a member of the investigator's trial staff who is authorized to initial CRF changes for the investigator. This authorization should be documented.

(r) Determining whether all adverse events (AEs) are appropriately reported within the time periods required by GCP, the protocol, the IRB/IEC, the sponsor, and the applicable regulatory requirement(s).

(s) Determining whether the local investigator is maintaining the essential documents

(t) Communicating deviations from the protocol, SOPs, GCP, and the applicable regulatory requirements to the local investigator and taking appropriate action designed to prevent recurrence of the detected deviations.

9.3 Direct Access to Data

The investigator will permit trial-related monitoring, audits, ethics committee review and regulatory inspections by providing direct access to source data/documents.

9.4 Confidentiality

All patient information will be kept in locked cabinets and will be available only to the treatment staff.

The patient's name and address will not be disclosed to the trial sponsor.

The patient's data/specimens will be identified by trial number and/or initials only. Individual patients will not be identified in the resulting publications and presentations from the trial. The trial will comply with the principles of the Data Protection Act of the country of the participating centre.

10. SAFETY REPORTING

10.1 Safety Reporting
Terms and definitions for adverse events

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a subject to whom a medicinal product has been administered including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	Any untoward and unintended response in a subject to an investigational medicinal product, which is related to any dose administered to that subject.
Unexpected Adverse Reaction (UAR)	An adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in question set out in: • The SPC for that product (for products with a marketing

	authorisation) • The Investigator's Brochure (IB) relating to the trial in question (for any other investigational product)
Serious Adverse Event (SAE) **Serious Adverse Reaction (SAR)** **Suspected Unexpected Serious Adverse Reaction (SUSAR)**	Respectively, any adverse event, adverse reaction or unexpected adverse reaction that: • results in death • is life-threatening* • requires hospitalisation or prolongation of existing hospitalisation** • results in persistent or significant disability or incapacity • consists of a congenital anomaly or birth defect • other important medical event(s)***

* The term 'life-threatening' in the definition of 'serious' refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

** Hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation. Hospitalisations for a pre-existing condition, including elective procedures that have not worsened, do not constitute an SAE.

*** Other events that may not result in death, are not life threatening, or do not require hospitalisation may be considered a serious adverse experience when, based upon appropriate medical judgement, the event may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed above (excluding new cancers or result of overdose).

Study site Responsibilities

All SAEs and grade III and IV AE must be reported within 48 hours by the Local Investigator by e-mail to the International Investigators. All other adverse events should be reported on the regular progress/follow-up reports in the CRF. The investigator should assess the SAE for the likelihood that that it is a response to a study drug.

Follow-up of SAEs: In the case of an SAE the subject must be followed-up until clinical recovery is complete and laboratory results have returned to normal, or until the event has stabilised. Follow-up may continue after completion of protocol treatment if necessary. Follow-up information is noted on another SAE form by ticking the box marked 'follow-up' and emailing to the international investigators as information becomes available. Extra, annotated information and/or copies of test results may be provided separately. The patient **must** be identified by trial number, date of birth and initials only. The patient's name **should not** be used on any correspondence.

- SUSARs which are fatal or life-threatening must be reported to the appropriate regulatory bodies and if required to the local ethics committee not later than 7 days after the investigators are first aware of the reaction. Any additional relevant information must be reported within a further 8 days.
- SUSARs that are not fatal or life-threatening must be reported to the appropriate regulatory bodies and if required to the local ethics committee within 15 days of the PI first becoming aware of the reaction.

The International Investigators (or a delegate) will evaluate all SAEs received for seriousness, expectedness and causality. Investigator reports of suspected SARs will be reviewed immediately and those that are SUSARs identified and reported to regulatory authorities. The causality assessment given by the local investigator cannot be overruled and in the case of disagreement, both opinions will be provided in subsequent reports.

Adverse events – Guidelines on inclusions and exclusions

Adverse events include	Adverse events do not include
a) an exacerbation of a pre-existing illness b) an increase in frequency or intensity of a pre-existing episodic event/condition c) a condition (even though it may have been present prior to the start of the trial) detected after trial drug administration d) continuous persistent disease or symptoms present at baseline that worsens following the administration of the study/trial treatment	a) medical or surgical procedures- the condition which leads to the procedure is the adverse event b) pre-existing disease or conditions present before treatment that do not worsen c) situations where an untoward medical occurrence has occurred e.g. cosmetic elective surgery d) overdose of medication without signs or symptoms e) the disease being treated or associated symptoms/signs unless more severe than expected for the patient's condition

10.2 Severity/grading of adverse events

This will be according to the modified DAIDS Classification filed in the Trial Master File.

10.3 Relationship to trial treatment

When reporting on serious adverse events, the trial investigator will state whether they believe that the event is causally associated with any of the trial treatments and the strength of the causal relationship. They will also state whether the adverse event was expected and what if any action was taken.

CLASSIFICATION OF ADVERSE EVENTS BY RELATIONSHIP TO STUDY MEDICATION	
UNRELATED:	This category applies to those AEs that are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.).
UNLIKELY:	This category applies to those AEs that are judged to be unrelated to the test drug, but for which no extraneous cause may be found. An AE may be considered unlikely to be related to study medication if or when it meets 2 of the following criteria: (1) it does not follow a reasonable temporal sequence from administration of the test drug; (2) it could readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject; (3) it does not follow a known pattern of response to the test drug; or (4) it does not reappear or worsen when the drug is re-administered.
POSSIBLY:	This category applies to those AEs for which a connection with the test drug administration appears unlikely but cannot be ruled out with certainty. An AE may be considered possibly related if or when it meets 2 of the following criteria: (1) it follows a reasonable temporal sequence from administration of the drug; (2) it could not readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject; or (3) it follows a known pattern of response to the test drug.
PROBABLY:	This category applies to those AEs that the investigator feels with a high degree of certainty are related to the test drug. An AE may be considered probably related if or when it meets 3 of the following criteria: (1) it follows a reasonable temporal sequence from administration of the drug; (2) it could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject; (3) it disappears or decreases on cessation or reduction in dose (note that there are exceptions when an AE does not disappear upon discontinuation of the drug, yet drug-relatedness clearly exists; for example, as in bone marrow depression, fixed drug eruptions, or tardive dyskinesia); or (4) it follows a known pattern of response to the test drug.
DEFINITELY:	This category applies to those AEs that the investigator feels are incontrovertibly related to test drug. An AE may be assigned an attribution of definitely related if or when it meets all of the following criteria: (1) it follows a reasonable temporal sequence from administration of the drug; (2) it could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject; (3) it disappears or decreases on cessation or reduction in dose and recurs with re-exposure to drug (if rechallenge occurs); and (4) it follows a known pattern of response to the test drug.

10.4 Follow-up after adverse events

Patients may be either admitted to hospital or seen at intervals to monitor the progress, recovery and investigations of the adverse events. In the event treatment needs to be modified or changed, the Local Investigator should inform the International Investigators and agree on the new treatment.

For details of safety reporting, expected adverse events and flow chart for assessing and notifying adverse events see Appendix 3 and Appendix 5.

11. ETHICAL CONSIDERATIONS AND APPROVAL

11.1 Ethical considerations

The patients will, before being enrolled into the study, have the conditions of the study, as set out in the Patient Information Sheet **(Appendix 1)** explained to them. The information contained in the PIS will be translated into the local dialect. Literate patients will be asked to read the PIS and the illiterate patients will have the contents explained to them by the Local Investigator or Research Nurse. The patient will have the opportunity to discuss the PIS. Once the person taking consent is satisfied that the patient has understood the PIS and the consent form, the patient will be asked to sign the consent form. The top copy should be filed in the patient's study folder and the duplicate, together with a copy of the PIS, given to the patient.

The right of the patient to refuse to participate in the trial without giving reasons will be respected.

After the patient has entered the trial, the clinician will remain free to give alternative treatment to that specified in the protocol, at any stage, if he/she feels it to be in the best interest of the patient. However, the reason for doing so should be recorded and the patient will remain within the trial for the purpose of follow-up and data analysis according to the treatment option to which they have been allocated. Similarly, the patient will remain free to withdraw at any time from the protocol treatment and trial follow-up without giving reasons and without prejudicing his/her further treatment.

11.2 Ethical approval

The protocol will be submitted to the Ethics Review Committee (ERC) of the London School of Hygiene and Tropical Medicine. The protocol will also be submitted to the Medical Ethics Committee of each of the participating clinical site and/or country and enrolment to the study will start only after receiving the written agreement of the relevant body(ies).

The trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki from the World Medical Association.

http://www.wma.net/e/ethicsunit/helsinki.htm

12. REGULATORY APPROVAL

Regulatory approval will be obtained, as required, in the countries of the study sites.

13. INDEMNITY

All personnel involved in the trial will be expected to be indemnified by their employing authority. Patients will be indemnified, for non- negligent harm, through a separate policy taken out by St George's University of London.

14. FINANCE

Funding has been awarded the MRC (UK), and is also being sought from the ANRS (France).

15. TRIAL COMMITTEES

15.1 Trial Management Group (TMG)

A Trial Management Group (TMG) will be formed comprising the international and local Investigators, trial manager and statistician. The TMG will be responsible for the day-to-day running and management of the trial and will liaise at regular intervals.

15.2 Trial Steering Committee (TSC)

A Trial Steering Committee (TSC) will be constituted. The Chairman will be independent of the running of the trial.

A committee with an independent Chairman will be formed.
Their terms of reference will be:

1. to monitor and supervise the progress of the trial towards its interim and overall objectives;
2. to review at regular intervals relevant information from other sources (e.g. other related trials);
3. to consider the recommendations of the Independent Data Monitoring Committee;

The role of the TSC is to provide overall supervision for the trial and provide advice through its Independent Chairman. The ultimate decision for the continuation of the trial lies with the TSC.

15.3 Independent Data Monitoring Committee (IDMC)

There will be an Independent Data Monitoring Committee whose terms of reference will be as follows:

1. To review safety data, in particular all serious adverse events possibly attributable to the trial drugs, such as local reactions or unexpected deaths.

2. To monitor the conduct of the trial with respect to the ethical aspects of the trial.

3. To assess the results of any interim analyses with the possibility of advising the Trial Steering Committee (TSC) that the trial should be modified or discontinued.

16. PUBLICATION

The results from different centres will be analysed together and published as soon as possible. Individual Clinicians must not publish data concerning their patients that are directly relevant to questions posed by the study until the Trial Management Group has published its report.

The Trial Management Group will form the basis of the Writing Committee and will advise on the nature of publications. The names of all the investigators will be included in any publication in the authorship. Any authorship policy will be agreed by all the investigators before any publication. The members of the TSC and IDMC will be listed with their affiliations in the Acknowledgements/Appendix of the main publication.

17. PROTOCOL AMENDMENTS

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19. APPENDICES

Appendix 1: Patient Information sheet (nb PIS will be site specific, and will be modified depending on the standard therapy for crytococcal meningitis currently being given at that site)

A RANDOMISED CONTROLLED TRIAL OF ORAL FLUCONAZOLE PLUS FLUCYTOSINE VERSUS AMPHOTERICIN B-BASED THERAPY FOR ONE OR TWO WEEKS FOR INITIAL TREATMENT OF HIV-ASSOCIATED CRYPTOCOCCAL MENINGITIS.

Patient Information

You are invited to take part in this research trial. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take your time to read through the following information carefully and ask any questions you may have. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

This study is being done with the aim of trying to improve the treatment of cryptococcal meningitis at this hospital. Currently the usual treatment for this infection at this hospital is a tablet treatment with a drug called fluconazole **or** is an intravenous drug called amphotericin B given daily by infusion for 1-2 weeks, on its own or with fluconazole, followed by tablet treatment with fluconazole. In hospitals in Europe and the United States this intravenous treatment is usually given for 2 weeks and with flucytosine. In previous studies we have shown that the tablet treatment can be strengthened by adding a second drug flucytosine to the fluconazole to make a more effective oral treatment. Amphotericin B has side effects that dependent on the dose and duration of treatment, and it has been shown that one week of amphotericin B treatment has less side effects than 2 weeks. We want to see if the strengthened tablet combination or the one week of amphotericin B treatments are as good as the 2 week amphotericin B treatment currently used in some other countries. If so these treatment options can be introduced widely in other hospitals. If not, efforts will need to be made so that the 2 weeks of amphotericin B can be given safely in this hospital. In addition, we wish to see which is the best drug to give with amphotercin B – fluconazole or flucytosine.

Why have I been chosen ?

You have been chosen because you have this infection, cryptococcal meningitis.

Do I have to take part ?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of the care you receive.

What will happen to me if I take part ?

You will be assigned to receive either: 2 weeks of initial treatment with amphotericin 1 mg/kg/day plus either fluconazole or flucytosine; or 1 week of initial treatment with amphotericin 1 mg/kg/day plus either fluconazole or flucytosine; or 2 weeks of the combination of fluconazole and flucytosine tablets. After these initial treatments, all patients will be switched to tablet treatment with fluconazole. You will stay in hospital for 2 weeks and we will monitor your progress clinically and from blood tests. In addition, in order to measure accurately the rate at which the organisms causing your infection are killed by the treatment we will do a repeat lumbar puncture after 7 and 14 days. Some patients have such repeat lumbar puncture tests as part of their normal treatment because the pressure in the spinal fluid is high or because they are not responding as quickly as we would like to the treatment; but for patients participating in the trial, we will do this test for everyone after 7 and 14 days. As part of the study some of the blood and spinal fluid samples may be used for measurements of your immune function (for example levels of specific proteins) and of levels of the drugs (amphotericin B, fluconazole, flucytosine) that should help us to understand this infection better. Samples and isolates may be analysed outside of this country. Any such samples will have only an identification number and the results will be anonymous.

What are the possible disadvantages and risks of taking part ?

As part of the study we need to repeat the lumbar puncture 7 and 14 days after starting treatment. Lumbar puncture can sometimes cause soreness of the back and headache, although in many patients with cryptococcal meningitis lumbar punctures actually relieve the associated headache.

There is the possibility of side effects from the drugs used in the study.

The side effects of amphotercin B are well understood and usually depend on the duration of the treatment. It can cause impairment of kidney function and anaemia, which however almost always recover when the drug is stopped. One week instead of 2 weeks should be associated with significantly fewer side effects. However we need to know that there is no important decrease in the effectiveness of the treatment if it is only given for one instead of 2 weeks – that is one of the purposes of the trial

Fluconazole is generally very well tolerated but can cause disturbance of liver function, gastro-intestinal symptoms and rashes. At the higher dosage used in this study (1200 mg/d) for initial treatment it has been used before without evidence of increased side effects, although the number of patients treated in this way for this infection in studies is only between 100 and 200 patients. Evidence suggests the higher dose is also more effective than lower doses at killing the organism causing your infection.

Flucytosine can cause a reduction in white blood cells in your blood, that occasionally means the drug needs to be stopped, but at the dose and duration (2 weeks) to be used in this study this is relatively rare (in the order of 5% or one in 20 patients). Evidence suggests this drug makes the oral treatment much stronger and that any increased side effects are outweighed by the increase in the effectiveness of the treatment.

We will monitor all patients carefully and with blood tests for possible side effects and stop the study drugs if necessary.

What are the possible benefits of taking part ?

A possible benefit of taking part is that you may benefit from the new treatments being given if you are assigned to a new treatment and it turns out that this is more effective, or as effective but with less side effects or needing less intravenous treatments, than the treatment currently being used in your hospital. Of course we do not yet know if this will be the case. That is why we must do the trial. In addition, the information we get from this study may help us to treat patients with cryptococcosis better in the future.

What happens when the research study stops?

When the treatment part of the study is complete after 2 weeks, you will continue with oral tablet treatment in the usual way. You will continue to be seen in the hospital clinic as soon as you are able to leave hospital. At the follow up visits, your doctors, nurses, and counsellors will also talk to you about treatment for the HIV virus, which is the usual reason that patients get cryptococcal meningitis. At the follow up visits, we will continue to collect information about your progress for 10 weeks to compare the safety and effectiveness of the alternative initial treatments.

What if something goes wrong?

If you are harmed by taking part in this research project you will be cared for as clinically indicated until your condition improves or stabilises. All personnel involved in the trial will be expected to be indemnified by their employing authority. Patients will be indemnified through the policy of St. George's, University of London.

Confidentiality

All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you that leaves the hospital will have your name and address removed so that you cannot be recognised from it.

What will happen to the results of the research study?

The results from this study will be used to assess how the new treatments compare with the standard of 2 weeks intravenous treatment with amphotericin B used in some other countries and with current treatments being given in the hospitals in the study countries.

Who has reviewed the study ?

This study has been reviewed and approved by the Local Research Ethics Committees at Queen Elizabeth Hospital, Blantyre, and Central Hospital, Lilongwe, Malawi, University Teaching Hospital, Lusaka, Zambia, and Central Hospital, Yaounde, Cameroon, and at The London School of Hygiene and Tropical Medicine, University of North Carolina, and Institut Pasteur, France.

Contact for further Information

If you have any questions relating to this study or if you should have a research related injury or suffer additional medical problems while you are in the study, please talk to your study nurse or doctor in the first instance.

The 24-hour telephone number, through which you can reach your study doctor or another authorized person, is .

APPENDIX 2: CONSENT FORMS

A RANDOMISED CONTROLLED TRIAL OF ORAL FLUCONAZOLE PLUS FLUCYTOSINE VERSUS AMPHOTERICIN B-BASED THERAPY FOR ONE OR TWO WEEKS FOR INITIAL TREATMENT OF HIV-ASSOCIATED CRYPTOCOCCAL MENINGITIS.

(The patient or next of kin should complete the whole of this form themselves)
Please cross out as necessary

Have you read the patient/ relatives information sheet?	YES/ NO
Have you had an opportunity to ask questions and discuss the study?	YES/ NO
Have you received satisfactory answers to all of your questions?	YES/ NO
Have you received all of the information which you require?	YES/ NO

Who have you spoken to? DR /MR/ MS

Do you understand that you are free to withdraw from the study:

At any time
Without having to provide a reason
And without affecting your future medical care   YES/ NO

Do you agree to take part in this study?   YES/ NO

CONSENT

Your signature below indicates that you have voluntarily decided to participate in this study after having read and understood all the information in this form. A signed copy of this form will be made available for your personal records.

PATIENT NAME (Print)

PATIENT SIGNATURE   Date

NEXT OF KIN (name, signature) IF PATIENT UNABLE TO CONSENT   Date

WITNESS (name, signature) IF PATIENT THUMBPRINT   Date

INVESTIGATOR'S SIGNATURE   Date

Appendix 3: Management of expected adverse events and dosage adjustment

Amphotericin B:

Infusion related side effects – amphotericin B:

1. Increase infusion duration to 6 hours.

2. Premedication with paracetamol 500 mg or chlorpheniramine 4 mg.

3. In case of severe infusion related reaction: pethidine 25 mg iv. Slow intravenous injection.

Renal impairment:

Conversion factor mg/dl to micromol/L = 88.4

If creatinine rises up to 2,5 mg/dl (220 µmol/l):

1. Miss one dose. Check adequate hydration. Check creatinine next morning:

- if stable or improving: institute alternate day dosing (1 mg/kg q 48 hours)

- if creatinine is increasing do not give amphotericin B and check again after 24 hours: if stable or improving institute alternate day dosing as above

- if still increasing: stop amphotericin B and switch to fluconazole (800 mg) adjusting its dose for renal impairment.

AVOID other nephrotoxic agents such as aminoglycosides, NSAIDS if possible.

Hypokalemia:

Supplement potassium as required

If hypokaleamia check magnesium and supplement as required

Flucytosine:

Renal insufficiency

Creatinine clearance ml/min	Individual dose mg/kg	Dose interval hour
>40	25	6
40-20	25	12
20-10	25	24
<10	25	>24

Clearance:

$$\frac{(140- age) \times weight\ (kg)}{72 \times serum\ creat\ (mg/dl)} \quad (\times 0,85\ for\ women) \quad = ml/min$$

Bone marrow toxicity:
Platelets $< 50,000 \times 10^6$ /L or neutrophils $< 500 \times 10^6$ /L are exclusion criteria.

If during therapy

Grade III fall in platelets or neutrophils (Platelets $< 50,000 \times 10^6$ /L or neutrophils $< 750 \times 10^6$ /L)
Then take blood for drug level and monitor daily. If grade III is confirmed next day, halve dose
(50%); if grade IV levels develop, stop flucytosine, until level III, then 50% dose

Grade IV fall in platelets or neutrophils (Platelets $< 25,000 \times 10^6$ /L or neutrophils $< 500 \times 10^6$ /L)
Then take blood for drug level and stop flucytosine, until grade III level at which point resume at
50% dose

Fluconazole:

Renal insufficiency
    If renal function decreases
    - to 20-50 ml/min then reduce the dose by 50%.
    - to < 20 ml/min reduce dose by 25 %.

Liver function abnormality: (Perfect *et al.*, 1992)
    ALT (SGPT) 5 x upper limit (= 200) is an exclusion criterion
    If during therapy
    - ALT (SGPT) from normal to 5 x upper limit or
    - from abnormal baseline increases by 150
    Then, if possible, stop fluconazole and switch to amphotericin B.

Skin reactions:
    Stop fluconazole in case of bullous lesions or erythema multiforme.

If gastro-intestinal side effects (like nausea and vomiting):
    Divide dosage into smaller more frequent dosage schedule.

Drug Interactions
Fluconazole may increase levels of phenytoin, warfarin and sulfonylurea derivatives.
If concomitant use of warfarin: check INR.
If concomitant use of sulfonylurea derivatives there is risk of hypoglycaemia, so check
glucose levels more often.

Rifampicin will reduce the levels of fluconazole if the patient has been on it for >_2weeks. Increase
the dose of fluconazole by 50%, in consolidation and maintenance phases.

Fluconazole is contraindicated in combination with cisapride and the new drug class of antihistamines
such as terfenadine and astemizole. The independent data safety monitoring committee will sit
monthly to discuss and review adverse effects

Appendix 4: Management of raised CSF opening pressure

Patients with markedly raised initial opening pressure (>30 cm water) will have CSF drained (20-30 ml) at the time of lumbar puncture aiming to reduce the opening pressure to $\leq$ 20 cm and a repeat lumbar puncture the following day to help control opening pressure, consistent with current recommendations [1-3]. In addition, patients deteriorating clinically with symptoms or signs suggestive of raised intracranial pressure such as increasing headache, reduced conscious level, hypertension, or cranial neuropathies, will have repeat lumbar puncture to measure the opening pressure and, if >30 cm water, follow up lumbar punctures as above. CSF from repeat lumbar punctures will have quantitative culture done. If available, CT or MRI head scan should be done to exclude hydrocephalus and significant focal lesions in patients requiring CSF drainage and repeat LP .

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2. Graybill JR, Sobel J, Saag M, Van der Horst C, Powderly W, Cloud G, Rider L, Hamill R, Dismukes W. Diagnosis and management of increased intracranial pressure in patients with AIDS and cryptococcal meningitis. *Clin Infect Dis* 2000; 30:47-54.

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Appendix 5: Adverse event reporting plan and Flow chart of assessing and notifying of adverse events

MONITORING PLAN.

1. All serious adverse events including deaths and readmissions within 10 weeks and grade IV laboratory toxicity will be reported by the local investigators within 48 h to the international investigators.

2. All suspected unexpected (not usually associated with study drugs or part of the usual clinical spectrum of cryptococcal meningitis and late stage HIV infection) serious adverse reactions will be reported by the international investigators to the DSMB, and regulatory authority where required, within 7 working days.

3. Because of the high expected mortality of patients with HIV-associated cryptococcal meningitis in this setting (up to 50% at 10 weeks), the local investigators will prepare monthly cumulative reports of serious adverse events including deaths, readmissions, and adverse events, to be sent to the international investigators. Such cumulative reports will forwarded by them to the DSM after every 100 patients are enrolled or at least every 6 months.

A PHASE III, RANDOMISED, CONTROLLED TRIAL FOR THE TREATMENT OF HIV-ASSOCIATED CRYPTOCOCCAL MENINGITIS: ORAL FLUCONAZOLE PLUS FLUCYTOSINE OR ONE WEEK AMPHOTERICIN B-BASED THERAPY VS TWO WEEKS AMPHOTERICIN B-BASED THERAPY

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GENERAL INFORMATION

This document describes the ACTA (Advancing Cryptococcal meningitis Treatment for Africa) trial and provides information about procedures for entering patients into it. The protocol should not be used as an aide-memoire or guide for the treatment of other patients; every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to the registered investigators in the trial. Clinical problems relating to this trial should be referred to the Investigators.

- **Compliance**

The trial will be conducted in compliance with the protocol, ICH GCP Guidelines, and other regulatory requirements applying in the countries in which the trial will be conducted.

- **Sponsor (Malawi, Zambia, Tanzania and Cameroon sites)**

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CONTENTS

Abbreviations and Glossary

AE	Adverse event
Ag	Antigen
AIDS	Acquired Immune Deficiency Syndrome
ALT	Alanine transaminase
AmB	Amphotericin B
AR	Adverse reaction
ART/ARV	Anti-retroviral therapy/ Anti-retroviral
CF	Consent form
CRF	Case Report Form
CSF	Cerebrospinous fluid
EFA	Early Fungicidal Activity
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
IDMC	Independent Data Monitoring Committee
Int Inv	International Investigator(s)
IRIS	Immune Reconstitution Inflammatory Syndrome
PBMC	Peripheral Blood Mononuclear Cell
PI	Principal Investigator
PIS	Patient information Sheet
Plts	Platelets
PMN	polymorphonuclear leukocyte
SAE	Serious adverse event
SAR	Serious adverse reaction
SOP	Standard operating procedures
SUSAR	Suspected unexpected serious adverse reaction
TB	Tuberculosis
TSB	Total Serum Bilirubin
TSC	Trial Steering Committee
UAR	Unexpected adverse reaction
5FC	Flucytosine

1. SUMMARY

1.1 Lay summary

Cryptococcal meningitis is a leading cause of death in HIV-infected patients in Africa. With current treatments the mortality rates are very high, varying between 30% to over 50% at 10 weeks in different series. A study of the global burden of cryptococcal disease estimated 3-month mortality to be 70% and that cryptococcal disease was associated with 500,000 deaths per year in Sub-Saharan Africa.

The current standard for initial treatment is with 2 weeks of amphotericin B-based therapy in developed country settings. However in many settings in Africa, amphotericin B is not available or not used due to the requirements with amphotericin B therapy for in-hospital care with intravenous administration, close blood monitoring, and fluid and electrolyte supplements, and the associated extra medical, nursing, and laboratory costs. Instead, many centres have relied on fluconazole, a safe and well-tolerated oral treatment, available free of charge through a donation programme. However, fluconazole monotherapy is much less rapidly active in controlling infection, and is associated with high mortality and also the development of secondary resistance.

The combination of fluconazole with a second oral drug, flucytosine, has been shown to lead to much more rapid control of infection, and to be associated with fewer deaths than fluconazole alone in a small study. In addition, shorter 5-7 day courses of amphotericin B have been shown to be much better tolerated than 2 weeks amphotericin B, reducing the need for intensive monitoring of treatment, and the duration of hospitalization. Such short course amphotericin B would be much more easily implemented in the many centres in Africa and Asia currently using fluconazole, and may not be associated with any loss in efficacy compared with 2-week courses.

Therefore, this study will compare the best oral treatment, a combination of fluconazole and flucytosine, with a one week amphotericin B-based strategy, and with the standard of 2 weeks amphotericin B, in resource-limited settings where implementation of 2 weeks of amphotericin B would be difficult to sustain, and therefore would not be used unless shown to be superior to more readily implementable alternatives.

Additionally, fluconazole and flucytosine will be compared as additional drugs to be given with amphotericin B, in the 2 amphotericin B treatment strategies. Amphotericin B plus flucytosine is the standard combination used in developed country settings, but flucytosine is not currently available in much of the rest of the world. Fluconazole is freely available in Africa through a donation programme and is safer and requires less monitoring, and is also an effective second drug based on smaller, phase II studies. A recent trial from Vietnam showed no significant differences between fluconazole and flucytosine as second drugs in the main analysis although results after longer follow-up favoured flucytosine. Thus, whether fluconazole is an acceptable alternative to flucytosine remains to be determined. The study will address this question with greater numbers than in the Vietnam study, and in the African setting with timely initiation of antiretroviral therapy.

1.2 Abstract and summary of trial design

Type of design

An open label, phase III randomised non-inferiority trial to compare 3 alternative strategies for the initial treatment of HIV-associated cryptococcal meningitis. In addition, the 2 amphotericin B-based strategies are split so that, as a secondary aim, fluconazole and flucytosine will be compared as second drugs to give with amphotericin B in Africa.

Disease/patients studied

Patients diagnosed with a first episode of cryptococcal meningitis on basis of cerebrospinal fluid (CSF) India ink stain or cryptococcal antigen testing.
If not known, human immunodeficiency virus (HIV) seropositivity to be confirmed.

Trial interventions

Study Regimen 1: Fluconazole 1200 mg /d plus

flucytosine 25 mg/kg qds for 2 weeks.

Study Regimen 2: Amphotericin B (AmB) 1 mg/kg/d plus EITHER

2A: fluconazole 1200 mg /d, OR 2B: flucytosine 25 mg/kg qds, for **7** days

Study Regimen 3: Amphotericin B (AmB) 1 mg/kg/d plus EITHER

3A: fluconazole 1200 mg /d, OR 3B: flucytosine 25 mg/kg qds, for **14** days

In regimen 2, patients will receive fluconazole 1200 mg /d during the second week. In all arms, after 2 weeks, patients will receive fluconazole 800 mg/d until antiretroviral therapy (ART) started (at 28 days +/- 4 days after start antifungal therapy), and at 28 days +/- 4 days for patients on ART at presentation, then fluconazole 400 mg/d to complete 10 weeks treatment, and fluconazole 200mg/d thereafter.

1.3 Outcome measures

Primary outcome measure

- Mortality at 2 weeks by treatment group (regimen 1 and regimen 2 vs regimen 3)

Secondary outcome measures

- Mortality at 10 weeks by treatment group, as above
- Mortality at 2, 10 weeks by treatment group (regimens [2A + 3A] vs regimens [2B + 3B]; and 2A vs 2B, 3A vs 3B)
- Mortality at 2, 4, and 10 weeks by treatment group, as above, adjusted for site and other possible confounders including ART experience at presentation.
- The proportion of patients in each arm suffering clinical and laboratory-defined adverse events.

- Rate of clearance of infection by treatment group based on CSF quantitative cultures at baseline and days 7 and 14.
- The proportion of patients in each arm suffering pre-defined / paradoxical IRIS reactions to 10 weeks

Duration

Patients will be admitted and clinical response monitored daily for the first 1-2 weeks. Outpatient follow up will be at 2 (for any patient discharged before 2 weeks), 4, 6, 8 and 10 weeks after starting therapy.

Data recording

Data will be recorded on paper case record forms (CRFs), kept at the local centre, then entered into a central database using a Datafax-type system.

Sample Size and Funding

Using a non-inferiority design with a 10% non-inferiority margin and 5% type 1 error; and assuming 85% 2-week survival in the 2-week AmB arm (regimen 3), would require 157 patients per arm at 80% power, 184 per arm at 85% power, and 219 per arm at 90% power. 680 patients total (226 per arm) will be studied in order to achieve 90% power, allowing for 2% losses to follow-up at this early endpoint, based on prior experience, including at trial sites (Lilongwe [46])).

MRC (UK) have agreed to support the Malawi, Tanzania and Zambia sites (570 patients) and ANRS have agreed to support the Cameroon site (110 patients).

The numbers of patients treated with fluconazole or flucytosine in the amphotericin B arms will be a minimum of 226. In all scenarios, these numbers are well in excess of the 100 per arm in the Vietnam trial. 220 patients treated with each of the companion drugs would give power of 0.80 to detect an increase in 2-week survival to 90% with the most effective adjunctive (second drug) treatment from 80% with the least effective adjunctive treatment. The trial would provide sufficient data to base policy with regard which drug should be used with amphotericin B in Africa.

1.4 Ancillary studies/sub-studies

1. Semi-quantitative CrAg test analysis (Sites: Lilongwe, Cameroon (retrospectively Lusaka))

Objective:
To evaluate a new diagnostic test for cryptococcal meningitis using serum, plasma, whole blood, urine and CSF.

An alternative, semi-quantitative lateral flow immunodiagnostic test will be used in parallel with the current IMMY CrAg test. Patient inclusion will continue to be based only on the approved, established test. This study is proposed for conduct in the Lilongwe and Cameroon sites only (and retrospectively in Lusaka). In Lilongwe and Cameroon the test will be evaluated on fresh serum, plasma, whole blood, urine and CSF samples, collected at baseline. These samples will also be stored at -80°C for future CrAg titre analysis. In Lusaka, the test will be evaluated retrospectively on previously stored samples only. The study is an important evaluation of an alternative rapid diagnostic under

development for cryptococcal infection. This new test has the potential advantage of giving, in addition to a positive or negative result, an indication of antigen load, whether high or low, upon which, in the future, more individualized treatment might be based.

2. RNA sequencing of HIV from plasma and CSF samples (Sites: Lilongwe, in collaboration with UNC Project)

Objective:
To determine if the appearance of cryptococcal meningitis is associated with independent replication of HIV-1 in the CNS (i.e. the presence of an HIV-1 sequence population that is distinct from that found in the blood) and whether the independently replicating virus has evolved to become macrophage-tropic.

Background:
HIV-1 can replicate in the brain independently from its replication in blood and lymphoid tissue. Replication in the brain can result in the evolution of macrophage-tropic HIV-1 where the virus has adapted to grow in cells with low levels of CD4 (i.e. macrophages and/or microglia) as opposed to its normal state of needing the high levels of CD4 found on CD4+ T cells. The evolution of macrophage-tropic HIV-1 is most often associated with low levels of CD4+ T cells and is also associated with the onset of HIV-associated dementia. Cryptococcal meningitis shares many features with the evolution of macrophage-tropic HIV-1 in that they are both more likely to occur late in HIV-1 disease and both can be considered opportunistic CNS infections.

We propose to determine if the appearance of cryptococcal meningitis is associated with independent replication of HIV-1 in the CNS (i.e. the presence of an HIV-1 sequence population that is distinct from that found in the blood) and whether the independently replicating virus has evolved to become macrophage-tropic. It is possible that local HIV-1 replication in the CNS further diminishes the local immune monitoring capacity of the host that would otherwise limit the growth of Cryptococcus. A better understanding of the evolution of HIV in the CNS may have implications for the better management of cryptococcal and other late stage patients.

In addition:

- A PK/PD sub-study will be developed

- Cryptococcal fluconazole resistance study, FLURES, will be conducted in Tanzania.

- A cost-effectiveness analysis will be developed

- Cryptococcal isolates will be saved and substudies developed

- CSF samples will be saved and used in immunological and proteomic analyses

- PBMC will be saved for later host genetic studies of susceptibility to cryptococcal infection at other collaborating sites but not from the participants recruited in Malawi and Tanzania.

All isolates and samples will be fully anonymised. Isolates and samples will also be shared with members of the cryptococcal research community. Transfer of materials out of country will be subject to site specific material transfer agreements.

Flow diagram

Trial entry, randomisation, treatment and analysis

```
                    ┌──────────────────────────┐
                    │    ELIGIBLE PATIENTS     │
                    └──────────────────────────┘
                                │
                                ▼
                    ╱───────────────────────╲
                   (      RANDOMIZATION       )
                    ╲───────────────────────╱
              ┌─────────────┼─────────────┐
              ▼             ▼             ▼
```

STUDY REGIMEN 1	STUDY REGIMEN 2	STUDY REGIMEN 3
Fluconazole 1200 mg/d + 5-FC 100 mg/kg/d	AmB 1 mg/kg/day + Fluconazole 1200 mg/d *or* 5-FC 100 mg/kg/d	AmB 1 mg/kg/day + Fluconazole 1200 mg/d *or* 5-FC 100 mg/kg/d
14 days	**day 1-7,** Fluc 1200 mg/d **day 7-14**	**14 days**

FOLLOW UP TREATMENT
Fluconazole 800 mg/d until 28 days +/- 4 days (ART starts then for ART naïve patients), then 400 mg/d to 10 weeks, then 200 mg/d

PATIENT FOLLOW UP
Lumbar punctures day 1, 7, 14. Outpatient follow-up to 10 weeks

ANALYSIS OF OUTCOME MEASURES

2. BACKGROUND

2.1 Background and Relevant studies/trials

Cryptococcosis is a very common opportunistic infection in patients with late stage HIV infection, especially in Southern and East Africa and South and South East Asia [1-3]: for example, cryptococcosis accounts for 20% and 24% of AIDS-defining diagnoses in the North and Northeast of Thailand, respectively (corresponding figures for tuberculosis were 22% and 27%) [4], and cryptococcosis is the commonest cause of adult meningitis in many areas of Africa with high HIV prevalence [5,6]. Despite expansion of ART programmes, cases of cryptococcal meningitis have not decreased in many Africa centres, and are unlikely to in the near future, since as many HIV-infected individuals continue to enter stage IV disease, and therefore become vulnerable to development of cryptococcal disease, as are accessing ART [7].

Furthermore, treatment is unsatisfactory: 10 week mortality in SE Asia and Latin America has ranged between 19% and 43% at 10 weeks [8-11]. In Africa where resources are most restricted, mortality has ranged from 24% at 10 weeks, in a trial in South Africa using amphotericin B, in which the patients were relatively selected, to 95% at 12 weeks in an unselected cohort treated with low dose fluconazole [12-18].

The combination of high incidence and difficulties with treatment mean cryptococcosis is a very common cause of death in AIDS patients in Africa and other parts of the developing world. For example, cryptococcosis accounted for 17% and 13% of all deaths in two cohorts of HIV-infected patients from Uganda [19,20] and 44% of all deaths in a series from South Africa [21]. For comparison, TB caused 6%, 5% and 13% of deaths, respectively, in these studies. A recent CDC analysis estimated that cryptococcal disease is associated with over 500,000 deaths per year in Sub-Saharan Africa alone [3].

A number of other factors combine to further increase the urgent need for improvement in the acute treatment of cryptococcal infection:
(1) Expansion of antitretroviral programmes in many areas raises the prospect of transforming the long-term prognosis of patients presenting with cryptococcal meningitis, *provided* they survive the acute phase of the illness. Unfortunately around 50% of patients do not currently survive long enough to benefit from antiretroviral treatment [15,16].
(2) Fluconazole is now widely available and affordable. It is effective and safe as maintenance treatment and its availability means gains in survival from improved initial management of cryptococcal meningitis will not be lost through lack of effective maintenance therapy.
(3) A large proportion of cases of cryptococcal disease represent the initial presentation of HIV infection and the AIDS-defining illness [10] and therefore without much more widespread and early testing for HIV infection only a proportion of cases are potentially preventable through early antiretroviral treatment (ART) or prophylactic strategies.

Fluconazole monotherapy
Many centers in Africa continue to rely on fluconazole, available free through the Pfizer donation program [22] and also in relatively inexpensive generic form. However, results with fluconazole monotherapy, especially at conventional dosages of up to 800 mg/d have been poor in Africa. In a series from Zambia the median survival with fluconazole 200 mg/d monotherapy was 19 days compared to 10 days in untreated patients [12]. Mortality with fluconazole 200 mg/d in a Ugandan trial was 40% in the first two weeks and 64% at two months [13]. In Cape Town, a retrospective

study with incomplete follow up, with a median time of follow up for discharged patients of just 36 days, and patients lost to follow up censored, nevertheless reported around 50% 10 week mortality with 200 or 400 mg/d [14]. This represents a minimum estimate, and the 10-week mortality in unselected patients treated with fluconazole at up to 800 mg/d is probably significantly more than 50%. In a small cohort from Uganda with complete follow up fluconazole at 800 mg/d was associated with a 60% 10 week mortality [18]. It was in the light of these data that investigators in a recent global burden study estimated the 3-month mortality associated with cryptococcal disease in Sub-Saharan Africa to be 70% [3].

In Cape Town, in a small group of patients, using serial quantitative cultures, fluconazole at 400 mg/d was shown to be essentially fungistatic, at least over the first 2 weeks of treatment [Figure 1B, 15]. While there is evidence for a dose-response effect with fluconazole [18, 23-27], and plasma concentrations of fluconazole in patients with fungal infection are known to increase linearly with doses up to 2 g/d [28], even with a dose of 1200 mg/d, the rate of clearance of infection is markedly less rapid than with amphotericin B-based treatment, even when this is for one week only (figure 1A and D, [15,18]):
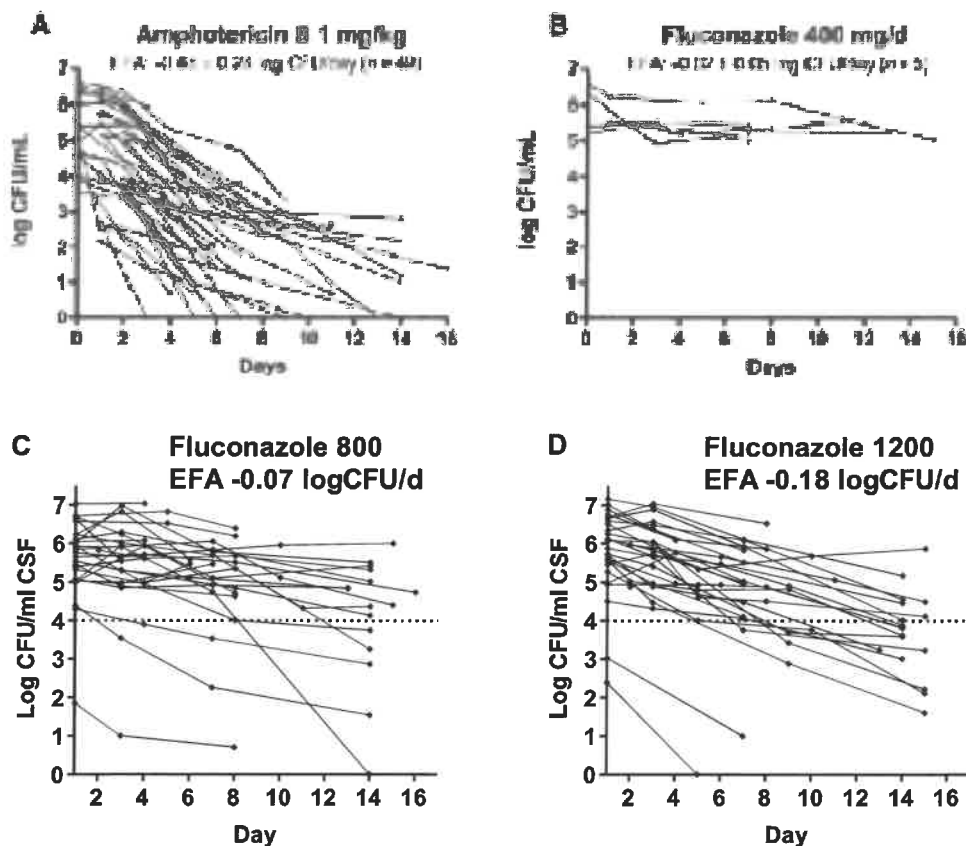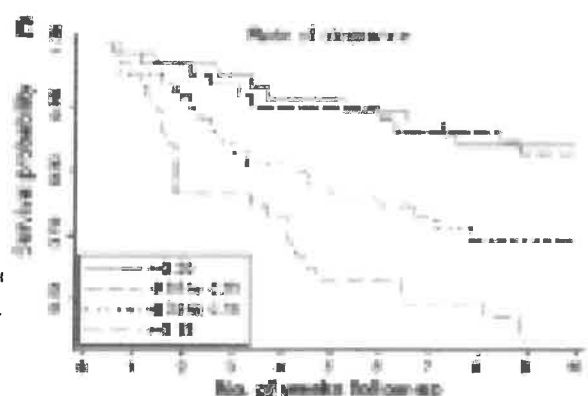

Figure 1: Rate of clearance data for amphotericin B at 1 mg/kg/d for 7 days (A), and for fluconazole at 400 mg/d (B), 800 mg/d (C), and 1200 mg/d (D), from [15] and [18].

Supporting the clinical relevance of such studies, rate of clearance of infection was recently shown to be independently associated with 2- and 10-week

mortality in a combined cohort that now totals over 300 patients (Figure 2, [29]):

Figure 2: Kaplan-Meier survival curves by rate of clearance of infection divided into quartiles (from [29])

In addition to inadequate antifungal activity, there are other reasons to avoid fluconazole monotherapy:

1. The prolonged period with a high viable organism load associated with fluconazole monotherapy may predispose to the development of secondary fluconazole resistance. Such resistance was a significant problem in Cape Town when initial therapy was with fluconazole [30], but disappeared following a switch to more-rapidly active amphotericin-B based therapy.

2. Prolonged active infection could also increase the risk of immune reconstitution reactions following introduction of ART. Although data on this point are lacking, of relevance, the risk of developing culture negative symptomatic relapse after ART was shown to be associated with the burden of infection, as assessed by serum cryptococcal antigen titer, prior to starting ART [31]. Consistent with an increase in the incidence and/or severity of IRIS when fluconazole monotherapy is used, in a recent study from Harare, Zimbabwe, fluconazole 800 mg/d with rapid initiation of ART was associated with very high mortality [32]. Given the high early mortality in patients waiting to access ART, delaying ART to avoid this problem, if fluconazole is used, is also not an attractive option.

In the light of these data, the Southern African HIV Clinicians Society concluded that amphotericin B-based treatment, even if shortened to one week, should be given, if at all possible, in preference to fluconazole [33]. Thus, the weight of evidence suggests fluconazole monotherapy is inadequate as induction treatment; and the question for the many centres still currently using fluconazole at up to 800 mg/d, is not whether this treatment is an appropriate option for the future; but which of the alternative regimens offers the best combination of efficacy and sustainability in a particular setting?

Standard 2-week amphotericin B induction

A 2 week initial induction phase with amphotericin B is widely accepted as the gold standard therapy for HIV-associated cryptococcal meningitis if resources and facilities allow [34]; largely based on a landmark study by van der Horst and colleagues, the rationale of which was to try to get early control of infection with rapidly fungicidal amphotericin B, with or without flucytosine, but then to avoid the toxicities associated with these drugs by switching to consolidation and then maintenance treatment with the better tolerated azoles [35]. The results, published over a decade ago, remain the best to date from a phase III trial. Overall 10-week mortality was between 10 and 23%.

Unfortunately in many centres in Africa, 2 weeks' induction therapy with amphotericin B may be difficult to sustain. In addition to the costs of the drug, which may be substantial in local terms [36], are the requirements for hospitalization, intravenous drug administration, including nursing time and expertise in siting and maintaining i/v access for a drug which causes considerable phlebitis, saline fluids, and electrolyte replacement, and regular, rapid and reliable laboratory monitoring for renal function, electrolytes, and hemoglobin.

However, while acknowledging the challenges of amphotericin B therapy, if it were shown that 2 weeks amphotericin B induction is superior to more readily implemented alternatives (see below), then the extra resources required could be justified. We have considerable experience in introducing

amphotericin B therapy in centres previously using oral fluconazole therapy. It is vital that amphotericin B therapy is monitored, and potassium replaced, as required, and that saline fluid supplementation is given, provided no contraindication, to reduce nephrotoxicity [37,38]. To reduce the need for oral potassium supplements that can exacerbate nausea, it has been our practice to add a small amount (20 mmol) of potassium to a daily 1 L of saline pre-hydration fluid. After starting collaborative work at Jooste Hospital, Cape Town, one week [15] and then 2 weeks amphotericin B [17] was introduced as the standard of care, in place of fluconazole. And in South Africa as a whole, following a reduction in the cost of amphotericin B [36], the proportion of patients treated with amphotericin B induction has progressively increased [Nelesh Govender, personal communication]. Of note, major donors have provided amphotericin B to countries with more resource limitation than South Africa including Uganda (PEPFAR), where 2 weeks amphotericin B is given in Mulago hospital [16], and Malawi, where sites for this study are located.

Short course amphotericin B induction

A one-week induction with amphotericin B would be much more easily implemented than a full, standard 2 weeks course, in resource limited settings, and may not be associated with a significant reduction in efficacy. A very significant reduction in the burden of infection in terms of the CSF CFU counts of between 3 and 4 logs can be achieved with 7 days of amphotericin B at 1 mg/kg/d [Figure 1A, 15]. Perhaps related to the long half life of amphotericin B, the rate of clearance of infection with 7 days amphotericin B, at 1 mg/kg/d, even if measured over 14 days, was not noticeably less rapid than when 14 days amphotericin B was given to patients at the same hospital: -0.48 log CFU/d with 7 days compared -0.45 and -0.56 log CFU/d with 0.7 and 1 mg/kg/d respectively, for 14 days [15,17].

In addition, renal impairment and anemia, which are dose-related, and usually manifest during the second week of induction [17], may be reduced, and iv access is much easier to maintain, and line infections may be reduced, with 7 rather than 14 days. In a recent cohort from Uganda, 5 days amphotericin B was extremely well tolerated and associated with a 26% 10 week mortality, less than that observed for prior cohorts at the same centre treated with high dose fluconazole (Muzoora, Taseera, Harrison et al, unpublished data). Again, rate of clearance, measured over 14 days, was impressive despite the short duration of amphotericin B (-0.30 log CFU/d). Of note, although during this study amphotericin B was fully monitored with 5 days, none of 30 patients required early discontinuation, and adjustments in potassium supplementation and fluids were very rare (Muzoora et al in preparation). This would suggest strongly that even if, in wider implementation, monitoring was not to a study standard, short-course amphotericin B could still be introduced and given safely.

Although small and underpowered, a randomized study from Thailand found induction with one week of amphotericin B not to be substantially inferior to two weeks [39].

Combination therapy with amphotericin B

The combination of amphotericin B plus flucytosine is recommended in international guidelines, based on consistent evidence for synergy in vitro, in animal models, and in clinical studies [34]. However, flucytosine is not currently available in much of the developing world. Fluconazole is freely available in Africa through a donation programme and is generally safer and requires less monitoring. Despite the antagonism between amphotericin B and azoles that has been observed in some systems [40], most animal model data suggest amphotericin B plus fluconazole is also a very effective combination against C. neoformans [41,42]. In Thailand, amphotericin B and fluconazole at 400 mg/d was more rapidly fungicidal than amphotericin B alone, although the difference did not reach statistical significance [10]. Amphotericin B plus fluconazole at 800 mg/d is more promising. In a recent phase II study by Pappas and colleagues, amphotericin B plus fluconazole at 800 mg/d was associated with the highest proportion of successful outcomes (combined endpoint of sterile CSF, survival, and neurological stability or improvement) at 2, 6, and 10 weeks, in comparison with

amphotericin B alone and amphotericin B plus fluconazole 400 mg/d [43]. A Phase III study comparing amphotericin B alone with amphotericin B plus flucytosine and with amphotericin B plus fluconazole 800 mg/d has recently been completed in Vietnam [ISRCTN95123928, www.controlled-trials.com, Day J et al, ICAAC 2011]. The data remain inconclusive with regard the comparison of flucytosine and fluconazole as second drugs: there were no significant mortality differences between fluconazole and flucytosine as second drugs in the main, 2 and 10 week analyses (HR for death at 10 wks, fluconazole vs flucytosine: 1.15 (0.7,1.9), p=0.6; adjusted HR 1.5 (0.9, 2.6) p=0.1), although results after longer 6 month follow-up favoured flucytosine (HR 1.4 (0.9,2.2), p=0.1, adjusted HR 1.8 (1.1, 2.9) p=0.01), and rates of clearance of infection were more rapid with flucytosine. Thus, whether fluconazole is an acceptable alternative to flucytosine remains to be determined. The study will address this question of the optimal drug to give with amphotericin B with greater numbers than in the Vietnam study and in the African setting with timely initiation of antiretroviral therapy: Patients randomized to each of the 2 amphotericin B arms will be assigned to receive either flucytosine or fluconazole as second agents.

An optimised oral regimen

In the recently published Infectious Diseases Society of America (IDSA) guideline [34], fluconazole 800mg/d is not recommended, even in resource-limited settings. Fluconazole monotherapy is not recommended; if amphotericin B is not available, the recommended oral regimen is fluconazole 1200 mg/d with flucytosine; and in absence flucytosine, fluconazole 1200 mg/d. This is based on:

1. Data showing a dose response effect for fluconazole going up at least to 1200 mg/d in 2 separate Phase II studies:

Longley et al [18], in Mbarara, Uganda found significantly more rapid rate of clearance of infection with fluconazole at 1200 compared with 800 mg/d, and also fewer deaths in high dose arm, although this was not statistically significant (Figure 1). Of note, rate of clearance of infection has been shown to be associated with survival at 2 and 10 weeks [29]. The 10 week mortality with 800 mg/d was 60%, emphasizing the need for more effective therapies. Milefchik et al. [27] in an earlier US study, using a combined clinical and culture conversion endpoint, and fluconazole dose escalation from 800 mg to 2 g/d, found the percentage of patients alive with a negative CSF culture at 10 weeks increased up to 1600 mg/d. These data build on earlier evidence for a dose response between 200 and 800 mg/d. Accepting the caveats of comparing across trials and the small numbers of patients in the higher dose cohorts, median time to CSF sterilization was 64 days with 200-400 mg/day [23], mean time to sterilization 41 days with 400mg/day [24], and median times 21 and 33 days with 800mg/day [25,26].

In terms of safety, 800 mg/d has been shown to be a safe in large randomized trials in this and other indications [43,44]; 1200 mg/d is given routinely by many experts in coccidioidal meningitis [45], and has been given to between 100 and 200 patients with cryptococcal meningitis in phase II studies [18, 46, Muzoora et al, Jackson et al, unpublished data]; and fluconazole doses up to 2 g have been given to small numbers of patients with cryptococcal meningitis and other fungal infections [27, 28]. The numbers treated at the highest doses are very small, but serious side effects seem not to be very frequent, at least up to 1600 mg. In Uganda and Lilongwe, there was no suggestion of increased toxicity, in particular, no liver function disturbance, at the 1200 mg/d dose, although the numbers involved (about 100 patients) means continued vigilance is needed.

2. Consistent evidence for the benefit of adding flucytosine to fluconazole in a combined regimen

Based on encouraging murine data [47], a cohort of patients was treated with fluconazole 400 mg/day plus flucytosine 150 mg/kg/day for 10 weeks [48]. The median time to CSF sterilization was

relatively short at 23 days. By 10 weeks at this dose, 28% of patients had side effects requiring discontinuation of flucytosine. However, over 95% of participants tolerated 2 weeks therapy. In Uganda, flucytosine for 2 weeks was well tolerated and additive with low dose fluconazole (200 mg/day) [13]. In a study by Larsen and colleagues, flucytosine 100 mg/kg/d was given for 4 weeks with increasing doses of fluconazole and again had a benefit (higher percent of patients alive with a negative CSF culture at 10 weeks) that was most pronounced with fluconazole at 800 – 1200 mg/day [27]. In that study, grade IV neutropenia ($<500 \times 10^9$/L) occurred in 18% of patients given flucytosine for 4 weeks, without evidence of increased infection.

In Malawi, subsequent to the fluconazole dosage study in Uganda, the fungicidal activity of fluconazole 1200 mg/d was compared with and without the addition of oral flucytosine 100 mg/kg/d for 2 weeks (Figure 3, [46]). Flucytosine led to a very significant further increase in EFA, to -0.28 log CFU/d (compared -0.11 log CFU/d with fluconazole monotherapy), to a level similar to amphotericin B alone at 0.7 mg/kg/d in Thailand (-0.31 log CFU/d, [10]). Of note this rate of clearance with oral combination therapy also approaches the level above which there is less evidence that a further increase in the rapidity of kill is associated with a further improvement in clinical outcome (please see Figure 2 [29]), further supporting the equipoise of comparing this oral therapy with amphotericin B–based induction treatment.

Although not powered for clinical endpoints, there were fewer deaths in the combination arm at 2 weeks that reached borderline significance (Figure 3, p=0.05 [46]). There were more episodes of neutropenia in the combination arm, although no increase in infection related serious adverse events.

Figure 3. Rate of clearance with fluconazole 1200 mg/d plus flucytosine 100 mg/kg/d in Lilongwe; and survival with this combination in comparison with fluconazole at 1200 mg/d alone, from [46]

This recent controlled trial is especially relevant – having been conducted in Malawi against a standard of fluconazole monotherapy. The rationale for using the rate of clearance endpoint is that these studies can be used to make some choices about drug dosages and regimens – as to which are the most promising to take forward to clinical endpoint trials.

In all the earlier studies above, flucytosine was given in higher dose and for longer than in recent studies and in this proposal. In the large MSG trial and in Thailand, flucytosine at 100 mg/kg/d for 2

weeks was very well tolerated with full blood count monitoring only, without real time flucytosine levels [10, 35]. The reasons for the lack of toxicity observed may be found in a PK substudy from Thailand in which levels of flucytosine in patients on oral flucytosine formulation at this dosage were well below those usually associated with bone marrow depression, although enough to stay above MIC for the whole dosing interval, ensuring maximum boosting of fungicidal activity [49]. In ongoing studies in South Africa and Malawi using 2 weeks of flucytosine with amphotericin B and/or fluconazole, 5% of 123 patients developed grade IV neutropenia in the first 2 weeks (authors' unpublished data). Thus, flucytosine at the historically low doses used now (100 mg/kg/d), is associated with a risk of neutropenia that requires monitoring – but is reversible and rarely requires drug discontinuation.

Flucytosine is not generally available at present in Sub Saharan Africa BUT there is no reason it should not be produced at low cost by generic manufacturers if data showed it to be an essential component of optimal therapy. Flucytosine is a simple molecule that is off patent, and used to be registered and marketed in South Africa [36]. Advocacy for increased access is already underway based on the Malawi combination oral therapy trial.

Thus, in this trial, amphotericin B-based therapy, of two weeks duration, will be compared with amphotericin B for one week and with an optimal oral regimen of fluconazole 1200 mg/d plus flucytosine. If either an optimal oral regimen or one week of amphotericin B prove to be non inferior to 2 week amphotericin B treatment, these treatment options could be introduced widely in other resource limited settings. If not, efforts will need to be made to improve medical and laboratory facilities and staffing so that 2 weeks of amphotericin B can be given safely in a wider variety of settings. Of note, a cost effectiveness sub-study will be developed to help in these policy decisions.

2.2 Risks and benefits

Phase II studies based on rate of clearance of infection have identified regimens (oral combination fluconazole plus flucytosine, and short course amphotericin B) that are much more rapidly fungicidal than oral fluconazole monotherapy [15,46], and of similar rapidity of action while being less toxic and easier to implement than 2 weeks induction with amphotericin B [10,17]. These alternatives would also potentially allow earlier discharge from hospital, and would be implementable in a much wider number of centres and than 2 weeks' induction with amphotericin B.

Rate of clearance of infection has been shown to be associated with clinical outcome at 2 and 10 weeks [29]. Thus, patients in the trial will benefit from more effective therapies than fluconazole, still currently used in many centres in Africa, including one of the study sites, Blantyre in Malawi.

The important side effects of amphotericin B are predictable, dose-dependent, and reversible. A one week course should be much better tolerated than 2 weeks. While 2 weeks of amphotercin B is the standard induction therapy in HIV-associated cryptococcal meningitis in settings without resource restriction, whether the side effects associated with this duration (anaemia and renal impairment), although reversible, may result in the alternative regimens being studied being as effective in terms of clinical endpoints is the question being addressed by the study. A small and underpowered study suggested no loss of efficacy with a shorter induction with one week of amphotericin B [39].

As discussed above, flucytosine carries a risk of neutropenia that requires monitoring, but at the dose and duration to be used is well tolerated. Data from phase II studies suggest any increase in side effects due flucytosine is more than balanced by superior antifungal efficacy, with a strong trend in favour of its addition to fluconazole, in terms of clinical endpoints [46].

Fluconazole has been used in small numbers of patients up to 2 g/d [27,28]. At 1200 mg/d it has been used in several phase II studies of cryptococcal meningitis [18,46], and has also been used in other serious systemic fungal infections, notably coccidioidal meningitis [45]. 1200 mg/d has not been found to be associated with any increase in serious side effects, in particular no increase in liver function abnormalities, compared to lower doses [18,46]. Azole antifungal drugs have been associated with QT prolongation. In a prior trial using 800 mg/d no significant prolongation of QT interval was reported [50]. Paired ECG at baseline and day 7 will be recorded for the first 120 patients (includes 60 on fluconazole at 1200 mg/d). These will be analysed to decide whether further ECG monitoring is required. Of note, 1200 mg/d has recently been adopted as the recommended dose for cryptococcal meningitis in Malawi.

Therefore, serious toxicity is not expected with the short course and dose of flucytosine and the short duration of relatively high dose fluconazole to be used in this study. Patients in the trial will be closely monitored for adverse events and the Independent Data Monitoring Committee (IDMC) will review safety and efficacy data regularly.

All patients who enroll in the study will benefit indirectly as it is well-established that the outcome for patients enrolled in clinical trials is almost always better than patients in routine care. Because cryptococcal meningitis is often the initial presentation of HIV infection, without improvements in antifungal therapy, cryptococcosis will remain a leading cause of death of HIV-infected patients in Africa and Asia even as antiretroviral therapy becomes more widely available.

2.3 Population

The study population will be HIV-seropositive patients with cryptococcal meningitis, at the participating centre, who fulfil the inclusion/exclusion criteria outlined below.
See **section 3** for full details.

2.4 Rationale and objectives

The principal aim of the trial is:-

To determine if alternative induction regimens of
1. Fluconazole 1200 mg/d plus flucytosine 25 mg/kg qds for 2 weeks; or
2. An amphotericin B-based strategy for 7 days [Amphotericin B (AmB) 1 mg/kg/d plus fluconazole 1200mg/day, or flucytosine 25 mg/kg qds, for 7 days],
are non-inferior to

3. An amphotericin B-based strategy for 14 days [Amphotericin B (AmB) 1 mg/kg/d plus fluconazole1200mg/day, or flucytosine 25 mg/kg qds, for 14 days].

for initial treatment of HIV-associated cryptococcal meningitis.

Secondary Aims:

* To compare flucytosine and fluconazole as additional drugs given with amphotericin B
* To determine the relative tolerability of these regimes.
* To determine the rate of clearance of infection with these regimens, based on quantitative cultures at baseline and day 7 and day 14.

- To determine the proportion of patients in each arm suffering pre-defined IRIS reactions to 10 weeks.

2.5 Selection of Centres

The study will be conducted at Kamuzu Central Hospital, Lilongwe, Zomba Central Hospital, Zomba and Queen Elizabeth Hospital, Blantyre, Malawi; at University Teaching Hospital Lusaka, Zambia; at Central Hospital, Yaounde, and General Hospital, Douala, Cameroon and Muhimbili and Mwananyamala municipal hospitals, Dar Es Salaam, Tanzania.

In Lilongwe, 40 patients were recently enrolled in a study with very similar inclusion/exclusion criteria in 10 months (4 per month) [46]. Since then patient numbers have increased. Queen Elizabeth Hospital, Blantyre serves a significantly larger population (approx. 1 million) than Lilongwe, sees an average of 14 culture positive cases per month and enrolled 60 patients in 8 months in a recent observational cohort (7.5 per month), and is expected to enrolled 6 patients per month. At University Teaching Hospital Lusaka, recruitment was estimated at 4 per month. Thus, it was originally estimated that a minimum of 168 patients (42 months x 4) could be enrolled at Lilongwe and Lusaka, and 252 at Blantyre in 3.5 years (total 588 patients; giving a total of over 570 patients. It was estimated that 110 patients could be enrolled in Central Hospital, Yaounde and General Hospital, Douala in 2-3 years. Because of slower than expected initial enrollment due to initial exclusion of ART-experienced patients, additional sites in Dar Es Salaam, Tanzania and Zomba, Malawi are proposed with an expected recruitment of up to 150 patients at each site. In addition, recruitment in Blantyre has been running well and it is expected that up to 300 patients may be enrolled at this site in 3.5 years.

The trial will be conducted according to GCP with an expert independent external data safety monitoring committee. Ethical and, if appropriate, regulatory approval will be obtained in countries where the study sites are located and in the UK and France.

3. SELECTION OF PATIENTS

3.1 Patient inclusion criteria

1. Consecutive patients $\geq$ 18 yrs with a first episode of cryptococcal meningitis on basis CSF India ink and/or CSF cryptococcal antigen.
2. Willing to agree to HIV testing
3. Willing to consent to participate in the study.

3.2 Patient exclusion criteria

A patient will not be eligible for entry to the study if:

1. Pregnancy or lactation.
2. Previous serious reaction to study drugs
3. Concomitant medication that is contraindicated with any study drugs.
4. Received >1 dose of Amphotericin B or Fluconazole therapy within 2 weeks of screening
5. Received > 1 cryptococcal treatment dose (up to 1200 mg) of fluconazole or > 7 days low dose (200 mg) fluconazole within 2 weeks of screening.

3.3 Number and source of patients

For selection of centres refer to **section 2.5**

680 patients will be enrolled (226 per arm) to give 90% power. The intake will take place over a 42 month period, and follow up will be for 10 weeks after the start of treatment. The total duration of the study will be 54 months.

4. ENROLMENT & RANDOMISATION PROCEDURE

Patients over 18 years of age with a first episode of cryptococcal meningitis diagnosed on the basis of CSF India ink or cryptococcal antigen test at the local laboratory will be assessed for eligibility for inclusion in the trial. Their details will be recorded in a Screening Register. Patients will be enrolled into the study based on the criteria of eligibility outlined in **sections 3.1 and 3.2**. For those found to be ineligible the reason for non-inclusion should be recorded on the Screening Register.

Patients found to be eligible on assessment of clinical and routine laboratory data will be invited to complete screening (pregnancy test for women of childbearing age) and enter the trial, if eligible. They will be given a Patient Information Sheet (PIS) about the trial and asked if they are willing to agree to participate in the study. Any information entered into the database for the trial, or sent to the laboratory will be identified by a number and their initials but not by name. They will be free to withdraw from the study at any time and if they do so this will not jeopardise their future care. If the staff are satisfied that the patient understands the above information, and is willing to continue, they will be asked to indicate their consent either by signature or by thumbprint (if the volunteer is illiterate) on the consent form. Illiterate volunteers will be asked to have a witness present (friend, family or another member of staff independent of the study team) to witness the discussion and thumbprint consent. If the volunteer is illiterate and declines to have a witness present, this will be recorded on the informed consent form. Participants will be given a copy of the signed/thumb-printed consent form and an information sheet to take away if they wish. Patients with altered mental status who are unable to consent will be enrolled into the study if their next of kin gives informed consent on their behalf. As soon as the patient's mental status improves they will be given a PIS and consent obtained as above, with care taken to ensure they understand that they are free to withdraw from the study and if they do so this will not jeopardise their future care.

Patients will be randomised individually using a computer-generated programme. Patients will be randomised by site.

5. TREATMENT OF PATIENTS

5.1 Baseline assessment

i) Baseline data – clinical:

Age, Sex, Significant past medical history, Drug history.
Symptoms and duration
Full Examination
Visual acuity (if and once able to co-operate with examination)

ii) Laboratory:

- Full blood count, urea and electrolytes, ALT
- HIV serology – if status not already known
- CD4 count
- Viral load (in ART experienced patients only, at sites where resources are available at time of sample collection)
- Magnesium levels (Lusaka site only)
- Urine: - pregnancy test if indicated. Analysis for proteinuria. Cryptococcal antigen
- Chest X-ray, if clinically indicated
- CSF: opening pressure, cell count and differential, protein, glucose, India ink, cryptococcal antigen titre (IMMY CrAg), semi-quantitative CrAg test (Lilongwe, Cameroon), quantitative fungal culture, organism counts by haemocytometer (at baseline, optional by site), (note CSF will be frozen for later immune parameter analysis, titre levels and viral sequencing).
- ECG for first 120 patients (40 per regimen).

C. neoformans isolates will be stored in TSB and 15% glycerol at minus 80°C.
Additional sample storage for valuable ancillary studies depend on specific site approvals, and site-specific patient information and consent.

5.2 Antifungal drugs

Antifungal drugs
Study Regimen 1: Fluconazole 1200mg/d plus

flucytosine 25 mg/kg qds for 2 weeks.

Study Regimen 2: Amphotericin B (AmB) 1 mg/kg/d plus

EITHER 2A: fluconazole 1200mg/d, OR 2B: flucytosine 25 mg/kg qds, for 7 days

Study Regimen 3: Amphotericin B (AmB) 1 mg/kg/d plus

EITHER 3A: fluconazole 1200mg/d OR 3B: flucytosine 25 mg/kg qds, for 14 days

In regimen 2, patients will receive fluconazole 1200 mg/d during the second week. In all arms, after 2 weeks, patients will receive fluconazole 800 mg/d until ART started (at 28 days +/- 4 days after start antifungal therapy), and at 28 days +/- 4 days for patients presenting on ART, then fluconazole 400 mg/d to complete 10 weeks treatment, and fluconazole 200mg/d thereafter.

Patients with baseline blood tests showing:
1. ALT>5 times upper limit of normal. or
2. PMNs<500x10^6/L , or Plts<50,000x10^6/L.

Will be withdrawn from the study, in view of the possibility of exacerbation of liver function abnormality with fluconazole and bone marrow depression with flucytosine. The numbers of such patients is likely to be very small. Treatment of such patients will be in conjunction with routine hospital services, but the study team will provide drugs and monitoring support for amphotericin B therapy for at least 7 days

3. Creat >2.5 mg/dl (225 µmol/L), will be rehydrated and creatinine repeated. If in AmB strategies:
   a) If repeat Creatinine falling and below 2.5 mg/dl (225 µmol/L), continue as per protocol (daily dosing, 1 mg/kg, paying close attention adequate hydration)

   b) If repeat Creatinine rising, patients will be withdrawn from the study, and treated with high dose fluconazole (1200 mg/d, adjusted for renal function) in conjunction with routine hospital services, and consistent with local guidelines

   c) if stable or improving but creatinine still above 2.5 mg/dl: institute alternate day AmB dosing (1 mg/kg q 48 hours). Monitor and act as per Appendix 3, Management of expected toxicities.

Management of raised CSF opening pressure:
Will be repeat daily lumbar punctures, consistent with guidelines. See **Appendix 4.**

Antiretroviral Treatment
Patients will start ART 28 days +/- 4 days after initiation of antifungal therapy,, consistent with current guidelines [33,34]. ART will conform to the treatment protocols in use in the ongoing programme of ART at study sites. Currently, this consists of nevirapine or efavirenz plus either stavudine and lamivudine or combivir or tenofovir.

Source of drugs
Amphotericin B (Fungizone, BMS) and fluconazole (Diflucan, Pfizer or Fluconazole generic WHO qualified manufacturer) will be obtained locally, if possible. Flucytosine (Meda Pharmaceuticals or Sigmapharm) will be imported for the study.

5.3 Treatment schedules

Details of Drug Dosages
The doses of drugs to be given to each patient are shown below and are based on the weight of the patient at the time of starting treatment.

Initial treatment (to be given for the first 2 weeks)

Amphotericin B:

1mg/kg/day. Patients will be given 1 L of Normal Saline (plus 20 mmol KCl) per day to help reduce amphotericin B nephrotoxicity (provided no contraindication – such as cardiac failure) [33]. Once reconstituted, amphotericin B will be shielded from UV degradation (however, solution is stable for 24 hours in indoor light).

Flucytosine:

Weight of patient	PO	(tablets)
30-39 kg	500-1000-500-1000	(1-2-1-2)
40-49 kg	1000 mg q 6 hours	(2-2-2-2)
50-59 kg	1000-1500-1000-1500	(2-3-2-3)
60-69 kg	1500 q 6 hours	(3-3-3-3)
70-79 kg	1500-2000-1500-2000	(3-4-3-4)

Fluconazole:

1200 mg/d for the first 2 weeks, or by NG tube if patient unable to swallow.

Continuation (maintenance) phase treatment

In all arms, after initial induction therapy, patients will receive fluconazole 800 mg/d until ART started (at 28 days +/- 4 days after start antifungal therapy), and at 28 days +/- 4 days for patients presenting on ART, then fluconazole 400 mg/d to complete 10 weeks treatment, and fluconazole 200mg/d thereafter.

See **Appendix 1** for dose adjustments in renal/hepatic insufficiency and management of expected adverse events.

5.4 Treatment Procedures

Initial intensive phase
Patients will be admitted to hospital for the initial intensive phase (first 7-14 days) of treatment, meaning drug administration can be directly observed and facilitating close clinical and laboratory monitoring.

Continuation phase
Patients will be followed in the outpatient clinic and given medication to take at home.

5.5 Drug Accountability.

Drug stocks will be regularly monitored and the remaining stocks checked against the amounts dispensed.

5.6 Measures of compliance and adherence

The initial induction treatment will be given in a directly observed hospital setting. Adherence to outpatient continuation treatment will be assessed by means of patient interview and tablet counts at follow up visits.

5.7 Non-trial treatment

Drugs not known to be contraindicated with the trial drugs will be permitted.

Medications not permitted/ precautions
Rifampicin – Increase dose of fluconazole by 50% (except for 1200 mg dose in first 2 weeks)
Warfarin – Check INR
Sulfonylurea derivatives – increased risk of hypoglycaemia, check glucose.
Fluconazole is contraindicated in combination with cisapride and the class of antihistamines including terfenadine and astemizole.

Data on concomitant medication
All non-trial treatment taken by the patient will be recorded at enrolment and follow up and in the event of an SAE occurring.

5.8 Dispensing

The drugs will be stored in the pharmacy. The trial staff will collect the amounts needed from the pharmacy. Detailed records of drugs dispensed and received will be maintained by both the pharmacist and the trial staff.

6. ASSESSMENTS AND PROCEDURES

6.1 Follow-Up Schedule

Patients will be admitted and clinical response monitored daily for the first 2 weeks. Outpatient follow up will be at 2 (if discharged before 2 weeks), 4, 6, 8 and 10 weeks after starting therapy.

6.2 Summary of Investigations during Treatment and Follow-Up

CLINICAL

Clinical response will be monitored daily for the first 2 weeks or until discharge; 2, 4, 6 and 8 weeks after discharge. Every effort will be made (for example with mobile telephone calls and financial help with travelling expenses) to obtain accurate and complete follow-up data for 10 weeks after the start of treatment. Particular attention will be paid to the possibility of immune reconstitution reactions after the patients have started ART. Follow up will be greatly helped by the fact that HIV clinics are already well-established at the sites, and all sites have trial experience.

Visual acuity by handheld LogMar chart - will be done as soon as feasible after study entry, on week 4 and week 10.

LABORATORY

Day 5, 9, 13:	FBC and Urea, creatinine, electrolytes
Day 3, 7, 11:	Urea, creatinine, electrolytes
Days 7, 13, 28:	ALT

At the time of monitoring blood tests, an extra 2.5 ml will be taken on up to 3 occasions in the first 2 weeks for drug levels.

Day 7: ECG for first 120 patients.
These paired ECGs at baseline and day 7 will then be examined for any evidence of QT prolongation with fluconazole at 1200 mg/d, and a decision made as to whether continued ECG monitoring is required.

Day 7, 14:   Follow up CSF examination
                   opening pressure,
                   quantitative fungal culture

C. neoformans isolates will be stored in TSB and 15% glycerol at minus 80°C at day 7 and 14. Additional sample storage for valuable ancillary studies depend on specific site approvals, and site-specific patient information and consent.

6.3 Procedures for assessing efficacy

Efficacy.

Survival will be recorded at 2, 4 and 10 weeks, as well as date of death or loss to follow up. Cox regression will be used to analyse mortality data. Analyses will be done on both an intention to treat and per protocol basis, and both unadjusted (primary endpoint) and adjusted for possible confounding factors including ART status at presentation.

Cryptococcal clearance rates will be calculated using a summary statistic for each patient, the rate of decrease in log CFU per ml CSF per day derived from the slope of the linear regression of log CFU against time for each patient, based on quantitative CSF cultures at baseline and on day 7 and 14. A linear regression model will be used to compare mean rates of decline or early fungicidal activity (EFA) for each treatment arm, giving summary differences with 95%CI and significance levels [14].

A full analytical plan will be drawn up prior to beginning analysis.

6.4 Procedures for assessing safety

Safety. Proportions of patients in each of the treatment arms suffering clinical and laboratory-defined side effects will be compared as well as the mean percent change from baseline of laboratory values in the treatment groups.

The frequency and severity of any immune reconstitution reactions, the factors associated with occurrence of reactions.
Logistic regression will be used to compare the proportion of patients developing pre-defined IRIS reactions in each of the arms.

Throughout this study patients will be closely monitored for signs and symptoms of drug toxicity. All toxicities leading to the study therapy being temporarily or permanently discontinued and all Grade 3 or 4 toxicity effects will require thorough investigation with relevant clinical and laboratory tests, as clinically indicated. These should be repeated as needed until final resolution or stabilization of the toxicity. All symptoms and laboratory findings will be graded according to severity using the modified Division of AIDS toxicity criteria. At the time of enrolment, if the patient already has a medical diagnosis whose signs or symptoms worsen during the study to a Grade 3 or 4, this is an adverse event that must be reported.

SAEs will be reported to International investigators as they occur.

For details of safety reporting, expected adverse events and flow chart for assessing and notifying adverse events see section 10 and Appendix 1.

6.5 Loss to follow-up

Every effort will be made (for example with mobile telephone calls and financial help with travelling expenses) to obtain accurate and complete follow-up data for 10 weeks after the start of treatment.

If a patient fails to attend, the research nurse will visit the home address and make every effort to persuade the patient to attend and continue antifungal and antiretroviral treatment.

6.6 Trial closure

The trial will be considered closed when the last patient has completed 10 weeks in the study and all follow-up and laboratory reports have been received. Early termination could occur if there is an unacceptable level of adverse events, occurring in any of the test arms.

7.   WITHDRAWAL OF PATIENTS

In consenting to the trial, patients are consenting to trial treatment, trial follow-up and data collection. If a patient wishes to withdraw from trial treatment, investigators should nevertheless explain the importance of remaining on trial follow-up, or failing this of allowing routine follow-up data to be used for trial purposes.

Patients may be withdrawn from a trial intervention for severe and intolerable adverse events, or if the patient withdraws consent. Follow-up should be continued unless the patient explicitly withdraws consent for follow-up. For patients moving within or outside the study area, every effort should be made for the patient to be followed up if at all possible.

8. STATISTICAL CONSIDERATIONS

8.1 Method of Randomisation

Patients will be randomised by site using a computer-generated programme. Details of treatment allocation are shown in **Section 5**.

8.2 Outcome Measures

Primary outcome measure

- Mortality at 2 weeks by treatment group (regimen 1 and regimen 2 vs regimen 3)

Secondary outcome measures

- Mortality at 10 weeks by treatment group, as above
- Mortality at 2, 10 weeks by treatment group (regimens [2A + 3A] vs regimens [2B + 3B]; and 2A vs 2B, 3A vs 3B)
- Mortality at 2, 4, and 10 weeks by treatment group, as above, adjusted for site and other possible confounders including ART status at presentation.
- The proportions of patients in different arms suffering clinical and laboratory-defined adverse events (grades III and IV)
- Rate of clearance of infection based on quantitative CSF cultures.

8.3 Sample Size

Using a non-inferiority design with a 10% non-inferiority margin and 5% type 1 error; and assuming 85% 2-week survival in the 2-week AmB arm (regimen 3), would require 157 patients per arm at 80% power, 184 per arm at 85% power, and 219 per arm at 90% power. 680 patients total (226 per arm) will be studied in order to achieve 90% power, allowing for 2% losses to follow-up at this early endpoint, based on prior experience, including at trial sites (Lilongwe [46])).

MRC (UK) have agreed to support the Malawi, Tanzania and Zambia sites (570 patients) and ANRS have agreed to support the Cameroon sites (110 patients).

The numbers of patients treated with fluconazole or flucytosine in the amphotericin B arms will be a minimum of 226. In all scenarios, these numbers are well in excess of the 100 per arm in the Vietnam trial. 220 patients treated with each of the companion drugs would give power of 0.80 to detect an increase in 2-week survival to 90% with the most effective adjunctive (second drug) treatment from 80% with the least effective adjunctive treatment. The trial would provide sufficient data to base policy with regard which drugs should be used with amphotericin B in Africa.

Interim Monitoring and Analyses

The Independent Data Monitoring Committee will monitor the primary endpoint on a regular basis as a measure of safety and will recommend cessation of any arm where there is evidence of inferiority. In the case of the comparison of fluconazole versus flucytosine, the inferior treatment will be terminated and the remainder of patients will receive the single treatment that is to be continued. In the case of one of the 3 strategies shown to be inferior, further enrolment to the inferior strategy arm will be terminated and new patients will be randomised to one of the two remaining strategies.

If the differences between any arms are borderline, then it is likely that Independent Data Monitoring Committee will recommend continuation of the trial as planned. However, this may result in the final analysis showing borderline non-inferiority which is difficult to interpret. This is more likely if the event rate turns out to be lower than expected. In this situation, continuation of the trial for a further short period could be justified. We therefore propose that an interim review is done when 2/3 of the planned recruitment has been done to determine the power of the trial given the findings up to that point. This interim review will be based on the primary endpoint and will be done at a stringent level (P<0.001) so as not to affect the final power of the trial. The review will be restricted to the 3-strategy comparison. The trial will not be stopped due to futility as our primary aim to show non-inferiority with tight confidence intervals.

8.4 Preliminary Analysis Plan (see also 6.3 and 6.4 above)

After data cleaning, analysis will proceed according to a pre-designed analysis plan. The primary analysis will be by both intention-to-treat and per protocol.

After the crude primary analysis has been performed, an adjusted analysis will be performed including covariates which may influence outcome.

A full analysis plan will be developed before the final analysis is conducted.

8.5 Analysis of adverse events

The primary endpoint for adverse events is the occurrence of an SAE or a Grade 4 adverse event.

9. TRIAL MONITORING

The purposes of trial monitoring are to verify that:

- The rights and well-being of human subjects are protected.
- The reported trial data are accurate, complete, and verifiable from source documents.
- The conduct of the trial is in compliance with the currently approved protocol/amendment(s), with GCP, and with the applicable regulatory requirement

9.1 Extent and nature of monitoring

The sites will be visited at regular intervals in order to monitor the conduct of the trial. These visits will be made by the Trial Monitor/Manager. Regular visits will also be made by the International Investigators and the Trial Statistician. The frequency of monitoring visits will be according to need but will be no less than every 6 months in each centre.

9.2 Site monitoring

At monitoring visits the data entered in the CRFs and database will be checked against available source data according to the procedures described in the trial monitoring plan filed in the Trial Master File. Data stored will be checked for missing or unusual values (range checks) and checked for consistency within participants over time. If any such problems are identified any data which are changed should be crossed through with a single line and initialled. Particular attention will be given to:

(a) Verifying, for the study drugs:

    (i) That storage times and conditions are acceptable, and that supplies are sufficient throughout the trial.

    (ii) That the study drugs are supplied only to subjects who are eligible to receive it and at the protocol specified dose(s).

    (iii) That subjects are provided with necessary instruction on properly taking study medication.

    (iv) That the receipt and use of study drugs at the trial sites are controlled and documented adequately.

(b) Verifying that the local investigator follows the approved protocol and all approved amendment(s), if any.

(c) Verifying that written informed consent was obtained before each subject's participation in the trial.

(d) Ensuring that the Principal Investigator has received the current Investigator's Brochure, all documents, and all trial supplies needed to conduct the trial properly and to comply with the applicable regulatory requirement(s).

(e) Ensuring that the local investigator and the investigator's trial staff are adequately informed about the trial.

(f) Verifying that the local investigator and the investigator's trial staff are performing the specified trial functions, in accordance with the protocol and any other written agreement

between the sponsor and the local investigator/institution, and have not delegated these functions to unauthorized individuals.

(g) Verifying that the local investigator is enrolling only eligible subjects.

(h) Reporting the subject recruitment rate.

(i) Verifying that source documents and other trial records are accurate, complete, kept up-to-date and maintained.

(j) Verifying that the local investigator provides all the required reports, notifications, applications, and submissions, and that these documents are accurate, complete, timely, legible, dated, and identify the trial.

(k) Checking the accuracy and completeness of the CRF entries, source documents and other trial-related records against each other. In particular:

    (1) The data required by the protocol are reported accurately on the CRFs and are consistent with the source documents.

    (2) Any dose and/or therapy modifications are well documented for each of the trial subjects.

    (3) Adverse events, concomitant medications and intercurrent illnesses are reported in accordance with the protocol on the CRFs.

    (4) Visits that the subjects fail to make, tests that are not conducted, and examinations that are not performed are clearly reported as such on the CRFs.

    (5) All withdrawals and dropouts of enrolled subjects from the trial are reported and explained on the CRFs.

    (6) Informing the local investigator of any CRF entry error, omission, or illegibility. Any corrections, additions, or deletions made, are dated, explained (if necessary), and initialled by the local investigator or by a member of the investigator's trial staff who is authorized to initial CRF changes for the investigator. This authorization should be documented.

    (7) Determining whether all adverse events (AEs) are appropriately reported within the time periods required by GCP, the protocol, the IRB/IEC, the sponsor, and the applicable regulatory requirement(s).

    (8) Determining whether the local investigator is maintaining the essential documents

    (9) Communicating deviations from the protocol, SOPs, GCP, and the applicable regulatory requirements to the local investigator and taking appropriate action designed to prevent recurrence of the detected deviations.

9.3 Direct Access to Data

The investigator will permit trial-related monitoring, audits, ethics committee review and regulatory inspections by providing direct access to source data/documents.

9.4 Confidentiality

All patient information will be kept in locked cabinets and will be available only to the treatment staff.

The patient's name and address will not be disclosed to the trial sponsor.
The patient's data/specimens will be identified by trial number and/or initials only. Individual patients will not be identified in the resulting publications and presentations from the trial. The trial will comply with the principles of the Data Protection Act of the country of the participating centre.

10. SAFETY REPORTING

10.1 Safety Reporting

Terms and definitions for adverse events

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a subject to whom a medicinal product has been administered including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	Any untoward and unintended response in a subject to an investigational medicinal product, which is related to any dose administered to that subject.
Unexpected Adverse Reaction (UAR)	An adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in question set out in: • The SPC for that product (for products with a marketing authorisation) • The Investigator's Brochure (IB) relating to the trial in question (for any other investigational product)
Serious Adverse Event (SAE) **Serious Adverse Reaction (SAR)** **Suspected Unexpected Serious Adverse Reaction (SUSAR)**	Respectively, any adverse event, adverse reaction or unexpected adverse reaction that: • results in death • is life-threatening* • requires hospitalisation or prolongation of existing hospitalisation** • results in persistent or significant disability or incapacity • consists of a congenital anomaly or birth defect • other important medical event(s)***

* The term 'life-threatening' in the definition of 'serious' refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

** Hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation. Hospitalisations for a pre-existing condition, including elective procedures that have not worsened, do not constitute an SAE.

*** Other events that may not result in death, are not life threatening, or do not require hospitalisation may be considered a serious adverse experience when, based upon appropriate medical judgement, the event may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed above (excluding new cancers or result of overdose).

Study site Responsibilities

All SAEs and grade III and IV AE must be reported within 48 hours by the Local Investigator by e-mail to the International Investigators. All other adverse events should be reported on the regular progress/follow-up reports in the CRF. The investigator should assess the SAE for the likelihood that that it is a response to a study drug.

Follow-up of SAEs: In the case of an SAE the subject must be followed-up until clinical recovery is complete and laboratory results have returned to normal, or until the event has stabilised. Follow-up may continue after completion of protocol treatment if necessary. Follow-up information is noted on another SAE form by ticking the box marked 'follow-up' and emailing to the international investigators as information becomes available. Extra, annotated information and/or copies of test results may be provided separately. The patient **must** be identified by trial number, date of birth and initials only. The patient's name **should not** be used on any correspondence.

- SUSARs which are fatal or life-threatening must be reported to the appropriate regulatory bodies and if required to the local ethics committee not later than 7 days after the investigators are first aware of the reaction. Any additional relevant information must be reported within a further 8 days.
- SUSARs that are not fatal or life-threatening must be reported to the appropriate regulatory bodies and if required to the local ethics committee within 15 days of the PI first becoming aware of the reaction.

The International Investigators (or a delegate) will evaluate all SAEs received for seriousness, expectedness and causality. Investigator reports of suspected SARs will be reviewed immediately and those that are SUSARs identified and reported to regulatory authorities. The causality assessment given by the local investigator cannot be overruled and in the case of disagreement, both opinions will be provided in subsequent reports.

Adverse events – Guidelines on inclusions and exclusions

Adverse events include	Adverse events do not include
a) an exacerbation of a pre-existing illness	a) medical or surgical procedures- the condition which leads to the procedure is the adverse event
b) an increase in frequency or intensity of a pre-existing episodic event/condition	
	b) pre-existing disease or conditions present before treatment that do not worsen
c) a condition (even though it may have been present prior to the start of the trial) detected after trial drug administration	c) situations where an untoward medical occurrence has occurred e.g. cosmetic elective surgery
d) continuous persistent disease or symptoms present at baseline that worsens following the administration of the study/trial treatment	d) overdose of medication without signs or symptoms
e) Any abnormal clinically significant event which occurs after trial drug administration	e) the disease being treated or associated symptoms/signs unless more severe than expected for the patient's condition

10.2 Severity/grading of adverse events

This will be according to the modified DAIDS Classification filed in the Trial Master File.

10.3 Relationship to trial treatment

When reporting on serious adverse events, the trial investigator will state whether they believe that the event is causally associated with any of the trial treatments and the strength of the causal relationship. They will also state whether the adverse event was expected and what if any action was taken.

CLASSIFICATION OF ADVERSE EVENTS BY RELATIONSHIP TO STUDY MEDICATION	
UNRELATED:	This category applies to those AEs that are clearly and incontrovertibly due to extraneous causes (disease, environment, etc.).
UNLIKELY:	This category applies to those AEs that are judged to be unrelated to the test drug, but for which no extraneous cause may be found. An AE may be considered unlikely to be related to study medication if or when it meets 2 of the following criteria: (1) it does not follow a reasonable temporal sequence from administration of the test drug; (2) it could readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject; (3) it does not follow a known pattern of response to the test drug; or (4) it does not reappear or worsen when the drug is re-administered.
POSSIBLY:	This category applies to those AEs for which a connection with the test drug administration appears unlikely but cannot be ruled out with certainty. An AE may be considered possibly related if or when it meets 2 of the following criteria: (1) it follows a reasonable temporal sequence from administration of the drug; (2) it could not readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject; or (3) it follows a known pattern of response to the test drug.
PROBABLY:	This category applies to those AEs that the investigator feels with a high degree of certainty are related to the test drug. An AE may be considered probably related if or when it meets 3 of the following criteria: (1) it follows a reasonable temporal sequence from administration of the drug; (2) it could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject; (3) it disappears or decreases on cessation or reduction in dose (note that there are exceptions when an AE does not disappear upon discontinuation of the drug, yet drug-relatedness clearly exists; for example, as in bone marrow depression, fixed drug eruptions, or tardive dyskinesia); or (4) it follows a known pattern of response to the test drug.
DEFINITELY:	This category applies to those AEs that the investigator feels are incontrovertibly related to test drug. An AE may be assigned an attribution of definitely related if or when it meets all of the following criteria: (1) it follows a reasonable temporal sequence from administration of the drug; (2) it could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject; (3) it disappears or decreases on cessation or reduction in dose and recurs with re-exposure to drug (if rechallenge occurs); and (4) it follows a known pattern of response to the test drug.

10.4 Follow-up after adverse events

Patients may be either admitted to hospital or seen at intervals to monitor the progress, recovery and investigations of the adverse events. In the event treatment needs to be modified or changed, the Local Investigator should inform the International Investigators and agree on the new treatment.

11. ETHICAL CONSIDERATIONS AND APPROVAL

11.1 Ethical considerations

The patients will, before being enrolled into the study, have the conditions of the study, as set out in the Patient Information Sheet explained to them. The information contained in the PIS will be translated into the local dialect. Literate patients will be asked to read the PIS and the illiterate patients will have the contents explained to them by the Local Investigator or Research Nurse. The patient will have the opportunity to discuss the PIS. Once the person taking consent is satisfied that the patient has understood the PIS and the consent form, the patient will be asked to sign the consent form. The top copy should be filed in the patient's study folder and the duplicate, together with a copy of the PIS, given to the patient.

The right of the patient to refuse to participate in the trial without giving reasons will be respected.

After the patient has entered the trial, the clinician will remain free to give alternative treatment to that specified in the protocol, at any stage, if he/she feels it to be in the best interest of the patient. However, the reason for doing so should be recorded and the patient will remain within the trial for the purpose of follow-up and data analysis according to the treatment option to which they have been allocated. Similarly, the patient will remain free to withdraw at any time from the protocol treatment and trial follow-up without giving reasons and without prejudicing his/her further treatment.

11.2 Ethical approval

The protocol will be submitted to the Ethics Review Committee (ERC) of the London School of Hygiene and Tropical Medicine. The protocol will also be submitted to the Medical Ethics Committee of each of the participating clinical site and/or country and enrolment to the study will start only after receiving the written agreement of the relevant body(ies).

The trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki from the World Medical Association.

http://www.wma.net/e/ethicsunit/helsinki.htm

12. REGULATORY APPROVAL

Regulatory approval will be obtained, as required, in the countries of the study sites.

13. INDEMNITY

All personnel involved in the trial will be expected to be indemnified by their employing authority. Patients will be indemnified, for non- negligent harm, through a separate policy taken out by St George's University of London.

The ANRS, which is sponsoring this study in Cameroon, has taken out public liability insurance for Cameroon.

14. FINANCE

Funding has been awarded the MRC (UK), and from the ANRS (France).

15. TRIAL COMMITTEES

15.1 Trial Management Group (TMG)

A Trial Management Group (TMG) will be formed comprising the international and local Investigators, trial manager and statistician. The TMG will be responsible for the day-to-day running and management of the trial and will liaise at regular intervals.

15.2 Trial Steering Committee (TSC)

A Trial Steering Committee (TSC) will be constituted. The Chairman will be independent of the running of the trial.

A committee with an independent Chairman will be formed.
Their terms of reference will be:

1. to monitor and supervise the progress of the trial towards its interim and overall objectives;
2. to review at regular intervals relevant information from other sources (e.g. other related trials);
3. to consider the recommendations of the Independent Data Monitoring Committee;

The role of the TSC is to provide overall supervision for the trial and provide advice through its Independent Chairman. The ultimate decision for the continuation of the trial lies with the TSC.

15.3 Independent Data Monitoring Committee (IDMC)

There will be an Independent Data Monitoring Committee whose terms of reference will be as follows:

1. To review safety data, in particular all serious adverse events possibly attributable to the trial drugs, such as local reactions or unexpected deaths.

2. To monitor the conduct of the trial with respect to the ethical aspects of the trial.

3. To assess the results of any interim analyses with the possibility of advising the Trial Steering Committee (TSC) that the trial should be modified or discontinued.

16. PUBLICATION

The results from different centres will be analysed together and published as soon as possible. Individual Clinicians must not publish data concerning their patients that are directly relevant to questions posed by the study until the Trial Management Group has published its report.

The Trial Management Group will form the basis of the Writing Committee and will advise on the nature of publications. The names of all the investigators will be included in any publication in the authorship. Any authorship policy will be agreed by all the investigators before any publication. The members of the TSC and IDMC will be listed with their affiliations in the Acknowledgements/Appendix of the main publication.

17. PROTOCOL AMENDMENTS

Version 1.0: 29 Dec 2011

Version 1.1: 15 March 2012

1.   Cover page, Authorised by: Protocol signed again to revise date of signature for Prof. Tom Harrison corrected from '29 December 2011' to '15 March 2012'

2.   General Information, Page 2: Sponsorship has been changed from 'London School of Hygiene and Tropical Medicine, London, United Kingdom' to 'St George's University of London, Cranmer Terrace, London, SW17 ORE, United Kingdom'.

3.   General Information, Page 2: Details of co-sponsorship between St George's University of London and ANRS for the Cameroon site, Yaoundé, have been included.

3.   Treatment Regimes with Fluconozole 1200mg od has been replaced by Fluconozole 600 bd throughout

4.   Requirement for a 14 day Lumbar Puncture (LP) has been included consistently throughout

5.   Pages 9 and 27: Additional power calculation for the secondary aim of comparing fluconazole and flucytosine as second drugs to give with amphotericin B, reflective of addition funding from ANRS, has been included: "220 patients treated with each of the companion drugs would give power of 0.80 to detect an increase in 2-week survival to 90% with the most effective adjunctive (second drug) treatment from 80% with the least effective adjunctive treatment."

6.   Appendix 1, Pages 42-45 : Patient information sheet: separate paragraphs have been added to clarify use of samples for further research and of blood samples for genetic analysis, and the consent form has been modified (Appendix 2, Pages 46-47) so that patients may or may not give separate consent for anonymised samples to be used for further research.

Version 1.2: 9th May 2012

1. Cover page, Authorised by: Protocol signed again to revise date of signature for Prof. Tom Harrison corrected from '15 March 2012' to '5 May 2012'. ANRS Sponsor signature and data added. Title has also been updated.
2. Cover page, Agence National de Recherche sur le SIDA et les hepatites virales (ANRS) added as co-sponsors for the Cameroonian site on title page.
3. Page 2, Contact details for St George's University of London and ANRS added. Acronym also added (ACTA).
4. Page 3, Sponsor representatives added
5. Page 7, Description of study design modified to emphasise 3 strategy structure.
6. Pages 8, 18 & 21, Fluconazole administration changes from Fluconazole 600mg bd to Fluconazole 1200mg/day.
7. Page 8, 10, 22, 24, Start of ART therapy changed from 2-4 weeks to 28 days +/- 4 days after start of antifungal treatment.
8. Page 9, Sample size and funding, definitive sample size, 680 patients with MRC (UK) and ANRS funding clarified.
9. Page 10, Flow diagram, details of strategy 2 detailed further.
10. Page 18, Study sites, Details of four definitive trial sites outlined.
11. Page 20, Patient exclusion criteria, ALT>5 times upper limit of normal or PMNs<500x10^6/L , Creat >2.5 mg/dl (225 µmol/L), or Plts<50,000x10^6/L removed from exclusion criteria on study enrolment.
12. Page 20, Number and source of patients, definitive total of 680 patients defined.
13. Page 20, Patients with ALT>5 times upper limit of normal or PMNs<500x10^6/L, or Plts<50,000x10^6/L. to be excluded from study.
14. Page 21, Enrolment and Randomisation, patients will be randomised by site only and not by altered mental status.
15. Page 23, Source of drugs, added in Sigmapharm as a manufacturer of flucytosine.
16. Page 23, Management of patients with Creat >2.5 mg/dl (225 µmol/L) defined.
17. Page 26, ALT will also be taken on Day 28. Visual acuity tested at enrolment and week 4.
18. Page 29, Sample Size, Final sample size defined.
19. Page 30, Interim monitoring and analyses, section rewritten by Prof Shabbar Jaffar.
20. Page 37, Indemnity, Clarification of ANRS indemnity clarified.
21. Page 45, Appendix 1, This has been updated so that it is more easily understandable using readability scores. A paragraph detailing the reimbursement given for transport to follow-up visits has also been included.
22. Page 49, Appendix 2, The title has been updated.
23. Page 51, Appendix 3, Management of Creat>2.5 mg/dl (225 µmol/L) outlined.

Version 1.3: 4th Sept 2012

1. Cover page, Authorised by: Protocol signed again to revise date of signature for Prof. Tom Harrison corrected from '5 May 2012' to '4 Sept 2012'.
2. Cover page, ANRS reference number and ISRCTN number updated correctly.
3. Page 2, Email and telephone details added for Prof Tom Harrison.
4. Members of study consortium updated and Duoala address added.
5. Page 23, Source of drugs, Fluconazole from Cipla added.
6. Page 37, Indemnity, ANRS indemnity clarified.
7. Page 18, Selection of centres, General Hospital, Douala added.
8. Page 34, Adverse events, Any abnormal clinically significant event which occurs after trial drug administration added to table

Version 1.4: 1st June 2013

1. Page 2, Members of study consortium updated and Duoala address added
2. Page 8, Disease/Patient studied, ' confirmed by CSF culture' removed to be consistent.
3. Page 8, Trial Interventions. Page 10, Flow diagram. Page 22 Antifungal drugs. Page 24 Fluconazole; clarification made for when to decrease fluconazole dose for patients on ARVs as they will no longer be excluded.
4. Page 9, Data recording, 'access- data base' removed.
5. Page 9, Ancillary studies, clarification added that 'transfer of material out of country will be subject to site specific material transfer agreements'.
6. Page 20, Inclusion/Exclusion criteria: >1 dose of FLU/AmB within 2 weeks of enrolment included here. ARV naïve removed.
7. Page 22, Laboratory, Viral load removed.
8. Page 22, Page 24, 600mg bd FLU changed to 1200mg/d for consistency.
. Page 26, Visual acuity, Snellen changed to LogMar and acuity test at week 10 added. At baseline acuity tested as close to baseline as feasible.
10. Page 26, Laboratory, cell count, differential and glucose at Day 7 and 14 removed.
11. Page 29, Section 8.1, randomisation by abnormal mental status removed and randomisation by site added
12. Page 29 and Page 8. Outcome measures, updated to reflect ARV patient inclusion ie '...confounders including ART status on presentation'.
13. Page 32, Formatting updating from section K.
14. Page 52, Renal impairment, creatinine changed from 220 to 225 for consistency.
15. Page 52, Renal impairment, magnesium added as well as potassium for supplementation for hypokalaemia.

Version 1.4_Blantyre: 1st June 2013

1) Amendment of **Section 1.4 Ancillary studies/sub-studies p.9 Protocol**
This section now reads:
"PBMC will be saved for later host genetic studies of susceptibility to cryptococcal infection at other collaborating sites but not from the participants recruited in Malawi."
2) Removal of **Will any genetic tests be done?p48 Appendix 1 Patient Information Sheet**
"Yes. Some blood will be used to look at host genetics and the expression of genes that are linked to the immune response to infection. This will help us to understand meningitis infection better."
This section has been removed.
3) Amendment of **Who has reviewed the study?p49 Appendix 1 Patient Information Sheet**
This section now reads:
"This study has been reviewed and approved by the Local Research Ethics Committees at the University of Malawi College of Medicine, Blantyre, and National Health Sciences Research Committee, Lilongwe, Malawi; University Teaching Hospital, Lusaka, Zambia; Central Hospital, Yaoundé, Cameroon; at The London School of Hygiene and Tropical Medicine; The Pasteur Institute, France.

Version 1.4.1_Blantyre: 27th June 2014

1. Page 20, Inclusion/exclusion criteria: Received > 1 cryptococcal treatment dose (up to 1200 mg) of fluconazole or > 7 days low dose (200 mg) fluconazole within 2 weeks of screening added.

1. Header/Footer: ACTA logo added, version number updated to V2.0.
2. Page 1, Version number updated to 2.0 and date updated to 5th May 2015.
3. Page 1, Tanzania added to sites sponsored by SGUL.
4. Page 2, Tanzania added to sites sponsored by SGUL. Sponsor contact name and address added. SGUL address updated. Page 1/2, Inserm-ANRS abbreviation corrected.
5. Page 3, Mary Pierse and Newton Kalata added to members of consortium in Blantyre (Kate Gaskell, Camilla Rothe and Theresa Allain removed). Aggrey Mweeba added in Lusaka (Aaron Mujujati removed). Consortium members for a new sites in Tanzania and Zomba also added. Trial manager address updated, SGUL sponsor representative updated to Debbie Rolfe and Inserm-ANRS sponsor representative updated to Paula Garcia. Page 6, TSB added to abbreviations list.
6. Page 9, Sample size and funding: Tanzania added as country supported by MRC.
7. Page 9/10, Section 1.4, Details for semi-quantitative CrAg evaluation ancillary study added for conduct in Lilongwe and Cameroon (retrospectively only in Lusaka).
8. Page 10, details of HIV RNA sequencing ancillary study added (Lilongwe only). Tanzania included as site where PBMCs will NOT be saved for genetic analysis. FLURES study in Tanzania noted.
9. Page 20, Section 2.5, Centres in Dar Es Salaam and Zomba included with recruitment up to 150 patients and expected numbers to be recruited in Blantyre up to 300.
10. Page 21, Section 3.2, Patient exclusion criteria updated to clarify the fluconazole treatment dose required for exclusion: 'Received >1 dose of Fluconazole therapy within 2 weeks of treatment' changed to 'Received > 1 cryptococcal treatment dose (up to 1200 mg) of fluconazole or > 7 days low dose (200 mg) fluconazole within 2 weeks of screening'.
11. Page 23, Section 5.1 (ii), and Page 26, Section 6.2, ECG collection updated from 60 to 120 patients (40 per regimen) following TMG investigator meeting in March 2014.
12. Page 23, Section 5.1, Viral loads to be taken from ART experienced patients only, at sites where resources available. Magnesium levels taken in Lusaka. Semi quantitative CrAg test at baseline in Lilongwe and Cameroon.
13. Page 23, Section 5.1, sentence added noting that sample saves at baseline will be dependant on site specific approvals and PIS/Consent forms at each site.
14. Page 24, Section 5.2, Source of drugs updated to clarify fluconazole manufacturer may be sourced from Pfizer or a generic WHO qualified manufacturer.
15. Page 31, Sample size, Tanzania site added as site supported by MRC.

Appendices: PIS and Consent forms removed as appendices. These are site specific and will be submitted as appropriate with the protocol for each site. Appendices 3 and 4 renamed as 1 and 2.

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19. APPENDICES

Appendix 1: Management of expected adverse events and dosage adjustment

Amphotericin B:

Infusion related side effects – amphotericin B:

1. Increase infusion duration to 6 hours.

2. Premedication with paracetamol 500 mg or chlorpheniramine 4 mg.

3. In case of severe infusion related reaction: pethidine 25 mg iv. Slow intravenous injection.

Renal impairment:

Conversion factor mg/dl to micromol/L = 88.4

If creatinine rises up to 2.5 mg/dl (225 µmol/l):

1. Miss one dose. Check adequate hydration. Check creatinine next morning:

- if stable or improving and creat < 2.5 mg/dl: restart daily dosing (1 mg/kg) paying close attention adequate hydration

- if stable or improving, but still above 2.5 mg/dl: institute alternate day dosing (1 mg/kg q 48 hours)

- if creatinine is increasing do not give amphotericin B and check again after 24 hours: if stable or improving institute daily or alternate day dosing as above

- if still increasing: stop amphotericin B and switch to fluconazole (1200 mg for first 2 weeks of antifungal therapy) adjusting its dose for renal impairment.

AVOID other nephrotoxic agents such as aminoglycosides, NSAIDS if possible.

Hypokalemia:

Supplement potassium and magnesium as required

Flucytosine:

Renal insufficiency

Creatinine clearance ml/min	Individual dose mg/kg	Dose interval hour
>40	25	6
40-20	25	12
20-10	25	24
<10	25	>24

Clearance:

$$\frac{(140\text{- age}) \times \text{weight (kg)}}{72 \times \text{serum creat (mg/dl)}} \quad (\times 0,85 \text{ for women}) \quad = \text{ml/min}$$

Bone marrow toxicity:
Platelets $< 50,000 \times 10^6$ /L or neutrophils $< 500 \times 10^6$ /L are early withdrawal criteria.

If during therapy

Grade III fall in platelets or neutrophils (Platelets $< 50,000 \times 10^6$ /L or neutrophils $< 750 \times 10^6$ /L)
Then take blood for drug level and monitor daily. If grade III is confirmed next day, halve dose (50%); if grade IV levels develop, stop flucytosine, until level III, then 50% dose

Grade IV fall in platelets or neutrophils (Platelets $< 25,000 \times 10^6$ /L or neutrophils $< 500 \times 10^6$ /L)
Then take blood for drug level and stop flucytosine, until grade III level at which point resume at 50% dose

Fluconazole:

Renal insufficiency
    If renal function decreases
    - to 20-50 ml/min then reduce the dose by 50%.
    - to < 20 ml/min reduce dose by 25 %.

Liver function abnormality: (Perfect *et al.*, 1992)
    ALT (SGPT) 5 x upper limit (= 200) is an early withdrawal criterion
    If during therapy
    - ALT (SGPT) from normal to 5 x upper limit or
    - from abnormal baseline increases by 150
    Then, if possible, stop fluconazole and switch to amphotericin B.

Skin reactions:
    Stop fluconazole in case of bullous lesions or erythema multiforme.

If gastro-intestinal side effects (like nausea and vomiting):
    Divide dosage into smaller more frequent dosage schedule.

Drug Interactions
Fluconazole may increase levels of phenytoin, warfarin and sulfonylurea derivatives.
If concomitant use of warfarin: check INR.
If concomitant use of sulfonylurea derivatives there is risk of hypoglycaemia, so check glucose levels more often.

Rifampicin will reduce the levels of fluconazole if the patient has been on it for >_2weeks. Increase the dose of fluconazole by 50%, in consolidation and maintenance phases.

Fluconazole is contraindicated in combination with cisapride and the new drug class of antihistamines such as terfenadine and astemizole. The independent data safety monitoring committee will sit monthly to discuss and review adverse effects

Appendix 2: Management of Raised CSF Opening Pressure

Measurement of CSF opening pressure should be performed at each lumbar puncture. Patients with markedly raised initial opening pressure (>30 cm water) will have CSF drained (20-30 ml) at the time of lumbar puncture aiming to reduce the opening pressure to $\leq$ 20 cm and a repeat lumbar puncture the following day to help control opening pressure, consistent with current recommendations [1-3]. In addition, patients deteriorating clinically with symptoms or signs suggestive of raised intracranial pressure such as increasing headache, reduced conscious level, hypertension, or cranial neuropathies, will have repeat lumbar puncture to measure the opening pressure and, if >30 cm water, follow up lumbar punctures as above. CSF from repeat lumbar punctures will have quantitative culture done. If available, CT or MRI head scan should be done to exclude hydrocephalus and significant focal lesions in patients requiring CSF drainage and repeat LP.

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ACTA Protocol: Summary of changes

Below is a full list of ACTA Protocol track changes from V1.0 to V2.0. When sites opened for recruitment V1.2 was in place.

Version 1.0: 29 Dec 2011

Version 1.1: 15 March 2012

1. Cover page, Authorised by: Protocol signed again to revise date of signature for Prof. Tom Harrison corrected from '29 December 2011' to '15 March 2012'

2. General Information, Page 2: Sponsorship has been changed from 'London School of Hygiene and Tropical Medicine, London, United Kingdom' to 'St George's University of London, Cranmer Terrace, London, SW17 ORE, United Kingdom'.

3. General Information, Page 2: Details of co-sponsorship between St George's University of London and ANRS for the Cameroon site, Yaoundé, have been included.

3. Treatment Regimes with Fluconozole 1200mg od has been replaced by Fluconozole 600 bd throughout

4. Requirement for a 14 day Lumbar Puncture (LP) has been included consistently throughout

5. Pages 9 and 27: Additional power calculation for the secondary aim of comparing fluconazole and flucytosine as second drugs to give with amphotericin B, reflective of addition funding from ANRS, has been included: "220 patients treated with each of the companion drugs would give power of 0.80 to detect an increase in 2-week survival to 90% with the most effective adjunctive (second drug) treatment from 80% with the least effective adjunctive treatment."

6. Appendix 1, Pages 42-45 : Patient information sheet: separate paragraphs have been added to clarify use of samples for further research and of blood samples for genetic analysis, and the consent form has been modified (Appendix 2, Pages 46-47) so that patients may or may not give separate consent for anonymised samples to be used for further research.

Version 1.2: 9th May 2012

1. Cover page, Authorised by: Protocol signed again to revise date of signature for Prof. Tom Harrison corrected from '15 March 2012' to '5 May 2012'. ANRS Sponsor signature and data added. Title has also been updated.
2. Cover page, Agence National de Recherche sur le SIDA et les hepatites virales (ANRS) added as co-sponsors for the Cameroonian site on title page.
3. Page 2, Contact details for St George's University of London and ANRS added. Acronym also added (ACTA).
4. Page 3, Sponsor representatives added

5. Page 7, Description of study design modified to emphasise 3 strategy structure.
6. Pages 8, 18 & 21, Fluconazole administration changes from Fluconazole 600mg bd to Fluconazole 1200mg/day.
7. Page 8, 10, 22, 24, Start of ART therapy changed from 2-4 weeks to 28 days +/- 4 days after start of antifungal treatment.
8. Page 9, Sample size and funding, definitive sample size, 680 patients with MRC (UK) and ANRS funding clarified.
9. Page 10, Flow diagram, details of strategy 2 detailed further.
10. Page 18, Study sites, Details of four definitive trial sites outlined.
11. Page 20, Patient exclusion criteria, ALT>5 times upper limit of normal or PMNs<500x10^6/L , Creat >2.5 mg/dl (225 µmol/L), or Plts<50,000x10^6/L removed from exclusion criteria on study enrolment.
12. Page 20, Number and source of patients, definitive total of 680 patients defined.
13. Page 20, Patients with ALT>5 times upper limit of normal or PMNs<500x10^6/L, or Plts<50,000x10^6/L. to be excluded from study.
14. Page 21, Enrolment and Randomisation, patients will be randomised by site only and not by altered mental status.
15. Page 23, Source of drugs, added in Sigmapharm as a manufacturer of flucytosine.
16. Page 23, Management of patients with Creat >2.5 mg/dl (225 µmol/L) defined.
17. Page 26, ALT will also be taken on Day 28. Visual acuity tested at enrolment and week 4.
18. Page 29, Sample Size, Final sample size defined.
19. Page 30, Interim monitoring and analyses, section rewritten by Prof Shabbar Jaffar.
20. Page 37, Indemnity, Clarification of ANRS indemnity clarified.
21. Page 45, Appendix 1, This has been updated so that it is more easily understood using readability scores. A paragraph detailing the reimbursement given for transport to follow-up visits has also been included.
22. Page 49, Appendix 2, The title has been updated.
23. Page 51, Appendix 3, Management of Creat>2.5 mg/dl (225 µmol/L) outlined.

Version 1.3: 4th Sept 2012

1. Cover page, Authorised by: Protocol signed again to revise date of signature for Prof. Tom Harrison corrected from '5 May 2012' to '4 Sept 2012'.
2. Cover page, ANRS reference number and ISRCTN number updated correctly.
3. Page 2, Email and telephone details added for Prof Tom Harrison.
4. Members of study consortium updated and Duoala address added.
5. Page 23, Source of drugs, Fluconazole from Cipla added.
6. Page 37, Indemnity, ANRS indemnity clarified.
7. Page 18, Selection of centres, General Hospital, Douala added.
8. Page 34, Adverse events, Any abnormal clinically significant event which occurs after trial drug administration added to table

Version 1.4: 1st June 2013

1. Page 2, Members of study consortium updated and Duoala address added
2. Page 8, Disease/Patient studied, 'confirmed by CSF culture' removed to be consistent.
3. Page 8, Trial Interventions. Page 10, Flow diagram. Page 22 Antifungal drugs. Page 24

Fluconazole; clarification made for when to decrease fluconazole dose for patients on ARVs as they will no longer be excluded.

4. Page 9, Data recording, 'access- data base' removed.

5. Page 9, Ancillary studies, clarification added that 'transfer of material out of country will be subject to site specific material transfer agreements'.

6. Page 20, Inclusion/Exclusion criteria: >1 dose of FLU/AmB within 2 weeks of enrolment included here. ARV naïve removed.

7. Page 22, Laboratory, Viral load removed.

8. Page 22, Page 24, 600mg bd FLU changed to 1200mg/d for consistency.

. Page 26, Visual acuity, Snellen changed to LogMar and acuity test at week 10 added. At baseline acuity tested as close to baseline as feasible.

10. Page 26, Laboratory, cell count, differential and glucose at Day 7 and 14 removed.

11. Page 29, Section 8.1, randomisation by abnormal mental status removed and randomisation by site added

12. Page 29 and Page 8. Outcome measures, updated to reflect ARV patient inclusion ie '...confounders including ART status on presentation'.

13. Page 32, Formatting updating from section K.

14. Page 52, Renal impairment, creatinine changed from 220 to 225 for consistency.

15. Page 52, Renal impairment, magnesium added as well as potassium for supplementation for hypokalaemia.

Version 1.4_Blantyre: 1st June 2013

1. Amendment of Section 1.4 Ancillary studies/sub-studies p.9 Protocol
This section now reads:
"PBMC will be saved for later host genetic studies of susceptibility to cryptococcal infection at other collaborating sites but not from the participants recruited in Malawi."

2. Removal of Will any genetic tests be done?p48 Appendix 1 Patient Information Sheet "Yes. Some blood will be used to look at host genetics and the expression of genes that are linked to the immune response to infection. This will help us to understand meningitis infection better." This section has been removed.

3. Amendment of Who has reviewed the study?p49 Appendix 1 Patient Information Sheet. This section now reads:
"This study has been reviewed and approved by the Local Research Ethics Committees at the University of Malawi College of Medicine, Blantyre, and National Health Sciences Research Committee, Lilongwe, Malawi; University Teaching Hospital, Lusaka, Zambia; Central Hospital, Yaoundé, Cameroon; at The London School of Hygiene and Tropical Medicine; The Pasteur Institute, France."

Version 1.4.1_Blantyre: 27th June 2014

1. Page 20, Inclusion/exclusion criteria: Received > 1 cryptococcal treatment dose (up to 1200 mg) of fluconazole or > 7 days low dose (200 mg) fluconazole within 2 weeks of screening added.

Version 2.0: 5th May 2015

1. Header/Footer: ACTA logo added, version number updated to V2.0.
2. Page 1, Version number updated to 2.0 and date updated to 5th May 2015.
3. Page 1, Tanzania added to sites sponsored by SGUL.

4. Page 2, Tanzania added to sites sponsored by SGUL. Sponsor contact name and address added. SGUL address updated. Page 1/2, Inserm-ANRS abbreviation corrected.
5. Page 3, Mary Pierse and Newton Kalata added to members of consortium in Blantyre (Kate Gaskell, Camilla Rothe and Theresa Allain removed). Aggrey Mweeba added in Lusaka (Aaron Mujujati removed). Consortium members for a new sites in Tanzania and Zomba also added. Trial manager address updated, SGUL sponsor representative updated to Debbie Rolfe and Inserm-ANRS sponsor representative updated to Paula Garcia. Page 6, TSB added to abbreviations list.
6. Page 9, Sample size and funding: Tanzania added as country supported by MRC.
7. Page 9/10, Section 1.4, Details for semi-quantitative CrAg evaluation ancillary study added for conduct in Lilongwe and Cameroon (retrospectively only in Lusaka).
8. Page 10, details of HIV RNA sequencing ancillary study added (Lilongwe only). Tanzania included as site where PBMCs will NOT be saved for genetic analysis. FLURES study in Tanzania noted.
9. Page 20, Section 2.5, Centres in Dar Es Salaam and Zomba included with recruitment up to 150 patients and expected numbers to be recruited in Blantyre up to 300.
10. Page 21, Section 3.2, Patient exclusion criteria updated to clarify the fluconazole treatment dose required for exclusion: 'Received >1 dose of Fluconazole therapy within 2 weeks of treatment' changed to 'Received > 1 cryptococcal treatment dose (up to 1200 mg) of fluconazole or > 7 days low dose (200 mg) fluconazole within 2 weeks of screening'.
11. Page 23, Section 5.1 (ii), and Page 26, Section 6.2, ECG collection updated from 60 to 120 patients (40 per regimen) following TMG investigator meeting in March 2014.
12. Page 23, Section 5.1, Viral loads to be taken from ART experienced patients only, at sites where resources available. Magnesium levels taken in Lusaka. Semi quantitative CrAg test at baseline in Lilongwe and Cameroon.
13. Page 23, Section 5.1, sentence added noting that sample saves at baseline will be dependant on site specific approvals and PIS/Consent forms at each site.
14. Page 24, Section 5.2, Source of drugs updated to clarify fluconazole manufacturer may be sourced from Pfizer or a generic WHO qualified manufacturer.
15. Page 31, Sample size, Tanzania site added as site supported by MRC.

Appendices: PIS and Consent forms removed as appendices. These are site specific and will be submitted as appropriate with the protocol for each site. Appendices 3 and 4 renamed as 1 and 2.

A PHASE III, RANDOMISED, CONTROLLED TRIAL FOR THE TREATMENT OF HIV-ASSOCIATED CRYPTOCOCCAL MENINGITIS: ORAL FLUCONAZOLE PLUS FLUCYTOSINE OR ONE WEEK AMPHOTERICIN B-BASED THERAPY VS TWO WEEKS AMPHOTERICIN B-BASED THERAPY

STATISTICAL ANALYSIS PLAN

ISRCTN registration number: ISRCTN45035509

ANRS Reference: ANRS12275

Chief Investigator: Prof Tom Harrison

Trial statisticians: Prof Duolao Wang, Prof Shabbar Jaffar

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ABBREVIATIONS

Abbreviation	Explanation
AE	Adverse Event
Ag	Antigen
AIDS	Acquired Immune Deficiency Syndrome
AmB	Amphotericin B
AR	Adverse reaction
ART/ARV	Anti-retroviral therapy/ Anti-retroviral
HIV	Human Immunodeficiency Virus
ITT	Intent-to-treat
IDMC	Independent Data Monitoring Committee
IRIS	Immune Reconstitution Inflammatory Syndrome
PP	Per-protocol
SAP	Statistical Analysis Plan
TB	Tuberculosis

1. INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to define the outcome variables, statistical methods, and analysis strategies to address the study's objectives in the randomised, controlled trial for the treatment of HIV-associated cryptococcal meningitis: oral fluconazole plus flucytosine or one week amphotericin B-based therapy vs two weeks amphotericin B-based therapy: the ACTA trial.

2. STUDY OBJECTIVES AND OUTCOMES

2.1. Study Objectives

2.1.1. Primary Objective

To determine (separately) the effects of the oral and 7-day amphotericin-based therapy when compared with the recommended gold standard of 14-day amphotericin-based therapy with respect to all-cause mortality up to two weeks after start of treatment.

2.1.2 Secondary Objectives

To make these comparisons between the trial arms in terms of mortality at 2, 4, and 10 weeks; the rate of clearance of infection; and incidence of serious adverse events.

To determine the effects of the oral regimen against the best available amphotericin / adjunct therapy

2.2. Outcomes

2.2.1. Primary outcome

The occurrence of death from any cause, at anytime up to and including day 14 (i.e. 2-week mortality).

Two-week mortality was chosen as the primary endpoint rather than 10-week mortality because deaths within two weeks are more likely due to CM than at 10 weeks. Ten week mortality is an important secondary endpoint (below).

2.2.2. Secondary outcomes

Efficacy:

- Death from any cause at anytime up to and including day 70 (i.e. 10-week mortality).
- Time from randomization to the occurrence of death from any cause at the end of day 70.
- Death from any cause, at anytime up to and including day 28 (i.e. 4-week mortality).
- Time from randomization to the occurrence of death from any cause at the end of day 28.

- Changes in log CSF fungal count over 14 days. Both the absolute median (IQR) at each of the time points and the rate of decrease in log CSF fungal count will be presented.

Safety:

- Occurrence of suffering clinical and laboratory-defined grade 3 and 4 adverse events to 10 weeks.

2.2.3. Case ascertainment and case definitions

(1). Deaths

Most deaths up to and including day 14 will occur in hospital because participants are hospitalised. However, participants who discharge themselves (or are discharged for whatever reason) will be contacted in the community, including by home visit if needed, if they do not return to hospital at day 14. Exhaustive attempts will be made through patient and next of kin contact and home details in order to ascertain the two-week survival status.

Participants will have regular clinic appointments following discharge and will continue to be followed up until day 70. Those who do not attend are telephoned the same day. If they cannot be contacted, a home visit is made to encourage re-attendance and continued treatment. In prior studies on cryptococcal meningitis in Africa, the lost-to follow up rate has been kept at $\leq 3\%$ at 10 weeks.

Details of participants lost to follow-up will be sent to an independent outcome review committee who will classify the participant as likely died or likely alive and estimate the date of death.

(2). CSF fungal count
Quantitative cultures of CSF are done as in prior studies [xx]. CSF is serially diluted 10 fold, with careful mixing at all stages, and from each dilution, 100 microL plated onto each half of a Sabourauds dextrose agar plates, and incubated at 30^0C. After 2- 3 days, colonies are counted by trained laboratory staff blinded to treatment assignment. Plates are re-incubated until, on daily inspection no new colonies are seen. Negative plates are kept for at least 7 days. Counts are taken from the plate with the least number of colonies, but a total of at least 30 colonies. The average of the counts on each half of the plate is taken and multiplied up to give the CFU count per ml of CSF.

(3). Clinical and laboratory-defined grade 3 and 4 adverse events.
These are reported to the trial management group and entered into the database in real time. DAIDS criteria are used.

(4). IRIS reactions
All suspected, clinically-significant IRIS reactions are reported to the trial management group through the SAE reports, which includes IRIS as a pre-defined cause of adverse

event and re-admission, and through CRFs completed at weeks 6, 8, and 10 of follow up. Of note, trial follow up is only for 10 weeks so that only early IRIS events will be identified. Suspected IRIS events will be reviewed at study conclusion, blinded to treatment assignment, by a single TMG member and an independent expert.

(5). Cause of deaths
Most deaths occur in hospital, either during the initial 2 week admission or between weeks 2 and 10 after re-admission. In either case, full information is available.

A much smaller number of deaths occur at home during the 10 week follow up. In this case, relatives are telephoned and visits to the patients address are carried out to see relatives if they cannot be contacted. Relatives are asked about symptoms and circumstance prior to death and an assessment of likely cause of death made.

Staff at study sites complete a death form with presumed cause(s) and narrative, which excludes treatment assignment. A single TMG member, who is not involved in SAE reporting, assesses cause of death, blinded to treatment assignment. If there is disagreement between site assessment of cause of death and the TMG assessment, an expert independent opinion is sought, again blind to treatment arm.

These data are being collected for safety analyses for the DMC. As discussed above, they may be included in exploratory analyses, or dropped altogether from analyses.

Additional Safety Variables:

No safety data are collected in this study.

Primary Analytical Subset:

Intent-to-treat (ITT) for the primary outcome, and the secondary outcomes analyses.

Per-protocol (PP) for supplementary analyses.

3.   STUDY DESIGN

3.1.   Design

The trial is an open-label, phase III randomised controlled non-inferiority trial. It is powered to show non-inferiority between the two experimental arms (oral regimen and the amphotericin based 7-day therapy) and the control (the amphotericin based 14-day therapy). Adult patients with a first episode of cryptococcal meningitis who fulfil the eligibility criteria as outlined in the protocol are invited to join the trial consecutively.

3.2.   Trial Sites

The trial is being currently being conducted in 5 African sites: Kamuzu Central Hospital, Lilongwe, and Queen Elizabeth Hospital, Blantyre, Malawi; at University Teaching

Hospital Lusaka, Zambia; at The Hopital General in Douala; and at Central Hospital, Yaounde, Cameroon.

Towards the end of 2014, it became clear that recruitment was slower than expected and mortality higher than expected. Therefore, in January 2015, approval was received to expand recruitment to two sites in Tanzania. It is anticipated that in April 2015 recruitment at two further sites in Dar es Salaam will begin.

3.3. Treatments

Trial arms:

Oral: Fluconazole 1200 mg /d plus flucytosine 25 mg/kg qds for 2 weeks
AmB7: Amphotericin B (AmB) 1 mg/kg/d for 7 days
AmB14: Amphotericin B (AmB) 1 mg/kg/d for 14 days

Adjunct treatment: In both the **AmB7** and **AmB14** arms, participants will receive an adjunct treatment of either fluconazole 1200 mg /d or flucytosine 25 mg/kg qds. This is a sub-study and its analysis will be the subject of a second separate analytical plan. In the **AmB7** arm, fluconazole is given at 1200 mg/d in the second week.

All participants are asked to be hospitalised for 14 days. On rare occasions participants may be discharged earlier if on a 7 day regimen, are well enough to do so and the team are confident the participant will return for follow up monitoring bloods. Following 14 day discharge, participants are scheduled to return at 2, 4, 6, 8 and 10 weeks. After day 14, fluconazole is given at 800 mg/d until ART is started, then at 400 mg/d until 10 weeks, and 200 mg/d thereafter. ART is initiated at week 4.

3.4. Randomisation

Randomisation to **Oral, Amb7** or **Amb14** is stratified by site. At each site, randomisation is done in blocks of sizes 18, 24 and 30. The size of each block is determined randomly. Within each block, 1/3 of participants are randomly assigned to each of the **Oral, Amb7** and **Amb14** strategies. The randomisation was done by Dr Victoria Simms of LSHTM using Stata software version 13. For each site a sequence of study IDs and treatment allocations was generated. This was done by creating a random variable and ordering on that variable. The allocations were put into sealed opaque envelopes and the study ID was written on the outside. When a patient is enrolled they are given a study number sequentially and the envelope corresponding to that number is opened to reveal their treatment allocation.

3.5. Sample Size

Using a non-inferiority design with a 10% non-inferiority margin and 5% type 1 error; and assuming 85% 2-week survival with the **AmB14** strategy, would require 157 patients allocated to each strategy at 80% power, 184 per strategy at 85% power, and 219 per strategy at 90% power. Based on these calculations the recruitment target was 680

participants (226 per strategy) in order to achieve 90% power, allowing for 2% losses to follow-up.

4.   ANALYSIS POPULATIONS

4.1.   Population Data Sets

Two populations will be considered in the analysis as follows:

Intent-to-Treat population

Intent to treat (ITT) will be defined at the moment the randomisation is performed. For the primary outcome analysis in this trial, patients will be followed with their ITT arm. Patient without a measurement of primary endpoint due to losses to follow-up will be excluded from the ITT analysis. In analyses referring to a specific number of days, the randomisation day will be considered day 0.

Per-protocol population

Per-protocol (PP) population is based on the treatment actually received. This population will be used for the supportive analyses. There is no single CRF question that determines the Per-protocol arm, and the arm will be determined by PI, data manager and the trial statistician.

4.2.   Analysis Close Date

The analysis close date is the date on which the last patient completed 10-week follow up.

Last contact date (also referred to as Trial reference end date): the date of the last trial related procedure. For survival subjects it is defined as the maximum of
- Date of last office visit (scheduled or unscheduled visit)
- Date of the last follow-up contact (including last date on subject survival status recorded
- Date of the last known adverse event (AE) status or lab results reported on the AE or lab case report from (CRF) pages, respectively

4.3. Data cleaning

The data will then be checked to ensure that there are no erroneous entries and that all missing data is properly coded. Any changes will be made on the Datafax database.

4.4. Data download

For each time point, once all data have been inputted and checked, the database will be locked and a data download request made. The data will be downloaded into SAS, SPSS STATA formats for statistical analyses.

5. STATISTICAL ANALYSES

5.1. Primary Outcome Analysis

5.1.1. ITT analysis of primary outcome

The primary outcome (occurrence of death from any cause at anytime up to and including day 14) is a binary outcome and will be summarised by number (%) of patients died by treatment group. The statistical test will be performed as a one-sided non-inferiority test, using the non-inferiority margin $\Delta = 0.10$.

Hypothesis: Let π_2 denote the mortality rate at 2 weeks in the **Oral** arm, and let π_1 denote the mortality rate at 2 weeks in the **AmB14** arm. The hypothesis test is

H_0: $\pi_2 - \pi_1 \geq \Delta$ versus the alternative H_A: $\pi_2 - \pi_1 < \Delta$

A generalised linear model (GLM) will be used to test the above hypothesis. In the GLM model, the occurrence of death at 2 weeks will be treated as the response variable following a binomial distribution and the treatment as fixed effect, and identity link function will be used. From this model, a point estimate in the mortality rate difference $(\pi_2 - \pi_1)$ and its one-sided 95% upper limit (two-sided 90% upper limit) for the comparison between **Oral** arm and the **AmB14** arm will be estimated. **Oral** treatment will be judged not inferior to the **AmB14** treatment if the upper confidence limit is less than Δ, where $\Delta= 10\%$, the predetermined non-inferiority margin.

The comparison between **AmB7** arm and the **AmB14** will be made in a similar way.

The GLM model will be estimated using SAS GENMOD. The primary analysis will be based on ITT population and the following suggested additional analyses will be supportive.

If the above identity-binomial regression model does not converge, a simple Z-test (appromixate normal distribution) for comparing two proportions will be used:

$$Z = \frac{P_1 - P_2}{SE(P_1 - P_2)}$$

$$SE(P_1 - P_2) = \sqrt{[P(1-P)(1/n_1 + 1/n_2)]}$$

Where $P = \dfrac{n_1 P_1 + n_2 P_2}{n_1 + n_2}$ and $SE(P_1 - P_2)$ is the standard error of $P_1 - P_2$.

5.1.2. Per-protocol analysis of primary outcome

The main conclusions in the clinical report will be based on the ITT analysis of the primary outcome. An additional analysis of the primary outcome will also be presented using the per-protocol population.

5.1.3. Sensitivity analyses of primary outcome

Sensititivity to losses to follow-up

For the primary endpoint, a sensitivity analysis will be done (comparing the **Oral** arm to the **AmB14** arm and the **AmB7** arm to the **AmB14** arm) in which all patients lost to follow up in the first 14 days will be classified as dead or alive by the independent outcome review committee.

A sensititivity analysis of 10 week mortality will also be carried out. Participants lost to follow will be classified as either dead or alive - according to the decision made by an independent outcome review committee and will be based on available data on the patient.

An analysis of the primary endpoint adjusted for site, age, sex, GCS, CD4 count, and CFU at baseline will be done comparing the **Oral** arm to the **AmB14** arm and the **AmB7** arm to the **AmB14** arm. This will be done to correct for any baseline imbalances between the arms after randomisation.

Sensititivity to imbalance in baseline covariates

The observed treatment effect comparing the **Oral** arm to the **AmB14** arm and the **AmB7** arm to the **AmB14** arm regarding the primary outcome may be confounded due to the imbalances of some baseline characteristics of patients which are associated with the primary outcome. To control for such potential confounding factors, the following covariates at baseline will be introduced into the GLM model:

1. Site (7 sites: Kamuzu Central Hospital, Lilongwe, and Queen Elizabeth Hospital, Blantyre, Malawi; University Teaching Hospital Lusaka, Zambia; The Hopital General in Douala; Central Hospital, Yaounde, Cameroon; and two hospitals in Dar es Salaam, Tanzania);
2. Age (<median, ≥median);
3. GCS (<15, ≥15);
4. CD4 (<median, ≥median);
5. CFU (<median, ≥median)

From the above model, the adjusted point estimate and 95% one-sided upper limit comparing the **Oral** arm to the **AmB14** arm and the **AmB7** arm to the **AmB14** arm will be derived

The above identity-binomial GLM model may not converge when all covariates are introduced into the model simultaneously. If this occurs, the adjusted regression model will be established by removing a covariate one by one starting from the last covariate (CFU) in the above list until the model conveges. If the covariate-adjusted identity-normal GLM model can not be established even for the last one covariate (Site), linear regression model will be employed from which the mortality difference and its 95% one-sided upper limit will be derived.

5.2. Secondary Outcome Analysis

5.2.1 Analysis of binary outcomes

Death from any cause at anytime up to and including day 70 (i.e. 10-week mortality) and day 28 will be treated as a binary outcome and will be summarised by number (%) of patients with event by treatment group and analysed in a similar way as the primary endpoint by means of GLM model. The point estimate rate difference and its one-sided 95% upper limit (two-sided 90% upper limit) for the comparison between **Oral** arm and the **AmB14** arm, and the comparison between **AmB7** arm and the **AmB14** will be estimated. **Oral** treatment or **AmB7** arm will be concluded not inferior to the **AmB14** treatment if the upper confidence limit is less than Δ, where $\Delta = 10\%$, the predetermined non-inferiority margin.

The analysis of other binary outcomes such as clinical and laboratory-defined grade 3 and 4 adverse events will also use GLM model with treatment as fixed effect. Relative risks, odds ratios and risk differences with their two-sided 95% confidence intervals comparing the **Oral** arm to the **AmB14** arm and the **AmB7** arm to the **AmB14** arm will be derived from the GLM models with binomial distribution, and log, logit and identity link functions, respectively.

5.2.2 Analysis of time-to-event outcomes

Mortality at day 70 and day 28 will also be analysed as time-to-event outcomes (eg, time from randomization to the occurrence of death from any cause at the end of day 70 and day 28) and will be summarised by number (%) of patient with event, person-years, and incidence rate.

The trial arms will be compared using the log-rank test, as a two-sided test. The Kaplan-Meier plots will be drawn to describe the process of event occurrence by treatment arms. Cox regression model will be used to derive hazard ratio and its 2-sided 95% confidence interval for comparing the **Oral** arm and the **AmB14** arm, and comparing the **AmB7** arm and the **AmB14** arm.

Patients not dead will be censored on the analysis close date, or the last date known alive, whichever is earlier.
- A patient will be considered known alive on follow-up dates, adverse event dates, various exam dates, and all other entries that indicate the patient is alive on a certain date.
- Patients who withdraw from the trial will be considered alive on the withdrawal date.
- If a patient is reported as lost to follow-up on the study exit form, that date will be not be considered as a date on which the patient is known alive. All other information will be used in computing the censoring date.

5.2.3   Analysis of comtinuous outcomes

The continuous outcome such as biochemical markers will be summarised using number of subjects (n), mean, standard deviation (SD), minimum, and maximum by treatment group, and will be analysed by a GLM model with treatment as fixed effect and with normal distribution and identity link function. Difference in mean outcome with their two-sided 95% confidence intervals between the **Oral** arm and the **AmB14** arm, and between the **AmB7** arm and the **AmB14** arm will be derived from the GLM model.

5.2.4   Analysis of count outcomes

The count outcome such as CSF fungal count will be summarised using number of events and incidence rate by treatment group, and will be analysed by a GLM model with treatment as fixed effect and with Poisson distribution and log link function. Incidence rate ratio (IRR) comparing the **Oral** arm to the **AmB14** arm and the **AmB7** arm to the **AmB14** arm with their two-sided 95% confidence intervals will be derived from the GLM model.

5.2.5   Analysis of secondary outcomes with repeated measurements

The CSF fungal count are measured on baseline, day 1, day 7 and day 14. Changes in log CSF fungal count over 14 days from baseline will be summarised and analysed using a generalised estimating equation (GEE) model with treatment, day and interaction between treatment and day as fixed effects, log baseline measurement of fungal count as covariate, and subject as cluster effect. Ratio in geometric mean with their two-sided 95% confidence intervals in fungal count between the **Oral** arm and the **AmB14** arm, and between the **AmB7** arm and the **AmB14** arm will be derived from the GEE model.

Model assumption will be assessed using Q-Q plots for the model residuals. If the mormality assumption is seriously violated or there is a large proportion of zero values, Wilcoxon rank-sum test will be used to compare the group difference at each day.

5.2.6   Analysis of other secondary outcomes

Other statistical methods may be used if deemed necessary.

6.   SAFETY ANALYSES

6.1.   Safety Variables

Adverse events (AEs) will be summarised using the number of AEs, the number (%) of patients with AEs by treatment arms.

There are no measurements on safety data on vital signs, ECG, and blood laboratory data during the follow-up visits.

6.2.   Additional Safety Analyses

There will be no additional safety analysis.

7. GENERAL CONSIDERATIONS FOR DATA ANALYSES

SAS® (version 9.3) will be used to perform all data analyses and generate the majority of data displays. Stata or SPSS or S-Plus or R may also be used for some data analyses.

7.1. Multi-centre Studies

The data will be analysed on a combined-site basis. As the randomisation was performed centrally, not at investigator level, no stratification based on sites will be performed in the analysis.

7.2. Covariates Analyses

Covariate analyses will be performed on the primary outcome (See Section 5.1.3.)

7.3. Subgroup Analysis

Subgroup analyses will be performed for primary outcome variable. The subgroup variable will be the site, age, sex, GCS, CD4 count, and CFU at baseline.

7.4. Multiplicity

Multiplicity adjustment will not apply to the primary and secondary outcome analyses.

7.5. Other Data Considerations

7.5.1 Data Summaries

Continuous variables will be summarised according to number of subjects with non-missing data (n), mean, standard deviation (SD), median, minimum, and maximum. The confidence interval will be added on summaries of continuous effectiveness variables.

Categorical variables will be summarised according to the absolute frequency and percentage of subjects (%) in each category level. The denominator for the percentages is the number of subjects in the treatment arm with data available, unless noted otherwise. Event rates per 100 patient years will also be reported for time-to-event clinical outcomes and adverse events of special interest.

7.5.2 Graphical Displays

Mean values for some continuous outcomes by treatment and visit will be plotted.

8. REFERENCES

A PHASE III, RANDOMISED, CONTROLLED TRIAL FOR THE TREATMENT OF HIV-ASSOCIATED CRYPTOCOCCAL MENINGITIS: ORAL FLUCONAZOLE PLUS FLUCYTOSINE OR ONE WEEK AMPHOTERICIN B-BASED THERAPY VS TWO WEEKS AMPHOTERICIN B-BASED THERAPY

STATISTICAL ANALYSIS PLAN

FOR THE STUDY COMPARING AMPHOTERICIN B-BASED THERAPY ADJUNCT TREATMENTS: FLUCONAZOLE VERSUS FLUCYTOSINE.

ISRCTN registration number: ISRCTN45035509

ANRS Reference: ANRS12275

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ABBREVIATIONS

Abbreviation	Explanation
AE	Adverse Event
Ag	Antigen
AIDS	Acquired Immune Deficiency Syndrome
AmB	Amphotericin B
AR	Adverse reaction
ART/ARV	Anti-retroviral therapy/ Anti-retroviral
CM	Cryptococcal Meningitis
HIV	Human Immunodeficiency Virus
ITT	Intent-to-treat
mITT	Modified Intent-to-treat
IDMC	Independent Data Monitoring Committee
IRIS	Immune Reconstitution Inflammatory Syndrome
PP	Per-protocol
SAP	Statistical Analysis Plan
TB	Tuberculosis

1. INTRODUCTION

This document describes the statistical analysis of a phase III, randomised, controlled trial – the ACTA trial - to compare fluconazole versus flucytosine as adjunct treatments for either a 7-day or 14-day course of amphotericin B.

This is the second of two analysis plans from the ACTA trial. The first plan deals with the comparison of oral fluconazole plus flucytosine combination versus one week amphotericin b-based therapy versus two weeks amphotericin b-based therapy for the initial treatment of cryptococcal meningitis.

The one week and two week treatments amphotericin B based treatments are given with the adjunct of either flucytosine or fluconazole; this choice of adjunct is also randomised.

This analysis plan focuses on the comparison between the two adjunct treatments, fluconazole versus flucytosine, and the comparison between either the one or two week amphotericin B based treatments with each adjunct versus the combination oral regimen.

The data from the trial have been separated in this way as the volume of analyses and information are too great to describe in a single plan or in a single paper.

2. STUDY OBJECTIVES AND OUTCOMES

2.1. Study objectives

2.1.1. Primary objective

To determine the effects of fluconazole versus flucytosine when given as adjuncts to amphotericin-based therapy with respect to all-cause mortality.

The statistical analysis will determine whether one is superior to the other. It will be done at a 5% two-sided significance level.

2.1.2 Secondary objectives

a). To determine the effects of the oral regimen (fluconazole and flucytosine) compared with 7-day amphotericin B and 14-day amphotericin B separately for each adjunct. This will involve 4 comparisons:

    i)   Oral regimen versus 7-day amphotericin B combined with fluconazole adjunct

    ii)   Oral regimen versus 7-day amphotericin B combined with flucytosine adjunct

    iii) Oral regimen versus 14-day amphotericin B combined with fluconazole adjunct

    iv) Oral regimen versus 14-day amphotericin B combined with flucytosine adjunct

b) To determine the effects of each adjunct overall and the effects of each adjunct – amphotericin B combination on the rate of clearance of infection and incidence of serious (grade III and grade IV) adverse events.

2.2.   Outcomes

2.2.1. Primary outcome

The primary endpoint is defined as death from any cause at anytime up to and including the last day of follow-up (i.e. day 70) (from the time of randomisation).

In studies of cryptococcal meningitis management, primary endpoints are usually either 2-week or 10-week mortality. Both are crucially important.

Our primary endpoint for the adjunct treatments comparison differs from the primary endpoint for the comparison of the Oral verus Amb7 versus Amb14 initial treatment regimens, for which 2-week mortality was chosen as the primary endpoint.

The sample size for the comparison of Oral verus 7-day amphotericin B versus 14-day amphotericin B is dictated by a non-inferiority design for this comparison. It is likely that 2-week mortality is more specific to cryptococcal meningitis compared with a 10-week mortality outcome. This possible lower specificity for the 10-week timepoint would increase the possibility of demonstrating non-inferiority at this timepoint; therefore to be conservative, we chose 2 week as the primary endpoint for the comparison of oral versus Amb7 versus Amb14.

The present comparison of flucytosine versus fluconazole adjuncts is based on a superiority hypothesis. We have chosen as the primary endpoint, death at anytime up to 10 weeks. In other words, we propose to simply compare mortality between the two regimens over the course of our follow-up.

Because of the substantial follow-up time, the primary analysis will be done as a time-to-event analysis and the comparison will be made by a log-rank test

2.2.2. Secondary outcomes

Efficacy:

The occurrence of death from any cause from randomisation to any time up to the end of day 70. This is a binary event.

Death from any cause at anytime up to and including day 14 (i.e. 2-week mortality) (binary outcome).

Time from randomisation to the occurrence of death from any cause at the end of day 14 (survival time analysis).

Death from any cause, at anytime up to and including day 28 (i.e. 4-week mortality).

Time from randomisation to the occurrence of death from any cause at the end of day 28.

Log CSF fungal count at day 7 and 14.

Changes in log CSF fungal count at day 7 and 14.

Safety:

- Occurrence of suffering clinical and laboratory-defined grade 3 and 4 adverse events to 10 weeks.

2.2.3. Case ascertainment and case definitions

(1). Deaths

Most deaths up to and including day 14 will occur in hospital because participants are hospitalised. However, participants who discharge themselves (or are discharged for whatever reason) will be contacted in the community, including by home visit if needed, if they do not return to hospital at day 14. Exhaustive attempts will be made through participant and next of kin contact and home details in order to ascertain the two-week survival status.

Participants will have regular clinic appointments following discharge and will continue to be followed up until day 70. Those who do not attend are telephoned the same day. If they cannot be contacted, a home visit is made to encourage re-attendance and continued treatment. In prior studies on cryptococcal meningitis in Africa, the lost-to follow up rate has been kept at ≤3% at 10 weeks.

Details of participants lost to follow-up will be sent to an independent outcome review committee who will classify the participant as likely died or likely alive and estimate the date of death. Details of these will be analysed are further below. By the end of August 2015, only 5 participants had been lost to follow-up and of these just one participant was lost within 2 weeks.

(2). CSF fungal count

CSF is serially diluted 10 fold, with careful mixing at all stages, and from each dilution, 100 microL plated onto each half of a Sabouraud dextrose agar plate, and incubated at 30^0C. After 2-3 days, colonies are counted by trained laboratory staff blinded to treatment assignment. Plates are re-incubated until, on daily inspection no new colonies are seen. Negative plates are kept for at least 7 days. Counts are taken from the plate with the least number of colonies, but a total of at least 30 colonies. The average of the counts on each half of the plate is taken and multiplied up to give the CFU count per ml of CSF (Ref: Lancet 2004; 363:1764-67).

(3). Clinical and laboratory-defined grade 3 and 4 adverse events.

These are reported to the trial management group and entered into the database in real time. DAIDS criteria are used.

(4). IRIS reactions

All suspected, clinically-significant IRIS reactions are reported to the trial management group through the SAE reports, which includes IRIS as a pre-defined cause of adverse event and re-admission, and through CRFs completed at weeks 6, 8, and 10 of follow up. Of note, trial follow up is only for 10 weeks so that only early IRIS events will be identified. Suspected IRIS events will be reviewed, blinded to treatment assignment, by a single TMG member and an independent expert.

(5). Cause of deaths

Most deaths occur in hospital, either during the initial 2 weeks of admission or between weeks 2 and 10 after re-admission. In either case, information on these deaths is available and could be used to estimate the cause of death.

A much smaller number of deaths occur at home during the 10 week follow up. In this case, relatives are telephoned and visits to the participant's address are carried out to estimate the cause of death. Relatives are asked about symptoms and circumstance prior to death and an assessment of likely cause of death is made.

Staff at study sites complete a death form with presumed cause(s) and narrative, which excludes treatment assignment. A single TMG member, who is not involved in SAE reporting, assesses cause of death, blinded to treatment assignment. If there is disagreement between site

assessment of cause of death and the TMG assessment, an expert independent opinion is sought, again blind to treatment arm.

These data are being collected for safety analyses for the DMC. They provide crude estimates of possible causes of death. Clinical autopsies are not done in any of the settings where the trial is based. Consistent with other publications on cryptococcal meningitis, the causes of death will not be reported in the papers discussed in these analytical plan.

Additional safety variables:

Monitoring blood laboratory values. These will be used to calculate mean decreases in haemoglobin, neutrophil count, and potassium, and increases in creatinine and ALT, reflective of known laboratory side effects of the study drugs, over the first one and two weeks.

3. STUDY DESIGN

3.1. Design

The trial is an open-label, phase III randomised controlled trial. Adult patients with a first episode of cryptococcal meningitis who fulfil the eligibility criteria as outlined in the protocol are invited to join the trial consecutively and are randomised individually to fluconazole or flucytosine adjunct therapy. These particpants are also receiving either 7-day amphotericin B or 14-day amphotericin B (this comparison is also randomised – see the accompanying analytical plan describing the comparison of oral versus 7-day amphotericin B versus 14-day amphotericin B).

3.2. Trial sites

The trial is being conducted in African sites: Kamuzu Central Hospital, Lilongwe, and Queen Elizabeth Hospital, Blantyre, Malawi; at University Teaching Hospital Lusaka, Zambia; at The Hopital General in Douala; and at Central Hospital, Yaounde, Cameroon.

Towards the end of 2014, it became clear that recruitment was slower than expected. Therefore, in January 2015, approval was received to expand recruitment to two sites in Tanzania. Recruitment at the two further sites in Dar es Salaam began in August 2015. Recruitment at the Zambia site had been very slow and this was stopped in June 2015.

3.3. Treatments

Trial arms:

The primary treatment strategies are:

Oral:	Fluconazole 1200 mg /d plus flucytosine 25 mg/kg qds for 2 weeks
AmB7:	Amphotericin B (AmB) 1 mg/kg/d for 7 days
AmB14:	Amphotericin B (AmB) 1 mg/kg/d for 14 days

Adjunct treatment: In both the **AmB7** and **AmB14** arms, participants receive an adjunct treatment of either fluconazole 1200 mg/d or flucytosine 25 mg/kg qds. This is a randomised comparison. In the **AmB7** arm, fluconazole is given at 1200 mg/d in the second week.

All participants are asked to be hospitalised for 14 days. On rare occasions participants may be discharged earlier if he/she is on a 7-day regimen, is well enough, and the team are confident that he/she will return for follow up monitoring bloods.

Following day 14, participants are scheduled to return at 4, 6, 8 and 10 weeks. After day 14, fluconazole is given at 800 mg/d until ART is started, then at 400 mg/d until 10 weeks, and 200 mg/d thereafter. ART is initiated at week 4.

The list of regimens in the database as follows:

Regimen 1: Fluconazole (FLU) 1200 mg daily + flucytosine (5-FC) 25 mg/kg four times daily for 14 days
Regimen 2A: AmB 1 mg/kg/d +FLU 1200 mg daily for 7 days
Regimen 2B: AmB 1mg/kg/d +5-FC 25 mg/kg four times daily for 7 days
Regimen 3A: AmB 1 mg/kg/d + FLU 1200 mg daily for 14 days
Regimen 3B: AmB 1 mg/kg/d + 5-FC 25 mg/kg four times daily for 14 days

There will be two adjunct treatment groups:
Adjunct A: Fluconazole (Regimens 2A and 3A)
Adjunct B: Fucytosine (Regimens 2B+3B)

Thus, the adjunct treatments will compared across both amphotericin B arms.

3.4.   Randomisation

Randomisation is stratified by site. At each site, randomisation is done in blocks of sizes 18, 24 and 30. The size of each block is determined randomly. Within each block, participants are randomly assigned to the 5 different treatments, Oral, Amb7 + fluconazole, Amb7 + flycytosine, Amb14 + fluconazole and Amb14 + flycytosine with probabilities 1/3, 1/6, 1/6, 1/6 and 1/6 respectively. Thus, overall within each block, 1/3 of participants were randomly assigned to each of the **Oral, Amb7** and **Amb14** strategies. The randomisation was done by Dr Victoria Simms of LSHTM using Stata software version 13. For each site a sequence of study IDs and treatment allocations was generated. This was done by creating a random variable and ordering on that variable. The allocations were put into sealed opaque envelopes and the study ID was

written on the outside. When a participant is enrolled they are given a study number sequentially and the envelope corresponding to that number is opened to reveal their treatment allocation.

3.5.   Sample size

The trial aims to recruit 680 participants. A third of these will be allocated to the **Oral** arm and so will not be included in the analysis outlined in this analytical plan. We assume 2% loss to follow up, so therefore if recruitment targets are reached there will be about 452 subjects in this analysis, approximately half of whom will be randomly assigned to fluconazole and half to flucytosine.

The sample size was dictated by the main strategies: oral versus 7-day AmB versus 14 day AmB. For this comparison, if 2-week mortality was 40% in one adjunct treatment, then we would have 90% power to detect a relative difference of 35% or between the two treatments (i.e. 40% versus 26%), and about 80% power to detect a relative difference of 30% or more.

4.   ANALYSIS POPULATIONS

4.1.   Study population data sets

Intent-to-treat (ITT) will be defined at the moment the randomisation is performed. For the ITT analysis in this trial, participants will be followed with their ITT arms. Thus, for example, if a participant receives the wrong treatment or no treatment at all, he/she will be analysed according to the arm to which they were allocated. This will be the primary analysis for the trial.

A modified ITT (mITT) will be done where participants will be allocated according to the regimen they received as the first dose. It is unlikely that this number will exceed 2-3 participants out of the entire total. In this case, the findings of mITT will be near identical to ITT and only ITT will be presented.

Per protocol population will be defined on the basis of the first two weeks of treatment and follow-up as follows:

-   Those who received the treatments as according to the protocol and were alive at the end of 14 days will be included.

-   Those who died within 14 days and received the treatments as according to the protocol up to their time of death will also be included.

Participants will be **excluded from the per-protocol population** if they:

- Did not adhere to study treatment (took less than the recommended doses over recommended time period, without sound clinical reason). Participants in whom study drug is withheld, or stopped early, in accordance with the protocol, due to known toxicities, will be retained in the per-protocol analysis.

- Switched treatment. The treatment groups in the per-protocol analysis will be defined according to what the participant actually received (e.g. a participant was randomised to Amb7 but received Amb14 as according to the protocol for Amb14, then he/she will be allocated to the Amb14 arm for this analysis).

There is no single CRF question that determines the per-protocol population. This will be determined by PI, data manager and the trial statistician.

Safety population. This will be defined as all study participants who received at least one dose of any of the study interventions.

4.2.   Analysis close date

The analysis close date is the date on which the last participant completed 10-week follow-up.

Last contact date (also referred to as Trial reference end date): the date of the last trial related procedure. For survival subjects it is defined as the maximum of
- Date of last office visit (scheduled or unscheduled visit)
- Date of the last follow-up contact (including last date on subject survival status recorded)
- Date of the last known adverse event (AE) status or lab results reported on the AE or lab case report from (CRF) pages, respectively

Data cleaning

The data will then be checked to ensure that there are no erroneous entries and that all missing data is properly coded. Any changes will be made on the Datafax database.

4.3. Data download

For each time point, once all data have been computerised and checked, the database will be locked and a data download request made. The data will be downloaded into SAS and STATA formats for statistical analyses.

5.   STATISTICAL ANALYSES

5.1.   Primary outcome analysis

5.1.1.   ITT analysis of the primary outcome - the primary analysis

The primary outcome is the incidence rate of all-cause mortality from recruitment up to 10 weeks after recruitment. Kaplan-Meier curves showing survival according to study arm will be calculated, and the log-rank test will be used to compare the survival curves. This will be the primary comparison between the two treatments.

Person years of observation (PYO) will be estimated as the time from enrolment into the trial to the date of death, withdrawal, loss to follow up, or 10 weeks after recruitment. These will be used in the calculation of incident rates and 95% confidence intervals. Hazard ratios will be calculated from a Cox regression.

All comparisons (primary and secondary) will be done using a 5% two-sided significance level.

5.1.2.   Modified ITT analysis of the primary outcome

An analysis of the primary outcome will also be performed on the modified ITT population and stratified by AmB treatment (whether one or two weeks of amphotericin B). Statistical methods will be the same as used in the Section 5.1.1.

5.1.3.   Sensitivity analysis of the primary outcome

A sensitivity analysis of the primary endpoint will be performed. This will use the ITT population. All participants lost to follow-up during this time will be classified as dead. We will also have information from the independent review committee, which will classify the participant lost according to likely dead or likely alive and these data will be reported.

As mentioned above, the number lost to follow-up will likely be very small. The sensitivity analyses may give near identical results to each other, and indeed to the primary analysis. In this case, there might be little point in reporting the sensitivity analysis.

5.1.4.   Covariate adjusted analysis of the primary outcome

An analysis of the primary endpoint adjusted for site, age, sex, GCS, CD4 count, CFU, ART status, CSF opening pressure, prior TB, haemoglobin, creatanine at baseline and by length of AmB treatment will be done comparing the fluconazole and flucytosine adjuncts. This will be done to correct for any possible baseline imbalances between the arms after randomisation.

This will be done using Cox proportional hazards regression.

5.1.5. Further analysis of the primary outcome

Subgroup analyses will be performed. We will stratify by amphotericin B (7 or 14 days), age, sex, GCS, CD4 count, CFU, ART status, CSF opening pressure, prior TB, haemoglobin, and creatanine at baseline. Age, CD4 count, CFU, CSF opening pressure, haemoglobin, and creatanine will be categorised into two approximately equal sized categories (i.e. cut-off at the median). GCS will be categorised at <15 or 15 or more. ART status at baseline and prior TB are a simple yes/no categorical variable. Sex will be male or female.

In addition, we will stratify by disease severity, defining this as GCS<15 and CFU>median, which we have shown to be an important prognostic indicator in our past studies.

Comparisons between fluconazole and flucytosine for both the 2 and 10-week mortality endpoints will be analysed for each of these strata.

The fluconazole and flucytosine adjuncts when used with either 7-day or 14-day amphotericin B will be compared with the oral regimen. These comparisons will be as follows:

i). Oral combination versus 7-day amphotericin B with flucytosine adjunct
ii). Oral combination versus 7-day amphotericin B with fluconazole adjunct

iii). Oral combination versus 14-day amphotericin B with flucytosine adjunct
iv). Oral combination versus 14-day amphotericin B with fluconazole adjunct

These comparisons will be done for the primary endpoint and also stratified by the variables age, sex, GCS, CD4 count, CFU, ART status, CSF opening pressure, prior TB, haemoglobin, and creatanine at baseline, as above.

5.2. Secondary outcome analysis

The secondary outcomes will be analysed overall, stratified as above, and also, as above, we will compare the fluconazole and flucytosine adjuncts when used with either 7-day or 14-day amphotericin B with the oral regimen.

Survival time data will be analysed as above for the primary endpoint. Death will also be treated as a binary outcome and compared between groups using a GLM model. Relative risks, odds ratios and risk differences with their two-sided 95% confidence intervals comparing two treatment arms will be derived from the GLM models with binomial distribution, and log, logit

and identity link functions, respectively. In order to simplify presentation only the relative risks and risk differences will be reported.

The continuous outcome such as biochemical markers will be summarised using number of subjects (n), mean, standard deviation (SD), minimum, and maximum by treatment group, and will be analysed by a GLM model with treatment as fixed effect and with normal distribution and identity link function. Difference in mean outcome and mean differences with their two-sided 95% confidence intervals between two groups will be derived from the GLM model.

The CSF fungal count are measured on day 1, day 7 and day 14. CSF fungal count will be transformed using log(CSF fungal count +1) to avoid taking the log of zero. Two analyses will be done.

Changes in log CSF fungal count over 14 days from baseline will be summarised and analysed using a generalised estimating equation (GEE) model with treatment, day and interaction between treatment and day as fixed effects, log baseline measurement of fungal count as covariate, and subject as cluster effect. Ratio in geometric mean with their two-sided 95% confidence intervals in fungal count between two arms will be derived from the GEE model. Model assumption will be assessed using Q-Q plots for the model residuals.

We will also calculate the slope of CSF fungal count decline for each patient using simple linear regression as done previously by us (Ref: Lancet 2004; 363: 1764-67 and Clin Infect Dis 2014; 58: 736-45).

We will present the analysis from the GEE model alone unless the normality assumption in Q-Q plots is seriously violated. In this case, the analysis involving linear regressions of individual patient data will be presented. Wilcoxon rank-sum tests will be used to compare the slopes between groups.

6. SAFETY ANALYSES

6.1. Safety variables

Adverse events (AEs) will be summarised using the number of AEs, the number (%) of participants with AEs by treatment arms.

The substudy collecting ECG data has stopped (as planned and in accordance with IDMC and TSC approval) and will be analysed and reported separately.

Blood monitoring laboratory data, occurrence of suffering clinical and laboratory-defined grade 3 and 4 adverse events to 10 weeks will be analysed descriptively.

7. GENERAL CONSIDERATIONS FOR DATA ANALYSES

SAS® (version 9.3) will be used to perform all data analyses and generate the majority of data displays. STATA or S-Plus or R may also be used for some data analyses.

7.1. Stratified analysis

Inferential statistics for measuring treatment difference between flucytosine and flucytosine will be from stratified statistical models by length of AmB treatment: 7 day treatment regimen AmB7 and 14 day treatment regimen Amb14.

7.2. Multi-site consideration

The data analyses will be performed on a combined-site basis. Of note, the randomisation was performed centrally. There are 8 sites in the study: Lusaka, Zambia; Blantyre, Malawi; Lilongwe, Malawi; Yaounde, Cameroon; Muhimbili, Tanzania; Mwananymala, Tanzania; Zomba, Malawi; and Douala, Cameroon. Eight sites may be collapsed into 4 countries (Zambia, Malawi, Cameroon, and Tanzania) in the covariate adjustied analysis and subgroup analysis in order to produce converging and stable estimate of treatment effects.

7.3. Covariates analyses

Covariate analyses will be performed on the primary outcome and the key secondary outcomes, in particular the 10-week mortality outcome (See Section 5.1.3.) on the ITT population. Other covariate analyses will be performed if deemed necessary.

7.4. Subgroup analysis

Subgroup analyses will be performed for the primary and key secondary outcomes on the ITT population. The subgroup variable will be the site, age, sex, GCS, CD4 count, CFU, ART status , disease severity, CSF opening pressure, prior TB, haemoglobin, and creatanine as defined above for covariated adjusted analysis.

7.5. Multiplicity

Multiplicity adjustment will not apply to the primary and secondary outcome analyses.

7.6. Other data considerations

7.5.1 Data summaries

Continuous variables will be summarised according to number of subjects with non-missing data (n), mean, standard deviation (SD), median, minimum, and maximum. The confidence interval will be added on summaries of continuous effectiveness variables.

Categorical variables will be summarised according to the absolute frequency and percentage of subjects (%) in each category level. The denominator for the percentages is the number of subjects in the treatment arm with data available, unless noted otherwise. Event rates per 100 participant years will also be reported for time-to-event clinical outcomes and adverse events of special interest.

7.5.2 Graphical displays

Mean values for some continuous outcomes by treatment and visit will be plotted. Kaplan-Meier plots will be produced for displaying the time-to-event data.

8. REFERENCES

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FINAL VERSION

INTRODUCTION

A PHASE III, RANDOMISED, CONTROLLED TRIAL FOR THE TREATMENT OF HIV-ASSOCIATED CRYPTOCOCCAL MENINGITIS: I) ORAL FLUCONAZOLE PLUS FLUCYTOSINE OR ONE WEEK AMPHOTERICIN B-BASED THERAPY VS TWO WEEKS AMPHOTERICIN B-BASED THERAPY AS INDUCTION THERAPIES AND II) AMPHOTERICIN B-BASED THERAPY ADJUNCT TREATMENTS FLUCONAZOLE VERSUS FLUCYTOSINE.

THE ACTA TRIAL.

Overview

The ACTA trial compares two novel strategies for induction therapy for HIV-associated cryptococcal meningitis – namely i) an oral combination of fluconazole plus flucytosine, and ii) one week of amphotericin B-based therapy – against iii) the international standard of 2 weeks of amphotericin B based therapy. In addition, within the 2 amphotericin B-based strategies, fluconazole is compared with flucytosine as the adjunctive treatment given with amphotericin B for the duration of amphotericin B. For simplicity, the planned analyses are set out in 2 documents:

1) For the comparison of the novel induction strategies – using non inferiority criteria with respect to the international standard

2) For the comparison of the adjunctive treatments given with amphotericin B.

In consideration of how the results will be presented, possible scenarios include:

Case 1: If there is no significant difference between fluconazole and flucytosine as adjunctive treatments.

Case 2: If there is a significant difference (at the 5% two-sided significance level) between fluconazole and flucytosine as adjunctive treatments.

ACTA Trial

In either case, the same analyses will be conducted as laid out in the two analysis plans. However, presentation of the results may vary:

In Case 1, two papers are envisaged, one dealing with the comparison of strategies, and one with the comparison of adjunctive therapies.

In Case 2, comparison of the oral combination strategy with the amphotericin B based strategies is complicated by the difficulty of combining the different results obtained with either adjunctive fluconazole or adjunctive flucytosine in the amphotericin B based strategies.

For this reason, in this case, the 2 papers will likely consist: one dealing with the comparison of the two amphotericin B-based strategies – the effect of induction duration (1 vs 2 weeks) and of adjunctive therapy (fluconazole vs flucytosine); and one dealing with the comparison of the oral combination strategy with the two amphotericin B-based strategies. In this paper, the results of comparisons of oral induction treatment vs 1 week AmB induction (combined results with flucytosine and fluconazole) and with 2 week AmB induction (combined results with flucytosine and fluconazole) will be presented. However, emphasis will be given to the separate comparisons of oral therapy vs the other 4 treatments: amphotericin B plus fluconazole for one week; amphotericin B plus flucytosine for one week; amphotericin B plus fluconazole for two weeks; and, amphotericin B plus flucytosine for two weeks.

INDUCTION

A PHASE III, RANDOMISED, CONTROLLED TRIAL FOR THE TREATMENT OF HIV-ASSOCIATED CRYPTOCOCCAL MENINGITIS: THE ACTA TRIAL

STATISTICAL ANALYSIS PLAN

FOR THE STUDY COMPARING INDUCTION THERAPIES: ORAL FLUCONAZOLE PLUS FLUCYTOSINE OR ONE WEEK AMPHOTERICIN B-BASED THERAPY VS TWO WEEKS AMPHOTERICIN B-BASED THERAPY

ISRCTN registration number: ISRCTN45035509

ANRS Reference: ANRS12275

1. INTRODUCTION

The purpose of this Statistical Analysis Plan (SAP) is to define the outcome variables, statistical methods, and analysis strategies for the randomised, controlled trial for the treatment of HIV-associated cryptococcal meningitis. This first analytical plan relates to the comparison of the three strategies: oral fluconazole plus flucytosine or one week amphotericin B-based therapy vs two weeks amphotericin B-based therapy: the ACTA trial.

A separate analytical plan addresses the comparisons between the adjunct treatments – fluconazole versus flucytosine and how the oral combination compares with amphotericin B given for one or two weeks with each of these two adjuncts.

2. STUDY OBJECTIVES AND OUTCOMES

2.1. Study Objectives

2.1.1. Primary Objective

To determine (separately) the effects of the combination oral and 7-day amphotericin B based therapy when compared with the recommended gold standard of 14-day amphotericin B based therapy with respect to all-cause mortality up to two weeks after start of treatment.

The analysis will determine whether or not either the oral or the 7-day amphotericin B regimen are non-inferior to the 14-day amphotericin B.

2.1.2 Secondary Objectives

To make these comparisons between the trial arms in terms of mortality at 2, 4, and 10 weeks; the rate of clearance of infection; and incidence of serious (grade III and grade IV) adverse events.

2.2. Outcomes

2.2.1. Primary outcome

The primary endpoint is defined as the occurrence of death from any cause, at anytime up to and including day 14 (i.e. 2-week mortality). The primary endpoint is a binary outcome and will be achieved if a participant dies during the two-week follow-up.

The choice of two-week mortality, as opposed to 10-week mortality, is almost arbitrary. 10-week mortality is a vitally important endpoint in the evaluation of the different strategies. We did not choose two co-primary endpoints purely for statistical reasons (i.e. to maintain a conventional alpha=5% significance level).

2.2.2. Secondary outcomes

Efficacy:

- Death from any cause at anytime up to and including day 70 (i.e. 10-week mortality).
- Time from randomisation to the occurrence of death from any cause at the end of day 70.
- Time from randomisation to the occurrence of death from any cause at the end of day 14. This is a time-to-event outcome, taking into account the time at which a death event occurs. A participant who is lost to follow up will be included in the analysis at the censored time point.
- Death from any cause, at anytime up to and including day 28 (i.e. 4-week mortality).
- Time from randomisation to the occurrence of death from any cause at the end of day 28.
- Log CSF fungal count at day 7 and 14.
- Changes in log CSF fungal count at day 7 and 14.

Safety:

- Occurrence of suffering clinical and laboratory-defined grade 3 and 4 adverse events to 10 weeks.

2.2.3. Case ascertainment and case definitions

(1). Deaths

Most deaths up to and including day 14 will occur in hospital because participants are hospitalised. However, participants who discharge themselves (or are discharged for whatever reason) will be contacted in the community, including by home visit if needed, if they do not return to hospital at day 14. Exhaustive attempts will be made through participant and next of kin contact and home details in order to ascertain the two-week survival status.

Participants will have regular clinic appointments following discharge and will continue to be followed up until day 70. Those who do not attend are telephoned the same day. If they cannot be contacted, a home visit is made to encourage re-attendance and continued treatment. In prior studies on cryptococcal meningitis in Africa, the lost-to follow up rate has been kept at ≤3% at 10 weeks.

Details of participants lost to follow-up will be sent to a TMG member, not directly involved in patient management and blind to study arm, who will classify the participant as likely died or likely alive and estimate the date of death. These analyses are detailed further below. By the end of August 2015, only 5 participants had been lost to follow-up and of these just one participant was lost within 2 weeks.

(2). CSF fungal count

CSF is serially diluted 10 fold, with careful mixing at all stages, and from each dilution, 100 microL plated onto each half of a Sabouraud dextrose agar plate, and incubated at 30^0C. After 2- 3 days, colonies are counted by trained laboratory staff blinded to treatment assignment. Plates are re-incubated until, on daily inspection no new colonies are seen. Negative plates are kept for at least 7 days. Counts are taken from the plate with the least number of colonies, but a total of at least 30 colonies. The average of the counts on each half of the plate is taken and multiplied up to give the CFU count per ml of CSF (Ref: Lancet 2004; 363:1764-67).

(3). Clinical and laboratory-defined grade 3 and 4 adverse events.

These are reported to the trial management group and entered into the database in real time. DAIDS criteria are used. Some patients enter the trial with adverse events at baseline. These are only reported if there is deteriotation. For example if a patient moves from grade 3 adverse event to grade 4.

(4). IRIS reactions

All suspected, clinically-significant IRIS reactions are reported to the trial management group through the SAE reports, which includes IRIS as a pre-defined cause of adverse event and re-admission, and through CRFs completed at weeks 6, 8, and 10 of follow up. Of note, trial follow up is only for 10 weeks so that only early IRIS events will be identified. For the main paper, an IRIS event will be determined by the study doctor and included in the table of adverse events as a grade 4 AE.

(5). Cause of deaths

Most deaths occur in hospital, either during the initial 2 weeks of admission or between weeks 2 and 10 after re-admission. In either case, information on these deaths is available and could be used to estimate the cause of death.

A much smaller number of deaths occur at home during the 10 week follow up. In this case, relatives are telephoned and visits to the participant's address are carried out to estimate the cause of death. Relatives are asked about symptoms and circumstance prior to death and an assessment of likely cause of death is made.

Staff at study sites complete a death form with presumed cause(s) and narrative, which excludes treatment assignment. A single TMG member, who is not involved in SAE reporting, assesses cause of death, blinded to treatment assignment. If there is disagreement between site assessment of cause of death and the TMG assessment, an expert independent opinion is sought, again blind to treatment arm.

These data are being collected for safety analyses for the DMC. They provide crude estimates of possible causes of death. Clinical autopsies are not done in any of the settings where the trial is based. Consistent with other publications on cryptococcal meningitis, the

presumed causes of death will not be reported in the papers discussed in these analytical plan.

Additional Safety Variables:

Monitoring blood laboratory values. These will be used to calculate mean decreases in haemoglobin, neutrophil count, and potassium, and increases in creatinine and ALT, reflective of known laboratory side effects of the study drugs, over the first one and two weeks.

3.   STUDY DESIGN

3.1.   Design

The trial is an open-label, phase III randomised controlled non-inferiority trial. It is powered to show non-inferiority between the two experimental arms (oral regimen and the amphotericin based 7-day therapy) and the control (the amphotericin based 14-day therapy). Adult patients with a first episode of cryptococcal meningitis who fulfil the eligibility criteria as outlined in the protocol are invited to join the trial consecutively.

3.2.   Trial Sites

The trial is being conducted in African sites: Kamuzu Central Hospital, Lilongwe, and Queen Elizabeth Hospital, Blantyre, Malawi; at University Teaching Hospital Lusaka, Zambia; at The Hopital General in Douala; and at Central Hospital, Yaounde, Cameroon.

Towards the end of 2014, it became clear that recruitment was slower than expected. Therefore, in January 2015, approval was received to expand recruitment to sites in Tanzania (Dar es Salaam) and in Malawi (Zomba). Recruitment at the Zambia site was very slow and stopped in June 2015.

3.3.   Treatments

Trial arms:

The primary treatment strategies are:

Oral: Fluconazole 1200 mg /d plus flucytosine 25 mg/kg qds for 2 weeks
AmB7:   Amphotericin B (AmB) 1 mg/kg/d for 7 days
AmB14:   Amphotericin B (AmB) 1 mg/kg/d for 14 days

Adjunct treatment: In both the **AmB7** and **AmB14** arms, participants will receive an adjunct treatment of either fluconazole 1200 mg/d or flucytosine 25 mg/kg qds. This is also

randomised and its analysis will be the subject of a second separate analytical plan. In the **AmB7** arm, fluconazole is given at 1200 mg/d in the second week.

All participants are asked to be hospitalised for 14 days. On rare occasions participants may be discharged earlier if he/she is on a 7-day regimen, is well enough, and the team are confident that he/she will return for follow up monitoring bloods.

Following day 14, participants are scheduled to return at 4, 6, 8 and 10 weeks. After day 14, fluconazole is given at 800 mg/d until ART is started, then at 400 mg/d until 10 weeks, and 200 mg/d thereafter. ART is initiated at week 4.

3.4.   Randomisation

Randomisation is stratified by site. At each site, randomisation is done in blocks of sizes 18, 24 and 30. The size of each block is determined randomly. Within each block, participants are randomly assigned to the 5 different treatments, Oral, Amb7 + fluconazole, Amb7 + flycytosine, Amb14 + fluconazole and Amb14 + flycytosine with probabilities 1/3, 1/6, 1/6, 1/6 and 1/6 respectively. Thus, overall within each block, 1/3 of participants were randomly assigned to each of the **Oral, Amb7** and **Amb14** strategies. The randomisation was done by Dr Victoria Simms of LSHTM using Stata software version 13. For each site a sequence of study IDs and treatment allocations was generated. This was done by creating a random variable and ordering on that variable. The allocations were put into sealed opaque envelopes and the study ID was written on the outside. When a participant is enrolled they are given a study number sequentially and the envelope corresponding to that number is opened to reveal their treatment allocation.

3.5.   Sample Size

Using a non-inferiority design with a 10% non-inferiority margin and 5% one-sided type 1 error; and assuming 85% 2-week survival with the **AmB14** strategy, would require 157 participants allocated to each strategy at 80% power, 184 per strategy at 85% power, and 219 per strategy at 90% power. Based on these calculations the recruitment target was 680 participants (226 per strategy) in order to achieve 90% power, allowing for 2% losses to follow-up.

4.   ANALYSIS POPULATIONS

4.1.   Study population data sets

Intent-to-treat (ITT) will be defined at the moment the randomisation is performed. For the ITT analysis in this trial, participants will be followed with their ITT arms. Thus, for example, if a participant receives the wrong treatment or no treatment at all, he/she will be analysed according to the arm to which they were allocated. This will be the primary analysis for the trial.

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There are also a number of late exclusion criteria based on the results of baseline bloods. If *Alanine* transaminase (ALT) is >5 times upper limit of normal, polymorphonuclear leukocytes (PMNs) <500 x 106/L, or platelets <50,000x106/L participants are withdrawn from the study because of the possibility of exacerbation of liver function abnormality with fluconazole and bone marrow depression with flucytosine. In addition, if a repeat creatinine remains above 220 µmol/L, despite rehydration, participants are withdrawn from the study, and treated with high dose fluconazole (1200 mg/d, adjusted for renal function) in conjunction with routine medical management, and consistent with local guidelines. Individuals fulfilling the late exclusion criteria will be reported in the consort chart but will be removed from analyses. **These late exclusion criteria are not included in the initial exclusion criteria due to the need to initiate treatment urgently.**

We have considered a modified ITT (mITT), allocating participants according to the regimen they received as the first dose. However, this number will likely be only 2-3 participants out of the entire total. The findings of mITT will be near identical to ITT and therefore we will present only ITT results.

Per-protocol population

Per protocol population will be defined on the basis of the first two weeks of treatment and follow-up as follows:

- Those who received the treatments as according to the protocol and were alive at the end of 14 days will be included.

- Those who died within 14 days and received the treatments as according to the protocol up to their time of death will also be included.

Participants will be **excluded from the per-protocol population** if they:

- Did not adhere to per protocol treatment (i.e. missed >1 dose of study treatment during the first 2 weeks of treatment without sound clinical reason). Participants in whom study drug is withheld, or stopped early, in accordance with the protocol, due to known toxicities, will be retained in the per-protocol analysis.

- Switched treatment. The treatment groups in the per-protocol analysis will be defined according to what the participant actually received (e.g. a participant was randomised to Amb7 but received Amb14 as according to the protocol for Amb14, then he/she will be allocated to the Amb14 arm for this analysis).

Safety population

This will be defined as all study participants who received at least one dose of any of the study interventions.

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4.2.   Analysis Close Date

The analysis close date is the date on which the last participant completed 10-week follow-up.

Last contact date (also referred to as Trial reference end date): the date of the last trial related procedure. For survival subjects it is defined as the maximum of
- Date of last office visit (scheduled or unscheduled visit)
- Date of the last follow-up contact (including last date on subject survival status recorded)
- Date of the last known adverse event (AE) status or lab results reported on the AE or lab case report from (CRF) pages, respectively

Data cleaning

The data will then be checked to ensure that there are no erroneous entries and that all missing data is properly coded. Any changes will be made on the Datafax database.

4.3. Data download

For each time point, once all data have been inputted and checked, the database will be locked and a data download request made. The data will be downloaded into SAS and STATA formats for statistical analyses.

5.   STATISTICAL ANALYSES

5.1.   Primary Outcome Analysis

5.1.1.   ITT analysis of the primary outcome - the primary analysis

The primary outcome is a binary outcome: occurrence of death from any cause at anytime up to and including day 14. The primary analysis will be based on the ITT population as defined above.

The primary endpoint will be summarised by number (%) of participants that have died by treatment group. A formal statistical analysis will be performed as a one-sided non-inferiority test, using the non-inferiority margin $\Delta = 0.10$.

In the primary analysis, participants lost to follow-up will be included in the denominators but will not be considered as a death in the numerator (i.e. effectively censored). Thus, for example, if n=200 were randomised, and by two weeks 50 had died, 3 had been lost lost to follow-up and 147 were confirmed alive, then the proportion of deaths used for the primary analysis will be 50/200 (25%).

Sensitivity analysis will be conducted (described below). These will be part of secondary analyses.

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Our hypothesis is as follows:

Hypothesis: Let π_2 denote the mortality rate at 2 weeks in the **Oral** arm, and let π_1 denote the mortality rate at 2 weeks in the **AmB14** arm. The hypothesis test is

H_0: $\pi_2 - \pi_1 \geq \Delta$ versus the alternative H_A: $\pi_2 - \pi_1 < \Delta$

A generalised linear model (GLM) will be used to test the above hypothesis. In the GLM model, the occurrence of death at 2 weeks will be treated as the response variable following a binomial distribution and the treatment as fixed effect, and identity link function will be used. From this model, a point estimate in the 2-week mortality rate difference ($\pi_2 - \pi_1$) and its one-sided 95% upper limit (two-sided 90% upper limit) for the comparison between **Oral** arm and the **AmB14** arm will be estimated. **Oral** treatment will be judged not inferior to the **AmB14** treatment if the upper confidence limit is less than Δ, where $\Delta= 10\%$, the predetermined non-inferiority margin.

The comparison between **AmB7** arm and the **AmB14** will be made in the same way.

The GLM model will be estimated using SAS GENMOD.

A simple Z-test (appromixate normal distribution) for comparing two proportions will also be done as a crude simple analysis to confirm the findings from the GLM model. A two-sided 90% confidence interval will be calculated.

$$Z = \frac{P_1 - P_2}{SE(P_1 - P_2)}$$
$$SE(P_1 - P_2) = \sqrt{[P(1-P)(1/n_1 + 1/n_2)]}$$

Where $P = \dfrac{n_1 P_1 + n_2 P_2}{n_1 + n_2}$ and $SE(P_1 - P_2)$ is the standard error of $P_1 - P_2$.

The GLM will be the primary analysis. However, in the unlikely event that the GLM model does not converge, we will use the Z-test as the primary analysis.

5.1.2.   A per-protocol analysis of the primary outcome

A supportive analysis of the primary outcome will also be performed on the per-protocol population. Statistical methods will be the same as used in the Section 5.1.1.

5.1.3.   Sensitivity analysis of the primary outcome

A sensitivity analysis of the primary endpoint will be performed. This will use the ITT population to compare the **Oral** arm to the **AmB7** arm and to the **AmB14** arm during the first two weeks. All participants lost to follow-up during this time will be classified as dead. A

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TMG member, not directly involved in patient management and blind to study arm, will also classify the participant lost according to likely dead or likely alive and these data will be reported.

As mentioned above, the number lost to follow-up will likely be very small. The sensitivity analyses may give near identical results to the primary analysis. In this case, there might be little point in reporting the sensitivity analysis.

Our primary endpoint involves censoring those lost within 2 weeks, as discussed above. This is simple and consistent with the most appropriate assumption for 10-week mortality, a key secondary endpoint (it would not make sense to do the 10-week mortality endpoint classifying those lost as dead as many will not have died). Censoring at 2 weeks is also probably more accurate when the participant is lost close to day 14.

As mentioned above, by August 2015, only 1 patient had been lost to follow-up in the first 2 weeks (at day 5).

5.1.4. Covariate adjusted analysis of the primary outcome

An analysis of the primary endpoint adjusted for site, age, sex, GCS, CD4 count, CFU at baseline, and ART status at baseline will be done comparing the **Oral** arm to the **AmB14** arm and the **AmB7** arm to the **AmB14** arm. This will be done to correct for any possible baseline imbalances between the arms after randomisation.

From the above model, the adjusted point estimate and 95% one-sided upper limit comparing the **Oral** arm to the **AmB14** arm and the **AmB7** arm to the **AmB14** arm will be derived.

The above identity-binomial GLM model may not converge when all covariates are introduced into the model simultaneously. If this occurs, the adjusted regression model will be established by removing one or more of the covariates: site, age, sex, CD4 count, and ART status. If an identity-normal GLM model can not be established after adjusting for covariates, a simple linear regression model will be employed from which the mortality difference and its 95% one-sided upper limit will be derived.

5.1.5. Subgroup analysis of the primary outcome

Subgroup analyses will be performed. We will stratify by age, sex, GCS, CD4 count, CFU and ART status at baseline. Age, CD4 count and CFU will be categorised into two approximately equal sized categories (i.e. cut-off at the median). GCS will be categorised at <15 or 15 or more. ART status at baseline is a simple yes/no categorical variable. Sex will be male or female.

In addition, we will stratify by disease severity, defining this as GCS<15 <u>and</u> CFU>median, which we have shown to be an important prognostic indicator in our past studies.

Comparisons between treatment strategies for both the 2 and 10-week mortality endpoints

will be analysed for each of these strata.

5.1.6.   Superiority analysis of the primary outcome

Two-sided 95% confidence intervals for the difference in the 2-week mortality rate between the **Oral or Amb7** and **Amb14** will be derived. In addition, superiority analysis will be performed for comparison between **AmB14** and **Oral arm**, and comparison **AmB7** and **Oral arm**.

5.2.   Secondary Outcome Analysis

All secondary outcomes will be analysed as for a superiority designed trial and two-sided significance with alpha=5% and standard 95% CIs for the treatment differences in these outcomes between two treatment groups will be calculated and presented. Secondary outcome analyses will be based on the ITT population unless specified.

5.2.1   Analysis of binary outcomes

Death from any cause at anytime up to and including day 70 (i.e. 10-week mortality) and day 28 will be treated as a binary outcome and will be summarised by number (%) of participants with event by treatment group and analysed in a similar way as the primary endpoint by means of GLM model. The point estimate risk difference and its two-sided 95% upper limit for three pairwise comparisons (**Oral** arm vs. **AmB14** arm, **AmB7** arm vs **AmB14,** and **Oral** arm vs **AmB7** arm) will be estimated.

A sensitivity analysis of 10-week mortality will also be carried out. Participants lost to follow-up will be classified as dead as a worse case scenario. We will also report the likely outcome of these losses (i.e. likely dead or likely to be alive) as ascertained by a TMG member, not directly involved in patient management and blind to study arm.

The analysis of other binary outcomes such as clinical and laboratory-defined grade 3 and 4 adverse events will also use GLM model with treatment as fixed effect. Relative risks, odds ratios and risk differences with their two-sided 95% confidence intervals comparing two treatment arms will be derived from the GLM models with binomial distribution, and log, logit and identity link functions, respectively. In order to simplify presentation only the relative risks and risk differences will be reported.

5.2.2   Analysis of time-to-event outcomes

Mortality at day 14, 28 and 70 will also be analysed as time-to-event outcomes (e.g. time from randomisation to the occurrence of death from any cause at the end of day 14, 28 and 70) and will be summarised by the number (%) of participants with an event, person-years, and incidence rates by treatment arm.

The trial arms will be compared using the log-rank test, as a two-sided test. The Kaplan-Meier plots will be drawn to describe the timings of of deaths by treatment arms. Cox

regression model will be used to derive hazard ratio and its 2-sided 95% confidence interval for comparing two treatment groups.

In the first analysis, we will censor participants lost on the last date that they were known to be alive (i.e. they will be considered as alive up to that time point). A sensitivity analysis will be done assuming that all those lost died on the last date that they were known to be alive. In addition, we will report the findings from the TMG member, not directly involved in patient management and blind to study arm, of the likelihood that the person is dead or alive.

5.2.3   Analysis of continuous outcomes

The continuous outcome such as biochemical markers will be summarised using number of subjects (n), mean, standard deviation (SD), minimum, and maximum by treatment group, and will be analysed by a GLM model with treatment as fixed effect and with normal distribution and identity link function. Difference in mean outcome and mean differences with their two-sided 95% confidence intervals between two groups will be derived from the GLM model.

5.2.4   Analysis of count outcomes

We don't have any "count" data.

5.2.5   Analysis of secondary outcomes with repeated measurements

The CSF fungal count are measured on day 1, day 7 and day 14. CSF fungal count will be transformed using log(CSF fungal count +1) to avoid taking the log of zero. Two analyses will be done.

Changes in log CSF fungal count over 14 days from baseline will be summarised and analysed using a generalised estimating equation (GEE) model with treatment, day and interaction between treatment and day as fixed effects, log baseline measurement of fungal count as covariate, and subject as cluster effect. Ratio in geometric mean with their two-sided 95% confidence intervals in fungal count between two arms will be derived from the GEE model. Model assumption will be assessed using Q-Q plots for the model residuals.

We will also calculate the slope of CSF fungal count decline for each patient using simple linear regression as done previously by us (Ref: Lancet 2004; 363: 1764-67 and Clin Infect Dis 2014; 58: 736-45). All data points will be used except for sterile cultures at the second week LP (i.e. day 14 +/- 1 or 2 days maximum) if this value reduced the slope. In these cases CSF sterility would probably have been achieved some time between days 7 and 14, and use of the day 14 result would therefore underestimate the true slope.

Wilcoxon rank-sum tests will be used to compare the slopes between groups.

5.2.6   Analysis of other secondary outcomes

Other statistical methods may be used if deemed necessary.

5.3.   Exploratory Analysis

None are planned.

6.   SAFETY ANALYSES

6.1.   Safety Variables

Adverse events (AEs) will be summarised using the number of AEs, the number (%) of participants with AEs by treatment arms.

The substudy collecting ECG data has stopped (as planned and in accordance with IDMC and TSC approval) and will be analysed and reported separately.

Blood monitoring laboratory data, occurrence of suffering clinical and laboratory-defined grade 3 and 4 adverse events to 10 weeks will be analysed descriptively.

7.   GENERAL CONSIDERATIONS FOR DATA ANALYSES

SAS® (version 9.3) will be used to perform all data analyses and generate the majority of data displays. STATA or SPSS or S-Plus or R may also be used for some data analyses.

7.1.   Multi-site consideration

The data analyses will be performed on a combined-site basis. Of note, the randomisation was performed centrally. There are 9 sites in the study: Lusaka in Zambia; Blantyre, Lilongwe, Zomba in Malawi; Duuala, Yaounde in Cameroon; Muhimbili, Mwananymala, and Amana in Tanzania. Nine sites may be collapsed into 4 countries (Zambia, Malawi, Cameroon, and Tanzania) in the covariate adjusted analysis and subgroup analysis in order to produce converging and stable estimate of treatment effects.

7.2.   Covariates Analyses

Covariate analyses will be performed on the primary outcome and the key secondary outcomes, in particular the 10-week mortality outcome (See Section 5.1.3.) on the ITT population. Other covariate analyses will be performed if deemed necessary.

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7.3. Subgroup Analysis

Subgroup analyses will be performed for the primary and key secondary outcomes on the ITT population. The subgroup variable will be the site, age, sex, GCS, CD4 count, CFU, ART status and disease severity, as defined above for covariated adjusted analysis.

7.4. Multiplicity and missing values.

Multiplicity adjustment will not apply to the primary and secondary outcome analyses.

Missing baseline covariates will be imputed using simple imputation methods in the covariate adjusted analysis based on the covariate distributions. For a continuous variable, missing values will be imputed from random values from a normal distribution with mean and SD calculated from the sample. For a categorical variable, missing values will be imputed from random values from a uniform distribution with probabilities P_1, P_2, ..., and P_k from the sample. For a count data, missing values will be imputed from random values from a Poisson distribution with λ from the sample. Seed for the imputation is set as 128.

7.5. Other Data Considerations

7.5.1 Data Summaries

Continuous variables will be summarised according to number of subjects with non-missing data (n), mean, standard deviation (SD), median, minimum, and maximum. The confidence interval will be added on summaries of continuous effectiveness variables.

Categorical variables will be summarised according to the absolute frequency and percentage of subjects (%) in each category level. The denominator for the percentages is the number of subjects in the treatment arm with data available, unless noted otherwise. Event rates per 100 participant years will also be reported for time-to-event clinical outcomes and adverse events of special interest.

7.5.2 Graphical Displays

Mean values for some continuous outcomes by treatment and visit will be plotted. Kaplan-Meier plots will be produced for displaying the time-to-event data.

8. REFERENCES

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9.

ADJUNCT

A PHASE III, RANDOMISED, CONTROLLED TRIAL FOR THE TREATMENT OF HIV-ASSOCIATED CRYPTOCOCCAL MENINGITIS: THE ACTA TRIAL

STATISTICAL ANALYSIS PLAN

FOR THE STUDY COMPARING AMPHOTERICIN B-BASED THERAPY ADJUNCT TREATMENTS: FLUCONAZOLE VERSUS FLUCYTOSINE.

ISRCTN registration number: ISRCTN45035509

ANRS Reference: ANRS12275

10. INTRODUCTION

This document describes the statistical analysis of a phase III, randomised, controlled trial – the ACTA trial - to compare fluconazole versus flucytosine as adjunct treatments for either a 7-day or 14-day course of amphotericin B.

This is the second of two analysis plans from the ACTA trial. The first plan deals with the comparison of oral fluconazole plus flucytosine combination versus one week amphotericin b-based therapy versus two weeks amphotericin b-based therapy for the initial treatment of cryptococcal meningitis.

The one week and two week amphotericin B based treatments are given with the adjunct of either flucytosine or fluconazole; this choice of adjunct is also randomised.

This analysis plan focuses on the comparison between the two adjunct treatments, fluconazole versus flucytosine, and the comparison between either the one or two week amphotericin B based treatments with each adjunct versus the combination oral regimen.

The data from the trial have been separated in this way as the volume of analyses and information are too great to describe in a single plan or in a single paper.

11. STUDY OBJECTIVES AND OUTCOMES

11.1. Study objectives

11.1.1. Primary objective

To determine the effects of fluconazole versus flucytosine when given as adjuncts to amphotericin-based therapy with respect to all-cause mortality.

The statistical analysis will determine whether one is superior to the other. It will be done at a 5% two-sided significance level.

2.1.3 Secondary objectives

a). To determine the effects of the oral regimen (fluconazole and flucytosine) compared with 7-day amphotericin B and 14-day amphotericin B separately for each adjunct. This will involve 4 comparisons:

      i)   Oral regimen versus 7-day amphotericin B combined with fluconazole adjunct

     ii)   Oral regimen versus 7-day amphotericin B combined with flucytosine adjunct

     iii)   Oral regimen versus 14-day amphotericin B combined with fluconazole adjunct

     iv)   Oral regimen versus 14-day amphotericin B combined with flucytosine adjunct

b) To determine the effects of each adjunct overall and the effects of each adjunct – amphotericin B combination on the rate of clearance of infection and incidence of serious (grade III and grade IV) adverse events.

11.2. Outcomes

11.2.1. Primary outcome

The primary endpoint is defined as death from any cause at anytime up to and including the last day of follow-up (i.e. day 70) (from the time of randomisation).

In studies of cryptococcal meningitis management, primary endpoints are usually either 2-week or 10-week mortality. Both are crucially important.

Our primary endpoint for the adjunct treatments comparison differs from the primary endpoint for the comparison of the Oral verus Amb7 versus Amb14 initial treatment regimens, for which 2-week mortality was chosen as the primary endpoint.

The sample size for the comparison of Oral verus 7-day amphotericin B versus 14-day amphotericin B is dictated by a non-inferiority design for this comparison. It is likely that 2-week mortality is more specific to cryptococcal meningitis compared with a 10-week mortality outcome. This possible lower specificity for the 10-week timepoint would increase the possibility of demonstrating non-inferiority at this timepoint; therefore to be conservative, we chose 2 week as the primary endpoint for the comparison of oral versus Amb7 versus Amb14.

The present comparison of flucytosine versus fluconazole adjuncts is based on a superiority hypothesis. We have chosen as the primary endpoint, death at anytime up to 10 weeks. In other words, we propose to simply compare mortality between the two regimens over the course of our follow-up.

Because of the substantial follow-up time, the primary analysis will be done as a time-to-event analysis and the comparison will be made by a log-rank test.

11.2.2. Secondary outcomes

Efficacy:

The occurrence of death from any cause from randomisation to any time up to the end of day 70. This is a binary event.

Death from any cause at anytime up to and including day 14 (i.e. 2-week mortality) (binary outcome).

Time from randomisation to the occurrence of death from any cause at the end of day 14 (survival time analysis).

Death from any cause, at anytime up to and including day 28 (i.e. 4-week mortality).

Time from randomisation to the occurrence of death from any cause at the end of day 28.

Log CSF fungal count at day 7 and 14.

Changes in log CSF fungal count at day 7 and 14.

Safety:

- Occurrence of suffering clinical and laboratory-defined grade 3 and 4 adverse events to 10 weeks.

11.2.3. Case ascertainment and case definitions

(1). Deaths

Most deaths up to and including day 14 will occur in hospital because participants are hospitalised. However, participants who discharge themselves (or are discharged for whatever reason) will be contacted in the community, including by home visit if needed, if they do not return to hospital at day 14. Exhaustive attempts will be made through participant and next of kin contact and home details in order to ascertain the two-week survival status.

Participants will have regular clinic appointments following discharge and will continue to be followed up until day 70. Those who do not attend are telephoned the same day. If they cannot be contacted, a home visit is made to encourage re-attendance and continued treatment. In prior studies on cryptococcal meningitis in Africa, the lost-to follow up rate has been kept at $\leq$3% at 10 weeks.

Details of participants lost to follow-up will be sent to a TMG member, not directly involved in patient management and blind to study arm, who will classify the participant as likely died or likely alive and estimate the date of death. Details of these will be analysed are further below. By the end of August 2015, only 5 participants had been lost to follow-up and of these just one participant was lost within 2 weeks.

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(2). CSF fungal count

CSF is serially diluted 10 fold, with careful mixing at all stages, and from each dilution, 100 microL plated onto each half of a Sabouraud dextrose agar plate, and incubated at 30^{0}C. After 2- 3 days, colonies are counted by trained laboratory staff blinded to treatment assignment. Plates are re-incubated until, on daily inspection no new colonies are seen. Negative plates are kept for at least 7 days. Counts are taken from the plate with the least number of colonies, but a total of at least 30 colonies. The average of the counts on each half of the plate is taken and multiplied up to give the CFU count per ml of CSF (Ref: Lancet 2004; 363:1764-67).

(3). Clinical and laboratory-defined grade 3 and 4 adverse events.

These are reported to the trial management group and entered into the database in real time. DAIDS criteria are used.

(4). IRIS reactions

All suspected, clinically-significant IRIS reactions are reported to the trial management group through the SAE reports, which includes IRIS as a pre-defined cause of adverse event and re-admission, and through CRFs completed at weeks 6, 8, and 10 of follow up. Of note, trial follow up is only for 10 weeks so that only early IRIS events will be identified. For the main paper, an IRIS event will be determined by the study doctor and included in the table of adverse events as a grade 4 AE.

(5). Cause of deaths

Most deaths occur in hospital, either during the initial 2 weeks of admission or between weeks 2 and 10 after re-admission. In either case, information on these deaths is available and could be used to estimate the cause of death.

A much smaller number of deaths occur at home during the 10 week follow up. In this case, relatives are telephoned and visits to the participant's address are carried out to estimate the cause of death. Relatives are asked about symptoms and circumstance prior to death and an assessment of likely cause of death is made.

Staff at study sites complete a death form with presumed cause(s) and narrative, which excludes treatment assignment. A single TMG member, who is not involved in SAE reporting, assesses cause of death, blinded to treatment assignment. If there is disagreement between site assessment of cause of death and the TMG assessment, an expert independent opinion is sought, again blind to treatment arm.

These data are being collected for safety analyses for the DMC. They provide crude estimates of possible causes of death. Clinical autopsies are not done in any of the settings

where the trial is based. Consistent with other publications on cryptococcal meningitis, the causes of death will not be reported in the papers discussed in these analytical plan.

Additional safety variables:

Monitoring blood laboratory values. These will be used to calculate mean decreases in haemoglobium, neutrophil count, and potassium, and increases in creatinine and ALT, reflective of known laboratory side effects of the study drugs, over the first one and two weeks.

12. STUDY DESIGN

12.1. Design

The trial is an open-label, phase III randomised controlled trial. Adult patients with a first episode of cryptococcal meningitis who fulfil the eligibility criteria as outlined in the protocol are invited to join the trial consecutively and are randomised individually to fluconazole or flucytosine adjunct therapy. These particpants are also receiving either 7-day amphotericin B or 14-day amphotericin B (this comparison is also randomised – see the accompanying analytical plan describing the comparison of oral versus 7-day amphotericin B versus 14-day amphotericin B).

12.2. Trial sites

The trial is being conducted in African sites: Kamuzu Central Hospital, Lilongwe, and Queen Elizabeth Hospital, Blantyre, Malawi; at University Teaching Hospital Lusaka, Zambia; at The Hopital General in Douala; and at Central Hospital, Yaounde, Cameroon.

Towards the end of 2014, it became clear that recruitment was slower than expected. Therefore, in January 2015, approval was received to expand recruitment to sites in Tanzania and Malawi. Recruitment at the Zambia site had been very slow and this was stopped in June 2015.

12.3. Treatments

Trial arms:

The primary treatment strategies are:

Oral: Fluconazole 1200 mg /d plus flucytosine 25 mg/kg qds for 2 weeks
AmB7:   Amphotericin B (AmB) 1 mg/kg/d for 7 days
AmB14:   Amphotericin B (AmB) 1 mg/kg/d for 14 days

Adjunct treatment: In both the **AmB7** and **AmB14** arms, participants receive an adjunct treatment of either fluconazole 1200 mg/d or flucytosine 25 mg/kg qds. This is a

randomised comparison. In the **AmB7** arm, fluconazole is given at 1200 mg/d in the second week.

All participants are asked to be hospitalised for 14 days. On rare occasions participants may be discharged earlier if he/she is on a 7-day regimen, is well enough, and the team are confident that he/she will return for follow up monitoring bloods.

Following day 14, participants are scheduled to return at 4, 6, 8 and 10 weeks. After day 14, fluconazole is given at 800 mg/d until ART is started, then at 400 mg/d until 10 weeks, and 200 mg/d thereafter. ART is initiated at week 4.

The list of regimens in the database as follows:

Regimen 1: Fluconazole (FLU) 1200 mg daily + flucytosine (5-FC) 25 mg/kg four times daily for 14 days
Regimen 2A: AmB 1 mg/kg/d +FLU 1200 mg daily for 7 days
Regimen 2B: AmB 1mg/kg/d +5-FC 25 mg/kg four times daily for 7 days
Regimen 3A: AmB 1 mg/kg/d + FLU 1200 mg daily for 14 days
Regimen 3B: AmB 1 mg/kg/d + 5-FC 25 mg/kg four times daily for 14 days

There will be two adjunct treatment groups:
Adjunct A: Fluconazole (Regimens 2A and 3A)
Adjunct B: Fucytosine (Regimens 2B+3B)

Thus, the adjunct treatments will be compared across both amphotericin B arms.

12.4.   Randomisation

Randomisation is stratified by site. At each site, randomisation is done in blocks of sizes 18, 24 and 30. The size of each block is determined randomly. Within each block, participants are randomly assigned to the 5 different treatments, Oral, Amb7 + fluconazole, Amb7 + flycytosine, Amb14 + fluconazole and Amb14 + flycytosine with probabilities 1/3, 1/6, 1/6, 1/6 and 1/6 respectively. Thus, overall within each block, 1/3 of participants were randomly assigned to each of the **Oral**, **Amb7** and **Amb14** strategies. The randomisation was done by Dr Victoria Simms of LSHTM using Stata software version 13. For each site a sequence of study IDs and treatment allocations was generated. This was done by creating a random variable and ordering on that variable. The allocations were put into sealed opaque envelopes and the study ID was written on the outside. When a participant is enrolled they are given a study number sequentially and the envelope corresponding to that number is opened to reveal their treatment allocation.

12.5.   Sample size

The trial aims to recruit 680 participants. A third of these will be allocated to the **Oral** arm and so will not be included in the analysis outlined in this analytical plan. We assume 2% loss to follow up, so therefore if recruitment targets are reached there will be about 452

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subjects in this analysis, approximately half of whom will be randomly assigned to fluconazole and half to flucytosine.

The sample size was dictated by the main strategies: oral versus 7-day AmB versus 14 day AmB. For this comparison, if 10-week mortality was 40% in one adjunct treatment, then we would have 90% power to detect a relative difference of 35% or more between the two treatments (i.e. 40% versus 26%), and about 80% power to detect a relative difference of 30% or more.

13. ANALYSIS POPULATIONS

13.1. Study population data sets

Intent-to-treat (ITT) will be defined at the moment the randomisation is performed. For the ITT analysis in this trial, participants will be followed with their ITT arms. Thus, for example, if a participant receives the wrong treatment or no treatment at all, he/she will be analysed according to the arm to which they were allocated. This will be the primary analysis for the trial.

There are also a number of late exclusion criteria based on the results of baseline bloods. alanine transaminase (ALT) is >5 times upper limit of normal, polymorphonuclear leukocytes (PMNs) <500 x 106/L, or platelets <50,000x106/L participants are withdrawn from the study because of the possibility of exacerbation of liver function abnormality with fluconazole and bone marrow depression with flucytosine. In addition, if a repeat creatinine remains above 220 µmol/L, despite rehydration, participants are withdrawn from the study, and treated with high dose fluconazole (1200 mg/d, adjusted for renal function) in conjunction with routine medical management, and consistent with local guidelines. Individuals fulfilling the late exclusion criteria will be reported in the consort chart but will be removed from analyses. **These late exclusion criteria are not included in the initial exclusion criteria due to the need to initiate treatment urgently.**

We have considered a modified ITT (mITT), allocating participants according to the regimen they received as the first dose. However, this number will likely be only 2-3 participants out of the entire total. The findings of mITT will be near identical to ITT and therefore we will present only ITT results.

Per protocol population will be defined on the basis of the first two weeks of treatment and follow-up as follows:

- Those who received the treatments as according to the protocol and were alive at the end of 14 days will be included.

- Those who died within 14 days and received the treatments as according to the protocol up to their time of death will also be included.

Participants will be **excluded from the per-protocol population** if they:

- Did not adhere to the per protocol treatment (took less than the recommended doses over recommended time period (i.e. missed >1 dose of study treatment during the first 2 weeks of treatment without sound clinical reason). Participants in whom study drug is withheld, or stopped early, in accordance with the protocol, due to known toxicities, will be retained in the per-protocol analysis.

- Switched treatment. The treatment groups in the per-protocol analysis will be defined according to what the participant actually received (e.g. a participant was randomised to Amb7 but received Amb14 as according to the protocol for Amb14, then he/she will be allocated to the Amb14 arm for this analysis).

Safety population. This will be defined as all study participants who received at least one dose of any of the study interventions.

13.2.   Analysis close date

The analysis close date is the date on which the last participant completed 10-week follow-up.

Last contact date (also referred to as Trial reference end date): the date of the last trial related procedure. For survival subjects it is defined as the maximum of
- Date of last office visit (scheduled or unscheduled visit)
- Date of the last follow-up contact (including last date on subject survival status recorded)
- Date of the last known adverse event (AE) status or lab results reported on the AE or lab case report from (CRF) pages, respectively

Data cleaning

The data will then be checked to ensure that there are no erroneous entries and that all missing data is properly coded. Any changes will be made on the Datafax database.

13.3. Data download

For each time point, once all data have been computerised and checked, the database will be locked and a data download request made. The data will be downloaded into SAS and STATA formats for statistical analyses.

14. STATISTICAL ANALYSES

14.1. Primary outcome analysis

14.1.1. ITT analysis of the primary outcome - the primary analysis

The primary outcome is the incidence rate of all-cause mortality from recruitment up to 10 weeks after recruitment. Kaplan-Meier curves showing survival according to study arm will be calculated, and the log-rank test will be used to compare the survival curves. This will be the primary comparison between the two treatments.

Person years of observation (PYO) will be estimated as the time from enrolment into the trial to the date of death, withdrawal, loss to follow up, or 10 weeks after recruitment. These will be used in the calculation of incident rates and 95% confidence intervals. Hazard ratios will be calculated from a Cox regression.

All comparisons (primary and secondary) will be done using a 5% two-sided significance level.

14.1.2. Per protocol analysis of the primary outcome

An analysis of the primary outcome will also be performed on the PP population and stratified by AmB treatment (whether one or two weeks of amphotericin B). Statistical methods will be the same as used in the Section 5.1.1.

14.1.3. Sensitivity analysis of the primary outcome

A sensitivity analysis of the primary endpoint will be performed. This will use the ITT population. All participants lost to follow-up during this time will be classified as dead. We will also have information from the TMG member, not directly involved in patient management and blind to study arm,, who will classify the participant lost according to likely dead or likely alive and these data will be reported.

As mentioned above, the number lost to follow-up will likely be very small. The sensitivity analyses may give near identical results to each other, and indeed to the primary analysis. In this case, there might be little point in reporting the sensitivity analysis.

14.1.4. Covariate adjusted analysis of the primary outcome

An analysis of the primary endpoint adjusted for site, age, sex, GCS, CD4 count, CFU, ART status, and by length of AmB treatment will be done comparing the fluconazole and flucytosine adjuncts. This will be done to correct for any possible baseline imbalances between the arms after randomisation.

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This will be done using Cox proportional hazards regression. If the model is unstable, then one or more covariates will be dropped from the list: site, age, sex, CD4 count, ART status, and length of AmB treatment

14.1.5. Further analysis of the primary outcome

Subgroup analyses will be performed. We will stratify by amphotericin B (7 or 14 days), age, sex, GCS, CD4 count, CFU, ART status, CSF opening pressure, concurrent TB, haemoglobin, and creatinine at baseline. Age, CD4 count, CFU, CSF opening pressure, haemoglobin, and creatinine will be categorised into two approximately equal sized categories (i.e. cut-off at the median). GCS will be categorised at <15 or 15 or more. ART status at baseline and TB are a simple yes/no categorical variable. Sex will be male or female.

In addition, we will stratify by disease severity, defining this as GCS<15 and CFU>median, which we have shown to be an important prognostic indicator in our past studies.

Comparisons between fluconazole and flucytosine for both the 2 and 10-week mortality endpoints will be analysed for each of these strata.

The fluconazole and flucytosine adjuncts when used with either 7-day or 14-day amphotericin B will be compared with the oral regimen. These comparisons will be as follows:

i). Oral combination versus 7-day amphotericin B with flucytosine adjunct
ii). Oral combination versus 7-day amphotericin B with fluconazole adjunct

iii). Oral combination versus 14-day amphotericin B with flucytosine adjunct
iv). Oral combination versus 14-day amphotericin B with fluconazole adjunct

These comparisons will be done for the primary endpoint and also stratified by the variables age, sex, GCS, CD4 count, CFU, ART status, CSF opening pressure, TB, haemoglobin, and creatinine at baseline, as above.

14.2. Secondary outcome analysis

The secondary outcomes will be analysed overall, stratified as above, and also, as above, we will compare the fluconazole and flucytosine adjuncts when used with either 7-day or 14-day amphotericin B with the oral regimen.

Survival time data will be analysed as above for the primary endpoint. Death will also be treated as a binary outcome and compared between groups using a GLM model. Relative risks, odds ratios and risk differences with their two-sided 95% confidence intervals comparing two treatment arms will be derived from the GLM models with binomial

distribution, and log, logit and identity link functions, respectively. In order to simplify presentation only the relative risks and risk differences will be reported.

The continuous outcome such as biochemical markers will be summarised using number of subjects (n), mean, standard deviation (SD), minimum, and maximum by treatment group, and will be analysed by a GLM model with treatment as fixed effect and with normal distribution and identity link function. Difference in mean outcome and mean differences with their two-sided 95% confidence intervals between two groups will be derived from the GLM model.

The CSF fungal count are measured on day 1, day 7 and day 14. CSF fungal count will be transformed using log(CSF fungal count +1) to avoid taking the log of zero. Two analyses will be done.

Changes in log CSF fungal count over 14 days from baseline will be summarised and analysed using a generalised estimating equation (GEE) model with treatment, day and interaction between treatment and day as fixed effects, log baseline measurement of fungal count as covariate, and subject as cluster effect. Ratio in geometric mean with their two-sided 95% confidence intervals in fungal count between two arms will be derived from the GEE model. Model assumption will be assessed using Q-Q plots for the model residuals.

We will also calculate the slope of CSF fungal count decline for each patient using simple linear regression as done previously by us (Ref: Lancet 2004; 363: 1764-67 and Clin Infect Dis 2014; 58: 736-45).). All data points will be used except for sterile cultures at the second week LP (i.e. day 14 +/- 1 or 2 days maximum) if this value reduced the slope. In these cases CSF sterility would probably have been achieved some time between days 7 and 14, and use of the day 14 result would therefore underestimate the true slope. Wilcoxon rank-sum tests will be used to compare the slopes between groups.

15.   SAFETY ANALYSES

15.1.   Safety variables

Adverse events (AEs) will be summarised using the number of AEs, the number (%) of participants with AEs by treatment arms.

The substudy collecting ECG data has stopped (as planned and in accordance with IDMC and TSC approval) and will be analysed and reported separately.

Blood monitoring laboratory data, occurrence of suffering clinical and laboratory-defined grade 3 and 4 adverse events to 10 weeks will be analysed descriptively.

16.   GENERAL CONSIDERATIONS FOR DATA ANALYSES

SAS® (version 9.3) will be used to perform all data analyses and generate the majority of data displays. STATA or S-Plus or R may also be used for some data analyses.

16.1.   Stratified analysis

Inferential statistics for measuring treatment difference between flucytosine and flucytosine will be from stratified statistical models by length of AmB treatment: 7 day treatment regimen AmB7 and 14 day treatment regimen Amb14.

16.2.   Multi-site consideration

The data analyses will be performed on a combined-site basis. Of note, the randomisation was performed centrally. There are 9 sites in the study: Lusaka in Zambia; Blantyre, Lilongwe, Zomba in Malawi; Duuala, Yaounde in Cameroon; Muhimbili, Mwananymala, and Amana in Tanzania. Nine sites may be collapsed into 4 countries (Zambia, Malawi, Cameroon, and Tanzania) in the covariate adjustied analysis and subgroup analysis in order to produce converging and stable estimate of treatment effects.

16.3.   Covariates analyses

Covariate analyses will be performed on the primary outcome and the key secondary outcomes, in particular the 10-week mortality outcome (See Section 5.1.3.) on the ITT population. Other covariate analyses will be performed if deemed necessary.

16.4.   Subgroup analysis

Subgroup analyses will be performed for the primary and key secondary outcomes on the ITT population. The subgroup variable will be the site, age, sex, GCS, CD4 count, CFU, and ART as defined above for covariate adjusted analysis.

16.5.   Multiplicity and missing values.

Multiplicity adjustment will not apply to the primary and secondary outcome analyses.

Missing baseline covariates will be imputed using simple imputation methods in the covariate adjusted analysis based on the covariate distributions. For a continuous variable, missing values will be imputed from random values from a normal distribution with mean and SD calculated from the sample. For a categorical variable, missing values will be imputed from random values from a uniform distribution with probabilities P_1, P_2, ..., and P_k from the sample. For a count data, missing values will be imputed from random values from a Poisson distribution with λ from the sample. Seed for the imputation is set as 128.

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16.6. Other data considerations

7.5.3 Data summaries

Continuous variables will be summarised according to number of subjects with non-missing data (n), mean, standard deviation (SD), median, minimum, and maximum. The confidence interval will be added on summaries of continuous effectiveness variables.

Categorical variables will be summarised according to the absolute frequency and percentage of subjects (%) in each category level. The denominator for the percentages is the number of subjects in the treatment arm with data available, unless noted otherwise. Event rates per 100 participant years will also be reported for time-to-event clinical outcomes and adverse events of special interest.

7.5.4 Graphical displays

Mean values for some continuous outcomes by treatment and visit will be plotted. Kaplan-Meier plots will be produced for displaying the time-to-event data.

17. REFERENCES

Brouwer A E, Rajanuwong A, Chierakul W, Griffin G E, Larsen R A, White N J and Harrison T S 2004 Combination antifungal therapies for HIV-associated cryptococcal meningitis: a randomised trial *Lancet* **363** 1764-7.

Jarvis JN1, Bicanic T, Loyse A, Namarika D, Jackson A, Nussbaum JC, Longley N, Muzoora C, Phulusa J, Taseera K, Kanyembe C, Wilson D, Hosseinipour MC, Brouwer AE, Limmathurotsakul D, White N, van der Horst C, Wood R, Meintjes G, Bradley J, Jaffar S, Harrison T. Determinants of mortality in a combined cohort of 501 patients with HIV-associated Cryptococcal meningitis: implications for improving outcomes. *Clin Infect Dis*. 2014 Mar;58(5):736-45

Signatures, 24th February 2017

ACTA Trial

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ACTA Statistical analysis plan: Summary of changes

Two separate documents were originally created for the main study and adjunct analyses. These were combined, with an introductory overview, to create the final version of the analysis plan.

List of Track changes for Main study analysis plan

1. Table of contents, abbreviations and list of summary tables and figures were removed.
2. Pages 1 and 2 added giving an overview of the trial and envisaged papers.
3. Page 4, section 1 Introduction: Updated to reflect the two analysis plans: main analysis and sub-study.
4. Page 4, section 2.1.1 Primary objective: Sentence added: 'The analysis will determine whether or not either the oral or the 7-day amphotericin B regimen are non-inferior to the 14-day amphotericin B.'
5. Page 4, section 2.1.2 Secondary objectives: '(grade III and grade IV)' added for clarity.
6. Page 4, section 2.1.2 Secondary objectives: Sentence deleted "To determine the effects of the oral regimen against the best available amphotericin / adjunct therapy"
7. Page 4, section 2.2.1 Primary outcome: Section updated for clarity to justify choice of primary endpoint.
8. Page 5, section 2.2.2 Secondary outcomes: Analysis of CSF fungal burden split into 2 separate points and time to death at day 14 added and points clarified as was incorrectly omitted previously.
9. Page 5, section 2.2.3 Case ascertainment and case definitions: Updated to reflect TMG member adjudicating outcome for patients lost to follow-up.
10. Page 6, section 2.2.3 Case ascertainment and case definitions: Reference added for CSF fungal count section and sentence deleted, "Quantitative cultures of CSF are done as in prior studies".
11. Page 6, section 2.2.3 Case ascertainment and case definitions: Clarification made on patients presenting with AEs at baseline and deterioration of adverse events.
12. Page 6, section 2.2.3 Case ascertainment and case definitions: IRIS: The sentence 'Suspected IRIS events will be reviewed, blinded to treatment assignment, by a single TMG member and an independent expert' was removed and 'For the main paper, an IRIS event will be determined by the study doctor and included in the table of adverse events as a grade 4 AE.' added.
13. Page 6, section 2.2.3 Case ascertainment and case definitions: Cause of death: clarification added: 'information on these deaths is available and

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could be used to estimate the cause of death.' And section added to state that cause of death will be collected for DMC but will not be reported in the main paper in line with previous studies.

14. Page 7, section 2.2.3 Case ascertainment and case definitions: Additional safety variables: Additional safety variables were added.
15. Page 7, section 3.2 Trial sites: Clarification made to detail addition of trial sites after initial slow recruitment.
16. Page 8, section 3.4 Randomisation: Wording updated to increase clarity.
17. Page 8, Section 3.5 Sample size: the word "patients" changed to "participants".
18. Page 9, section 4.1 Study population data sets: Section detailing late exclusion criteria added and plan to conduct mITT analysis removed with justification included. ITT definition also clarified.
19. Page 9, section 4.1 Study population data sets: Per protocol definition more clearly defined.
20. Page 9, section 4.2 Data cleaning (formally section 4.3) added to section 4.2.
21. Page 10, section 5.1.1 ITT analysis of the primary outcome - the primary analysis: Detail added to describe how patients lost to follow-up will be dealt with in the ITT and sensitivity analyses.
22. Page 11 - section 5.1.1 ITT analysis of the primary outcome - the primary analysis: Wording updated/clarified for use of z-test if GLM does not converge.
23. Page 11, section 5.1.2 A per-protocol analysis of the primary outcome: sentence added 'Statistical methods will be the same as used in the Section 5.1.1.'
24. Page 12, section 5.1.3 Sensitivity analysis of the primary outcome: Wording updated for clarity and the following section added: 'A TMG member, not directly involved in patient management and blind to study arm, will also classify the participant lost according to likely dead or likely alive and these data will be reported. Our primary endpoint involves censoring those lost within 2 weeks, as discussed above. This is simple and consistent with the most appropriate assumption for 10-week mortality, a key secondary endpoint (it would not make sense to do the 10-week mortality endpoint classifying those lost as dead as many will not have died). Censoring at 2 weeks is also probably more accurate when the participant is lost close to day 14. As mentioned above, by August 2015, only 1 patient had been lost to follow-up in the first 2 weeks (at day 5).'
25. Page 12, section 5.1.4 Covariate adjusted analysis of the primary outcome: ART status at baseline added as previously omitted in error and wording of the section updated for clarity.
26. Page 12, section 5.1.5 Subgroup analyses of the primary outcome: More detailed section added.
27. Page 13, section 5.1.6 Superiority analysis of the primary outcome: Section added.
28. Page 13, section 5.2 Secondary outcomes. Introduction added to this section for clarity.

29. Page 13, section 5.2.1 Analysis of binary outcomes: Details of sensitivity analyses added

30. Page 14, section 5.2.2 Analysis of time-to-event outcomes: Clarification on sensitivity analysis made.

31. Page 14, section 5.2.4 Analysis of count outcomes: Section deleted and sentence 'We don't have any count data' added.

32. Page 14, section 5.2.5 analysis of secondary outcomes with repeated measures: More detail added on process for analyzing rate of clearance with reference added.

33. Page 15, section 5.3 Exploratory analysis: This section was added but also the sentence 'None are planned'.

34. Page 15, section 6.1 Safety variables: 2 sentences added 'The substudy collecting ECG data has stopped (as planned and in accordance with IDMC and TSC approval) and will be analysed and reported separately.' and 'Blood monitoring laboratory data, occurrence of suffering clinical and laboratory-defined grade 3 and 4 adverse events to 10 weeks will be analysed descriptively.' And additional safety analyses sentence removed as none had been planned.

35. Page 15, section 7.1 Multi-site consideration: Wording updated for clarity. Sentence 'no stratification based on sites will be performed in the analysis.' removed.

36. Page 15, section 7.2 Covariate analysis: Sentence added 'and the key secondary outcomes, in particular the 10-week mortality outcome (See Section 5.1.3.) on the ITT population. Other covariate analyses will be performed if deemed necessary.'

37. Page 16, section 7.3 Subgroup analysis: key secondary outcomes also added and ART status and disease severity (as defined above for covariate adjusted analysis) added to list for subgroup analyses.

38. Page 16, section 7.4 Multiplicity: Section added for dealing with missing data.

39. Page 18, section 7.5.2 Graphical displays: Sentence 'Kaplan- Meier plots will be produced for displaying the time-to-event data.' added

40. Page 16, section 8: 3 references added.

List of Track Changes for Adjunct analysis plan

1. Table of contents, abbreviations and list of summary tables and figures were removed

2. Page 21, section 11.2.3 Case ascertainment and case definitions: IRIS: The sentence 'Suspected IRIS events will be reviewed, blinded to treatment assignment, by a single TMG member and an independent expert' was removed and 'For the main paper, an IRIS event will be determined by the study doctor and included in the table of adverse events as a grade 4 AE.' added.

3. Page 22, section 12.2 Trial sites: Clarification made to detail addition of trial sites after initial slow recruitment.

4. Page 24, section 13.1 Study population data sets: Section detailing late exclusion criteria added and plan to conduct mITT analysis removed with justification included.
5. Page 24, section 13.1 Modified ITT analysis was removed and PP analysis of primary outcome added.
6. Page 25, section 13.1 Study population datasets: Sentence added '(i.e. missed >1 dose of study treatment during the first 2 weeks of treatment without sound clinical reason)' to clarify definition of PP population.
7. Page 25, section 13.1 Study population datasets: The sentence 'There is no single CRF question that determines the per-protocol population. This will be determined by PI, data manager and the trial statistician. ' was removed.
8. Page 26, section 14.1.2 Per protocol analysis of the primary outcome: This section was added and the mITT description removed.
9. Page 26, section 14.1.3 Sensitivity analysis of the primary outcome: Updated to clarify that TMG member blinded to study arm would classify outcome for patients lost to follow-up.
10. Page 26/27, section 4.1.4 Covariate adjusted analysis of the primary outcome: clarification made for procedure if model unstable and CSF opening pressure, prior TB, haemoglobin, creatinine at baseline removed as variables to adjust for as initial inclusion was an error.
11. Page 28, section 14.2 Secondary outcome analysis: clarification for analysis of CSF slopes included.
12. Page 29, section 16.2 Multi-site consideration: correction made to list of sites.
13. Page 29, section 16.4 Subgroup analysis: CSF opening pressure, prior TB, haemoglobin, creatinine at baseline removed as variables to adjust for.
14. Page 29, section 16.5 Multiplicity: Section added for dealing with missing data.