PROTOCOL - AMENDED

CryptoDex: A Randomized, Double Blind, Placebo-Controlled Phase III Trial of Adjunctive Dexamethasone in HIV infected Adults with Cryptococcal Meningitis

Funded by DfID, MRC(UK) and the Wellcome Trust (UK) through the Joint Global Health Trials Initiative

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1 OVERVIEW

A multi-centre double blind randomized placebo controlled trial of adjunctive treatment with dexamethasone in adults with HIV associated cryptococcal meningitis

Study Aim: To reduce mortality from cryptococcal meningitis in HIV infected in adult patients

Intervention: Dexamethasone in a reducing dose over the first 6 weeks of treatment

Randomisation: 1:1 study intervention versus placebo

Primary endpoint: Survival during the first 10 weeks following randomisation

Antifungal therapy: Amphotericin B 1mg/kg/day + fluconazole 800mg/day for 2 weeks, followed by fluconazole 800mg/day for 8 weeks, followed by secondary prophylaxis with fluconazole 200mg/day

Secondary endpoints: Survival during 6 months post-randomisation, disability, rates of fungal clearance, rates of visual impairment, rates of IRIS, rates of new AIDS defining illnesses, frequency of grade 3, grade 4 or serious adverse events, rates of raised intracranial pressure, time to new neurological events.

Sample Size: 880 patients (330 from Africa and 550 from Asia)

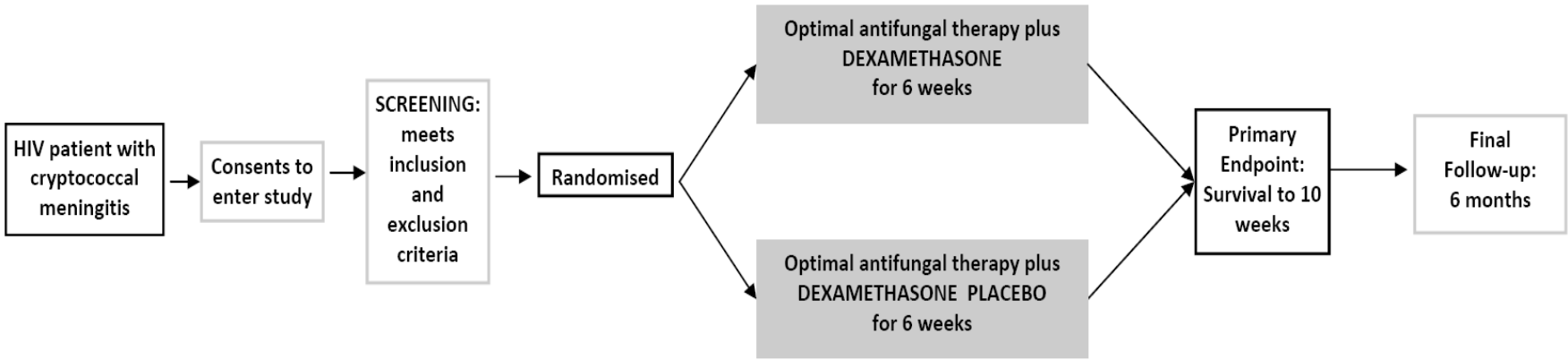
Participating Countries: Uganda, Malawi, Vietnam, Thailand, Indonesia, Laos

Study Sponsor: Oxford University

Study Funding: The UK Department for International Development, The Wellcome Trust (UK) and the Medical Research Council (UK) Joint Global Health Trials Scheme

Study Duration (recruitment and follow-up): 3 years

1.1 Trial flow diagram



1.2 Trial flow chart*

	Day 1: Study Entry	Day 3	Day 7	Day 11	Day 14	Day 21	Day 28	Day 42	Day 70	Day 182
Take informed consent	✓									
Clinical Assessment**	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
FBC (Hb, WCC, plt) 1mL	✓		✓		✓					
Na, K, Urea, creat, glu 2 mL	✓	✓	✓	✓	✓	✓#	✓#	✓#		
CD4 / CD8 count 2mL	✓									
HIV antibody 2mL	✓									
Blood cultures 5mL	✓									
CSF Opening pressure	✓	✓	✓		✓	If indicated	If indicated		If indicated	
Lateral Flow Antigen on CSF	✓									
CSF Gram stain, India Ink 0.5mL	✓	✓	✓		✓	If indicated	If indicated		If indicated	
CSF cell count, protein, glucose 1mL	✓	✓	✓		✓	If indicated	If indicated		If indicated	
CSF TB smear 6mL***	✓									
CSF Yeast Quant Count 1mL	✓	✓	✓		✓	If indicated	If indicated			
Store *C. neoformans* isolate****	✓									
Store CSF supernatant and pellet	✓	✓	✓		✓	If indicated	If indicated		If indicated	
Sputum TB smear*****	✓									
Chest X-ray***	✓									
Store blood plasma 4.5mL	✓									
Store blood cell pellet	✓									
Approximate blood volume mL	16.5	2	3	2	3	3	3	2		
Approximate CSF volume mL	8.5	2-5	2-5		2-5					

* Study drug is given daily from day 1 – day 42

** GCS Assessment is daily while an in-patient. When outpatient assessment can take place at the scheduled time + up to 5 days (eg 4 week assessment on day 28-33). Day 182 assessment may be by telephone.

***Optional if local resources are unavailable

****Also store any isolate where the quantitative culture assessment is higher than the previous assessment or relapse case

***** Perform sputum smear if patient can produce a sample

glucose only

NB: Blood volumes are estimates

The Problem

The problem addressed by this trial is the high death rate in HIV infected patients with cryptococcal meningitis (CM).

The Research Question

This trial aims to determine whether adjuvant therapy with dexamethasone in addition to standard antifungal therapy will reduce the mortality rate from cryptococcal meningitis in patients with HIV. In this double blind trial patients will be randomised to receive either dexamethasone or placebo in addition to optimal antifungal therapy.

2 BACKGROUND AND RATIONALE

2.1 Background

Cryptococcal meningitis (CM) is estimated to cause 625 000 deaths every year, most occurring within 3 months of diagnosis [1]. It is the leading cause of death in HIV patients in Asia and Africa affecting 3.2% of the HIV infected population per year [1]. The incidence in these regions is the highest in the world - in Africa, it is estimated there are more deaths due to CM than to tuberculosis [1]. The 90 day case fatality rate is up to 55% in Asia and 70% in Africa [1].

Despite improvements in access to HIV care, the WHO estimates that HIV/AIDS will be the leading cause of disease in middle and low income countries by 2015, and models suggest that even if 80% access to HIV treatment is achieved by 2012, there will be 6.5 million AIDS deaths p.a. by 2030 [2]. Thus, CM is likely to remain a significant health burden for the foreseeable future.

There has been no major advance in the treatment of cryptococcal meningitis since the 1970's. The mainstays of induction therapy are drugs that are over 50 years old – amphotericin B and flucytosine - although these are often poorly available where the disease burden is highest. While amphotericin therapy is undoubtedly superior to fluconazole monotherapy, amphotericin combination therapy has only recently been shown to reduce mortality when compared with amphotericin monotherapy. While effective antifungal therapy is key, adjunctive treatments, which have been seen to have dramatic effects on mortality in other neurological infections, are untested in cryptococcal meningitis. Given the high death rates in patients receiving current optimal treatment, and the lack of new agents on the horizon, adjuvant treatments offer the greatest potential to reduce mortality in CM.

This study aims to reduce the death rate from CM. The principal research question is: does adding dexamethasone to standard antifungal therapy for CM reduce mortality? In this double blind placebo-controlled trial (DBRCT) patients will be randomised to receive either dexamethasone or placebo. Dexamethasone is a cheap, readily available, and practicable intervention.

This is a multicentre study, recruiting patients in both Asia and Africa to ensure the relevance of the study results in the populations where the disease burden is highest.

2.2 Treatment of CM

Successful treatment of CM depends upon effective anti-fungal therapy and successful management of complications, notably raised intracranial pressure. Antifungal treatment schedules for cryptococcal meningitis are not globally uniform but are affected by drug availability, costs and human resources. The Infectious Diseases Society of America convenes an international panel to draw up treatment guidelines, most recently published in 2010, and the WHO currently has guidelines in development which will be specifically aimed at management in resource-poor countries. These conform closely to the IDSA guidelines. Treatment generally consists of a period of induction therapy using high dose or combination antifungal therapy (usually for 2 weeks), followed by a period of consolidation therapy of 8 weeks with fluconazole. After this time, provided the patient has responded to treatment, secondary prophylaxis using lower dose fluconazole is given to prevent disease relapse. It is generally considered safe to stop secondary prophylaxis if ARV therapy has resulted in suppression of the plasma viral load and there has been immune reconstitution with recovery of the CD4 cell count to > 100 cells/uL for at least 6 months).

Consistent with the local practices and the WHO and IDSA guidelines, in this study patients will receive anti-fungal therapy consisting of amphotericin B (1mg/kg/day) combined with fluconazole 800mg/day for 2 weeks, followed by fluconazole 800mg/day for a further 8 weeks before switching to secondary prophylaxis [3, 4].

2.3 Rationale for adjuvant treatment with dexamethasone

Several mechanisms exist through which dexamethasone may modify disease outcome in cryptococcal meningitis. Current IDSA guidelines suggest corticosteroids may be beneficial in cryptococcal meningitis in patients who have cryptococcomas with mass effect, acute respiratory distress syndrome or IRIS [4]. However, corticosteroids have never been tested in a randomized controlled trial. Of note, 150 of 381 patients in the ACTG trial of combination antifungal therapy in cryptococcal meningitis received steroids during the study. Graybill's (2000) post-hoc analysis of these patients has some serious limitations

(1997) [5, 6]. Steroids were prescribed at the discretion of the attending physician, and the reasons for prescription are unclear. Graybill's analysis was limited to just 41 of the 150 steroid recipients, and did not report their impact on mortality. Clinical success (defined as stabilization or improvement at 2 weeks) was lower in the 41 steroid recipients analysed (66% vs 86%). Apparently worse mycological outcomes in these 41 patients are impossible to interpret since no adjustments were made for the antifungal therapy received, fungal load or clinical severity at baseline, all of which are known to be important outcome predictors [7, 8]. The exclusion from the analysis of the vast majority of patients who received steroids means it is impossible to draw robust conclusions about their effect and underlines the need for a trial. In contrast with Graybill's paper, a study in HIV uninfected patients with *C. gattii* meningitis found a 10-fold reduction in blindness in those who received steroids [9]. Moreover, in tuberculous meningitis (TBM), a disease which shares pathophysiological features with CM, dexamethasone has been shown to improve outcome (RR of death, 0.69; 95 %CI 0.52-0.92; P=0.01) [10]. In this RCT, conducted in Vietnam, 545 patients were recruited of whom 98 were HIV infected. In addition to the reduced risk of death, there were fewer adverse events in patients receiving dexamethasone [10].

2.4 Potential mechanisms of action dexamethasone in CM

2.4.1 Anti-inflammatory action

Cryptococcal meningitis is a chronic granulomatous meningitis, with foci of inflammation in the basilar meninges, cerebral mass lesions, and a lymphocytic cerebrospinal fluid (CSF). The basilar meningitis leads to impairment of resorption of CSF and this, along with physical blockage of CSF drainage by yeast cells and conglomerations of capsule, is believed to contribute to the raised intracranial pressure seen in the disease [11]. The immune response in the mouse model mimics that seen in AIDS patients [12]. The effect of anti-inflammatory agents has been tested in this model. Reduction of inflammation prolongs survival in mice infected with a lethal dose of *C. neoformans* [13]. Dexamethasone increases median survival from 19 to 26 days ($p<0.05$), a benefit preserved when dexamethasone is combined with amphotericin B [14]. Dexamethasone does not interfere with the pharmacological properties of amphotericin or fluconazole [14, 15]. Moderation of the inflammatory response is postulated to be through microglial cells, key in the immune response to CM [16].

2.4.2 Reduction of cerebral oedema and brain swelling

Cerebral oedema is a key feature of CM, and cryptococcal capsule has been shown to directly induce cerebral oedema [17]. Vascular endothelial growth factor (VEGF) is a potent inducer of vascular permeability and angiogenesis which has been implicated in the pathogenesis of brain oedema [18, 19]. Elevated levels are seen in both the CSF and blood of HIV patients with cryptococcal meningitis, and cryptococcal capsule has been shown to induce VEGF production by monocytes, neutrophils and peripheral blood mononuclear cells in a dose dependent manner [18, 20]. In vitro this induction is significantly down-regulated by dexamethasone[18]. Down-regulation occurs at a range of concentrations consistent with those achieved in human dosing. A rat model of brain oedema suggests that the effect of steroids on oedema is mediated through the inhibition of VEGF [21]. Corticosteroids reduce vascular permeability and limit oedema and inhibition of VEGF may explain the beneficial effect of steroid therapy in TBM [10, 22].

2.4.3 Reduction of intracranial pressure

Raised intracranial pressure (ICP) is frequent in CM, and an important cause of mortality [5]. Dexamethasone reduces raised ICP in models of other brain infections [23-25]. The mechanisms are not clear, but through attenuation of inflammation, cerebral oedema and restoration of the blood brain barrier it is plausible that dexamethasone may reduce raised ICP in CM.

2.4.4 Modification of cerebral vasculitis

Cerebral vasculitis, frequently described in infections, is a recognised feature of CM [26-29]. The pathophysiology of neurological vasculitis is relatively well-understood: the neurological features arise principally through ischaemia and infarction secondary to inflammation [30]. In infections, the vascular insult may be mediated through any or all of vascular wall invasion, immune complexes, or cryoglobulins. Of these, both endothelial invasion and immune complex disease are described in cryptococcosis [31, 32].

Steroids are an important therapeutic option in primary cerebral vasculitides, and their beneficial effect in TBM may be through an anti-vasculitic action [30]. Attenuation of the vasculitis seen in CM may improve outcome.

2.5 Potential harms of Dexamethasone

Reported side effects of dexamethasone are well described and similar to other corticosteroids. Side effects include dysglycaemia, changes in mood, Cushing's-like syndrome, gastrointestinal bleeding, immunosuppression, hypertension and secondary hypoadrenalism. Side effects are more likely with higher doses (dexamethasone ≥16mg/kg/day) and longer courses of treatment [33]. Dexamethasone is frequently prescribed for patients with intracranial pathology including infectious diseases, and in addition data on adverse events are available from large RCTs of dexamethasone which have been completed in tuberculous meningitis (TBM) and acute bacterial meningitis (BM) [10, 34-36]. In the BM trials, the duration of steroid therapy was 4 days and in TBM 6 to 8 weeks. In all these trials, adverse events, including potentially life-threatening adverse events such as gastrointestinal bleeding, were rare and were no more common in patients receiving dexamethasone compared with placebo. In this study patients will receive dexamethasone 0.3mg/kg/day, reducing weekly over 6 weeks. This is the lower dose that was used in the TBM trial for patients with Grade I disease [10]. The risk and severity of any adverse events need to be considered in the context of the high mortality seen in cryptococcal disease. Notably dexamethasone has no mineralocorticoid effect and is not associated with hypokalaemia [37].

2.5.1 Immunosuppression

Corticosteroids are immunosuppressive. It is possible that they may slow yeast clearance from CSF, and increase risk of other infections. However, animal studies suggest dexamethasone improves yeast clearance from CSF [14]. Even if the rate of yeast clearance is reduced, this may not be detrimental. In this study we will measure the rate of clearance of yeast from CSF (SEE APPENDIX 3). Patients with CM are profoundly immunosuppressed – the median CD4 count in Vietnamese patients is 16 cells/μL (which compares with a median CD4 count of 42 in Vietnamese HIV patients with tuberculous meningitis) [38]. Given this, whether dexamethasone will significantly increase the risk of other opportunistic infections is not clear. The incidence of other OIs is a secondary endpoint of the study. Patients in the study will receive chemoprophylaxis against *Pneumocystis jirovecii* and other opportunistic infections in line with national guidelines. Patients will be screened for TB at study entry as part of normal care.

2.5.2 Secondary hypoadrenalism

Adrenal suppression is a recognized risk in patients receiving corticosteroid therapy for prolonged periods or in higher doses. With a treatment course of the length in this study, adrenal suppression will be short lived and is prevented through the gradual reduction in dose as stipulated in the protocol.

Patients must be provided with an information card detailing the importance of taking the steroids as per the study protocol, ad so that other doctors whom they attend will be aware of their prescription. Steroid information cards must be provided in the local language. Steroids will be administered in a reducing dose according to best medical practice.

2.5.3 Hyperglycaemia

Reversible hyperglycaemia is a recognized side effect of corticosteroids. Hyperglycaemia is uncommon in patients receiving dexamethasone in this dose, with elevated fasting glucose occurring in less than 1% of patients and equally frequently amongst TBM patients receiving active drug or placebo [10]. A review of the adverse effects of corticosteroids in severe sepsis found an increased absolute risk of hyperglycaemia of 5.6% [39].

2.5.4 Cushing's-like syndrome

A Cushing's type syndrome can develop in people receiving prolonged steroid therapy, with weight gain, redistribution of body fat and acne. However, in the dose and duration given in this study development of Cushing's syndrome is unlikely. Moreover, it is reversible on treatment cessation.

2.5.5 Gastrointestinal bleeding

Data from TBM, bacterial meningitis and sepsis suggest that corticosteroids do not increase the risk of gastrointestinal bleeding when used in these dosages and durations [10, 35, 36, 39].

2.5.6 Hypertension, salt retention and hypokalaemia

These effects of corticosteroids are due to their mineralocorticoid action. Since dexamethasone belongs to the glucocorticoid group, the risk of these side effects is negligible.

3 STUDY AIMS

3.1 Primary aim

To investigate the effect of dexamethasone adjunctive therapy on 10-week survival in adult HIV infected patients with cryptococcal meningitis

3.2 Secondary aims

The secondary aims are to determine the effect of adjuvant treatment with dexamethasone on survival at 6 months, disability at 10 weeks and 6 months, the rate of sterilisation of cerebrospinal fluid (CSF), the frequency of grades 3, 4 and serious adverse events, the incidence of IRIS, the incidence of other opportunistic infections, re-treatment for cryptococcal meningitis, and the presence of visual deficit at 10 weeks. In addition, the effect of steroids on survival by continent will be assessed.

4 ENDPOINTS

4.1 Primary endpoint

The primary outcome is overall survival until 10 weeks after randomisation.

4.2 Secondary endpoints

4.2.1 Survival until 6 months after randomization

Relapse occurs in cryptococcal meningitis. Most cases occur within 6 months of diagnosis. We will collect survival data at 6 months (alive vs date of death) to ensure that any survival benefit at 10 weeks is sustained at 6 months, and not negated by, for example, a higher relapse rate in patients receiving steroids.

4.2.2 Disability at 10 weeks and 6 months

Neurological disability will be assessed using the modified Rankin score and the Two Simple Questions and classified as good, intermediate, severe disability, or death, as previously described [10].

Disability is an expected consequence of cryptococcal meningitis, including blindness, deafness and other focal neurological deficits. In addition to reducing the death rate, patients receiving dexamethasone may also suffer less (or more) rates of these disabilities. The Rankin score and the Two Simple Questions are well validated measures of the degree of disability in stroke survivors, and have been used frequently to measure disability following neurological infections.

4.2.3 Rate of CSF sterilisation during the first 2 weeks

It is conceivable that dexamethasone could slow the rate of CSF sterilisation, although this is not seen in the animal model. In a subset of patients we will measure the repeated fungal burdens in CSF over the first 2 weeks of treatment, model the rate of decline and determine the effect of dexamethasone on fungal clearance. We will also determine whether the rate of CSF sterilisation is predictive of mortality in both study arms based on joint modeling of longitudinal fungal counts and mortality.

4.2.4 Adverse events

Comparison of the proportion of patients with any grade 3 or 4 adverse event and of serious adverse events between treatment groups will form an important part of the study analysis, in order to determine the safety of the intervention.

4.2.5 Rate of IRIS until 10 weeks

We will model the rate of IRIS over time with a cause-specific hazards model taking into account the competing risk of prior death. CM-related IRIS will be defined as per the recent proposed definition (see appendix 4) [40].

4.2.6 Time to new AIDS-defining illnesses or death until 10 weeks

AIDS-defining illnesses will be defined as per the WHO classification (see appendix 5)

4.2.7 Visual deficit at 10 weeks

Retrospective data from HIV uninfected patients with meningitis due to *C. gattii* suggest that steroids may have a profound effect reducing visual loss. 10% of Vietnamese HIV patients have visual impairment at 10 weeks *(JN Day, manuscript in submission)*. We will compare the incidence of blindness and other visual deficit between treatment groups. Visual deficit will be assessed using a simple 6 point scale (see appendix 6)

4.2.8 Time to new neurological event or death until 10 weeks

A neurological event is defined as a fall in Glasgow coma score by ≥2 points for ≥2 days from the highest previously recorded Glasgow coma score (including baseline) or the occurrence of any of the following adverse events: cerebellar symptoms, coma, hemiplegia, paraplegia, seizures, cerebral herniation, new onset blindness or deafness, or cranial nerve palsy.

4.2.9 Longitudinal measurements of intracranial pressure during the first 2 weeks

Intracranial pressure (ICP) will be measured at study entry, day 3, 7, and 14, and if clinically indicated (depending on local practice). The main outcomes are longitudinal ICP-measurements until day 14 and we will model the effect of dexamathasone on ICP based on a joint model for longitudinal and survival data.

Clinical need and local practice will determine the frequency of lumbar punctures post day 14 and clinician's diagnoses of raised ICP based upon the presence of headache, nausea, diurnal and postural variation, relief with lumbar puncture and presence of papilloedema. Thus, these measures will only be descriptively analyzed.

4.2.10 Antifungal treatment intensification or re-treatment for cryptococcal meningitis in the 6 months post-randomisation

Relapse occurs in patients with cryptococcal disease. All patients will be receiving either treatment doses of antifungal therapy or secondary prophylaxis doses during the 6 month period of follow-up. While all patients will be encouraged to return to the study hospital in the event of illness during the 6 month follow-up period, it is possible that, because of their location, some patients will undergo treatment elsewhere where diagnostic facilities may be less developed. This episode may only be identified at the 6 month follow-up period. For this reason, a pragmatic definition of relapse will be used. This is defined as either intensification of antifungal therapy above that according to the study antifungal schedule, or readmission for treatment of cryptococcal disease. The main outcome measure will be the cause-specific hazard of relapse taking into account the competing risk of death.

5 DESIGN

5.1 Study design

A randomized, double-blind, placebo-controlled trial with 2 parallel arms: dexamethasone versus placebo during the first 6 weeks of treatment. This is a multicentre study that will recruit patients across Asia and Africa, in Malawi, Uganda, Laos, Thailand, Indonesia and Vietnam. The study is pragmatic, designed to maintain relevance through trialing adjuvant treatment with dexamethasone in the context of the best standard of locally available care.

Dexamethasone Treatment Dose

Dexamethasone will be given in a reducing dose according to body weight:

Period	Dexamethasone Dose	Timing
Week 1	0.3 mg/kg iv	Once daily
Week 2	0.2 mg/kg iv	Once daily
Week 3	0.1 mg/kg po	Once daily
Week 4	3.0mg total/day po	Once daily
Week 5	2.0mg total/day po	Once daily
Week 6	1.0mg total/day po	Once daily
Week 7 onwards	Stops	

This dose is identical to the dose used in patients with Grade 1 tuberculous meningitis and has been shown to have a low rate of side effects [10]. Dexamethasone/placebo will be administered intravenously while the antifungal treatment is intravenous, and orally once antifungal treatment is administered orally. Treatment will be weight-dosed to the nearest half milligram. With the exception of the first dose, which should be given with the first dose of anti-fungal therapy, dexamethasone should be given in the morning.

6 STUDY POPULATION

All HIV infected adult patients with a diagnosis of cryptococcal meningitis presenting to the study centres will be eligible to enter the study, subject to meeting the inclusion/exclusion criteria.

6.1 Trial Location

The study will recruit patients at sites in Vietnam, Indonesia, Thailand, Laos, Uganda and Malawi.

6.2 Inclusion criteria

- Age ≥ 18 years
- HIV antibody positive
- Cryptococcal meningitis defined as a syndrome consistent with CM and one or more of:
    - positive CSF India ink (budding encapsulated yeasts),
    - *C. neoformans* cultured from CSF or blood,
    - positive cryptococcal antigen Lateral Flow Antigen Test (LFA) in CSF
- Informed consent to participate given by patient or acceptable representative

6.3 Exclusion criteria

- Pregnancy
- Active gastrointestinal bleeding (defined as vomiting blood or malaena stool in the previous week)
- Currently receiving treatment for cryptococcal meningitis and having received ≥ 1 week of anti-cryptococcal meningitis therapy
- Known allergy to dexamethasone
- Current steroid use defined as
    a) currently receiving the equivalent of prednisolone 40mg/day or more
    b) currently receiving steroid therapy (any dose) for more than 3 weeks (except topical steroids which are permitted)
- Concurrent condition for which corticosteroids are indicated because of proven benefit (such as severe *Pneumocystis* pneumonia (pO_2<70mmHg) or tuberculous meningitis).
- Renal failure (defined as creatinine >3*ULN, despite adequate hydration)

7 STUDY PROCEDURES

7.1 Recruitment

The target population for study screening is any patient known or suspected to have cryptococcal meningitis. To enter the study patients must be confirmed to have cryptococcal meningitis according to the definition in the inclusion criteria. Screening procedures will adapt to the standard of care of each setting to ensure that study related procedures are performed only after informed consent has been obtained. According to the clinical care of the treating hospital, patients suspected of having cryptococcal meningitis will undergo:

a) an IMMY lateral flow cryptococcal antigen test (LFA) on serum or plasma,

AND/OR

b) blood culture for *Cryptococcus*

AND/OR

c) a lumbar puncture with an IMMY lateral flow cryptococcal antigen test, and/or microscopic examination of CSF, and/or culture of CSF.

When the results of the IMMY lateral flow test (plasma, serum or CSF), or CSF microscopy, or blood or CSF culture are available study staff may consider if the patient should be approached regarding the study. Only patients 18 years or older who are not known to be pregnant and who have evidence of cryptococcal disease from one of the specified tests will be approached.

A study staff will invite the patient to discuss the details of the study. If the patient is judged by the staff to be unfit or unable to give informed consent, an acceptable representative will act on their behalf for the following procedures. The study staff will give the patient/representative a copy of the informed consent form and explain the details of the study including the study procedures, risks and benefits, financial and confidentiality considerations, treatment alternatives and how to obtain more information. The study staff will invite the patient/representative to ask questions and will endeavor to ensure that s/he understands the information given. The study staff will then ask the patient/representative to consider study participation. Those who refuse consent will be treated as per the best available standard care and will not have any study related procedures performed.

Those who consent to the study will sign and date two copies of the informed consent form. The study staff will also sign and date the two copies.

If the patient/representative is illiterate, a witness who is not a member of the study staff will be present during the informed consent discussion. The informed consent form will be read to the

patient/representative in the presence of the witness. If the patient/representative agrees to participate, the form will be signed and dated by the witness.

Consented patients will be screened for eligibility.

7.2 Screening

Only patients who are 18 years or older, who are not known to be pregnant, and who have at least one of positive CSF or blood/serum LFA test, positive blood or CSF culture, or positive CSF microscopy will be consented. Consented patients will undergo the following screening procedures/tests. In the case of an unconscious patient, information will be obtained from a knowledgeable relative or caregiver.

- Medical history will be taken including: 1) signs and symptoms consistent with cryptococcal meningitis, 2) allergy to dexamethasone, 3) history of corticosteroid use and anti-fungal therapy.
- Patient will be checked for signs of active gastrointestinal bleeding.
- All females of child bearing age will have a urine or blood pregnancy test.
- Creatinine level
- HIV status will be confirmed from clinical history or testing as per standard of care
- A lumbar puncture will be performed on all patients to obtain CSF. CSF will be tested by: 1) India ink stain or equivalent, 2) culture, 3) lateral flow cryptococcal antigen test. Cultured isolates will be stored for susbequent studies.
    - If a lumbar puncture was done recently (within 48 hours) for clinical care and volume of fresh CSF remains available for these tests (stored according to established SOPs), the lumbar puncture need not be repeated.
    - If the patient underwent a recent lumbar puncture, an additional puncture will be performed (provided there are no contra-indications) if any of the following are true: 1) there is uncertainty regarding the microbiological diagnosis, 2) raised intracranial pressure is suspected, 3) the previous puncture was >2 days prior and no effective treatment has been given, 4) it is required for standard care. If none of the above are true or if the patient refuses further lumbar puncture, they may be randomized without additional lumbar puncture provided they are eligible for the study.
    - If the lumbar puncture was not recent, it will be repeated (provided there are no contraindications) in order to confirm the diagnosis, and to determine CSF pressure and fungal burden.

All lumbar punctures require verbal or written consent according to local standard clinical practice. Failure to consent according to local practice is a contraindication to the procedure.
When all inclusion and exclusion criteria are verified, eligible patients will be randomized to treatment.
Patients who are determined to be ineligible will be withdrawn from the study and the reason recorded. Patients withdrawn from the study will be treated according to the best available standard care. Screening flow is illustrated in Appendix 12.

The number of patients who do meet inclusion/exclusion criteria, but are not enrolled to the study will be recorded.

7.3 Randomization

Randomization will be 1:1 to either dexamethasone or identical placebo. Patients will be stratified by hospital of enrolment. Enrolment logs specific to each site will be used to assign patients to the next available sequential number. The assigned number will correspond to a blinded, sealed, treatment pack containing dexamethasone for injection and dexamethasone tablets, or visually identical placebos. Treatment packs will be prepared centrally by an unblinded study pharmacist and distributed to the sites in batches as required. Only central study pharmacists who holds the master randomization list will know the contents of each pack. This list will be accessed only in the case of emergency unblinding authorized by an investigator or designee as per standard operating procedures. At each site we will use block randomization with variable block size. Stratification by site will minimize the effect of any differences in patient or health care characteristics by ensuring that nearly equal numbers of patients receive either of the two treatment arms at each site. Drug appearance and administration schedules will be identical to maintain blinding amongst the attending physicians and nurses.

8 PATIENT MANAGEMENT

8.1 Initial evaluation

On admission all patients will have a full clinical assessment and examination.

Study entry laboratory tests will be performed as per the study schedule in section 1.2.

A baseline chest radiograph will be performed. A CT or MRI brain scan will be performed if there is evidence of raised intracranial pressure or focal neurological abnormalities according to local practice and resources.

8.2 Other Treatment

8.2.1 Antifungal treatment

All patients will receive antifungal treatment consisting of 2 weeks of intravenous amphotericin B 1mg/kg/day combined with high dose fluconazole (800mg/day), followed by fluconazole 800mg/day for 8 weeks (10 weeks in total, (see appendix 8)). This is locally feasible and consistent with recent guidelines [3, 4]. Following the 10 week period of therapy all patients, provided they have responded to treatment, will receive long term secondary prophylaxis with fluconazole 200mg/day. Modification of antifungal therapy will be made according to the patient's needs and the judgment of the attending physician. Any changes to antifungal therapy will be recorded in the case record form. The cost of antifungal treatment (including secondary prophylaxis until 6 months after randomisation) will be covered by existing local financial support or trial finances.

8.2.2 Antiretroviral therapy

All patients must to be referred to local HIV services as soon as practicable, preferably while still admitted to hospital, to ensure that they have access to locally available HIV services including counseling and ARVs.

It is not clear when antiretroviral therapy should be started in cryptococcal meningitis. Large studies are currently underway in order to answer this question. Early initiation may offer faster immune reconstitution benefitting survival, or may increase the risk of IRIS and drug toxicities, adversely affecting survival. In the absence of reliable data, most physicians would recommend starting ARVs after 2-4 weeks antifungal treatment, provided the patient has made a good response. The date that ARVs are started (or stopped) in patients in the study will be recorded.

8.2.3 Opportunistic Infection Prophylaxis

Most patients will be profoundly immunosuppressed and should receive prophylactic therapy against other opportunistic infections, such as daily trimethoprim-sulfamethoxazole, in accordance with local guidelines and practices.

8.3 Hospital Admission

Intravenous amphotericin B is administered for 14 days necessitating hospital admission during this period.

8.4 Clinical monitoring

Patients will have daily GCS and review as per standard care until discharge from hospital. The decision to discharge patients from the hospital is at the attending physician's discretion and is based upon the clinical status of the patient. Following discharge patients will be seen weekly until 4 weeks, at 6 weeks and at 10 weeks. If the exact visit day is not feasible, scheduled visits can occur at up to 5 days following the stipulated time to account for weekend and holidays (for example, the 4 week review should occur at on day 28-33 following randomisation, week 10 visit on day 70-75). Patients will be monitored closely for

- Death - the date of death and cause will be recorded
- New neurological events (onset of new focal neurological signs or fall in Glasgow coma score of $\geq$ 2 points for $\geq$ 2 days, following > 7 days clinical stability or improvement after randomization)
- Drug-related adverse events
- New or recurrent AIDS defining illnesses (Appendix 10)
- Visual deficit (at week 10)
- IRIS
- Raised intracranial pressure (clinical or measured)

Uniform management of patients and recording of data will be ensured by the local study staff who will do clinical assessments daily while admitted and at follow-up visits.

Disability and mortality at 6 months and evidence of morbidity (including IRIS) since last seen (week 10) will be collected with either a structured telephone interview or an outpatient visit. Outpatient visits may occur in the patient's home when the patient cannot come to the hospital.

8.5 Laboratory monitoring

Inpatient laboratory monitoring will be as shown in the study schedule (section 1.2).

Other investigations may be performed as clinically indicated. Data for the following will be recorded when performed for routine clinical care:

- CSF, if neurological deterioration (Gram stain and routine culture, ZN stain and mycobacterial culture, India ink stain and fungal quantitative culture)
- Sputum, if symptomatic (routine culture, ZN stain)
- Urine culture, if urinary symptoms (urine culture)
- Stool culture, if prolonged diarrhoea (microscopy, culture and parasites)
- Blood cultures, if persistent fever
- Lymph node aspiration (routine and mycobacterial cultures)
- Blood glucose will be measured when CSF is examined or if hyperglycaemia is suspected

A window period of +/- 2 days outside of the scheduled laboratory tests will apply to all tests that are not baseline inclusion/exclusion criteria.

8.6 Imaging

A chest X-ray needs to be performed on study entry if it has not been done at the time of diagnosis. The result will be recorded in the CRF. Brain imaging is not mandated by the study. The decision to perform brain imaging will be according to local practice. Results of brain imaging when available will be recorded.

8.7 Management of adverse events

Possible side effects of dexamethasone are described in section 2.5.

Given the experience from tuberculous meningitis, gastrointestinal haemorrhage is extremely unlikely (it occurred more frequently in placebo recipients than in patients receiving dexamethasone in the large randomised controlled trial from Vietnam). Gastrointestinal haemorrhage will be treated as per local practices with cardiovascular support and proton pump inhibitors or H2 antagonists. Hyperglycaemia will be managed with insulin.

8.8 Stopping Study Drug and Unblinding Rules

The purpose of the randomised, double blind design of the trial is to protect the results from the potential influence of study staff or patient bias about which treatment is the most effective.

Occasionally it can be necessary to STOP the study intervention (placebo or dexamethasone) or to UNBLIND the patient's treatment allocation.

Note:

- *STOPPING* study drug does not necessitate *UNBLINDING* the treatment allocation. *UNBLINDING* treatment is not necessarily an indication to *STOP* treatment.
- STOPPING or UNBLINDING a patient's treatment does not mean that they have withdrawn from the study. Patients continue in the study and follow the protocol schedule for visits and investigations until its conclusion at 6 months of follow-up.

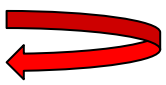
8.8.1 Stopping study drug

Due to the risk of corticosteroid-induced adrenal suppression, study drug must be stopped in a carefully controlled manner. There are few good quality data available to enable prediction of the degree of adrenal suppression that occurs with corticosteroid therapy. Therefore, conventional rules for management of corticosteroid withdrawal will be followed for study patients. Tapering the dose of corticosteroids reduces the risk of adrenal suppression. Study drug should only be stopped immediately in 2 circumstances:

1. if the patient has received 4 or fewer doses of study drug
2. if the study drug is being replaced by other corticosteroid therapy

In all other circumstances, study drug will be stopped using dose tapering by moving the patient's treatment forward on the dosing schedule as illustrated below: (for example, if they are in week 3 of treatment, they should be moved immediately onto the week 4 dose, and then continue treatment as per the dose tapering schedule with weekly reductions in the treatment dose).

Period	**Dexamethasone Dose**
Week 1	0.3 mg/kg iv
Week 2	0.2 mg/kg iv
Week 3	0.1 mg/kg po
Week 4	3.0mg total/day po
Week 5	2.0mg total/day po
Week 6	1.0mg total/day po
Week 7 onwards	Stops



Any changes in patient's medication must be recorded on the CRF and on the concomitant medication form.

8.8.2 Indications for stopping Study Drug/Placebo

The main indication to stop the study drug is where the patient develops a condition for which there is good evidence that steroids are beneficial and should be prescribed. Examples of this are:

1. **Tuberculous meningitis** - Dexamethasone has been demonstrated in a large randomized controlled trial to reduce the risk of death [10]. If a patient develops TB meningitis, the study drug should be stopped, and the patient should received the appropriate dosage of steroids according to the severity of their disease. This does not necessitate unblinding of treatment allocation.
2. **Intracranial space occupying lesion with mass effect -** For example, a primary brain tumour such as CNS lymphoma. Space occupying lesion with mass effect is generally considered to be an indication for steroid therapy. A typical dose would be dexamethasone 4mg four times daily. The study drug should be stopped in all patients, and the patient should receive corticosteroid treatment as per local guidelines. This does not necessitate unblinding of treatment allocation.

   Note: a suspected cryptococcomas is not an indication to alter the study drug – we do not know if dexamethasone is indicated in this case, and the study is testing this indication.
3. **Severe Pneumocystis Pneumonia (PCP) -** All patients in the study must receive PCP prophylaxis with co-trimoxazole - this reduces the risk of developing PCP. However, PCP may still occur. Microbiological confirmation of PCP is not always possible and the diagnosis is often made on clinical grounds. The chest X-ray typically shows bilateral interstitial,often perihilar, shadowing. The differential diagnosis of this appearance includes bacterial pneumonia and Acute Respiratory Distress Syndrome (ARDS) due to cryptococcosis. The gold standard for diagnosis of PCP is to demonstrate the presence of *Pneuomcystis jirovecii* in broncho-alveolar lavage washings or in induced sputum samples. Severe PCP is confirmed when there is a typical clinical syndrome AND the arterial blood oxygen partial pressure (pO2) is less than 70 mmHg (FiO2 ≥20%). Prednisolone (40mg twice daily for 5 days, 40mg once daily for 5 days, followed by 20mg/day for 11 days), in combination with high dose co-trimoxazole, has been shown to reduce the risk of death from severe PCP [41]. Where arterial blood gas measurement is not available, diagnosis of severe PCP should be made in accordance with local guidelines. In any patient in whom the diagnosis of severe PCP is made the study drug must be stopped immediately and the patient treated with prednisolone in conventional doses, and high dose co-trimoxazole as per local guidelines. This does not necessitate unblinding of treatment allocation.

Any changes to study medication will be recorded in the case record form.

8.8.3 Unblinding

Unblinding means revealing the identity of the study treatment (i.e. dexamethasone or placebo). Treatment allocation should only be unblinded if knowing the treatment that a patient has been allocated will result in a change in the patient's management. The need to unblind is likely to be rare during the study. Unblinding a patient's treatment allocation does not enable us to tell if a particular adverse event is due to the investigational agent – that conclusion can only be drawn with an analysis of all available data, either at an interim safety analysis (which is done by the independent data and safety monitoring board) or after the final analysis of the study.

8.8.4 Process for unblinding

The decision whether or not to unblind should be discussed with the lead country investigator or the principal investigator when possible. Unblinded treatment allocation should be held securely at each site and available at all times. The responsibility to approve unblinding will be assigned to dedicated staff at each site. Access to treatment allocation should only be given with the approval of one of these dedicated staff. Unblinding will be documented in the case record form.

8.9 Data on concomitant medications

At each visit, information on other medications, including start dates and reason for taking them, will be documented in the case record forms.

8.10 Withdrawal from the trial

Patients may voluntarily withdraw from the trial for any reason. If this occurs, the patient will be referred to standard clinical care facilities. The patient's withdrawal from the trial will not affect their access to the best standard of care within the local health system. With the agreement of the patient, clinical and laboratory assessment should be performed and recorded at the time of withdrawal. Patients may also decide to stop study treatment without withdrawing from the study, in which case treatment will be adjusted as described below and follow-up will continue as per the study schedule.
Provided no more than 4 days of study drug have been administered, if a patient wishes to withdraw study drug can be stopped immediately. If a patient has received 5 or more days of study drug, then it is possible that there will be adrenal suppression, and the dose of dexamethasone should be tapered.

Thus, if a patient wants to withdraw from the study, and has received 5 or more days of study drug, then the withdrawal phase of steroid dosing will be started immediately – the patient will be switched to the next dexamethasone dose in the steroid dosing sequence. For example, if on day 16 a patient wants to leave the study then they will be immediately switched to the next phase of steroid/placebo dose - 3mg/day - and the treatment tailed off according to the treatment schedule (Section 5.1). This continuation of treatment post-withdrawal will be fully explained in the informed consent form. Follow-up will be according to clinical need. All patients in the study will be provided with steroid information cards in their own language. These will detail the need to gradually reduce the dose if they are to be stopped, and to inform other health care workers that they are taking dexamethasone should they require treatment from a health care worker not involved with the trial. If the patient has an unscheduled period off treatment or not in follow-up this should be recorded in the case report forms.

9 DEFINITION AND ASSESSMENT OF ADVERSE EVENTS

9.1 Definition of Adverse Events

An adverse event (AE) is any undesirable event that occurs to a study participant during the course of the study whether or not that event is considered related to the study drug. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the study drug, whether or not considered related to the study drug.

Examples include:

- An increase in severity or frequency of a pre-existing abnormality or disorder (events that are marked by a change from the participant's baseline/entry status)
- All reactions from sensitivity or toxicity to study drug
- Injuries or accidents (e.g., for a fall secondary to dizziness, "dizziness" is the event and the injury secondary to the fall is the "outcome")
- New clinically significant abnormalities in clinical laboratory values, physiological testing or physical examination.

Stable chronic conditions, such as arthritis, which are present prior to clinical trial entry and do not worsen are not considered AEs and will be documented in the subject's clinical chart as medical history.

Clinical or laboratory events are considered adverse events only if they occur after the first dose of study treatment and before the patient completes trial participation. (See below for reporting of adverse events.)

9.2 Definition of Serious Adverse Events

An AE is considered to be "serious" if it results in one of the following outcomes

- Death,
- Life-threatening event (the subject was at immediate 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),
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity (a substantial disruption of a person's ability to conduct normal life functions),
- Congenital anomaly/birth defect
- Important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

9.3 Definition of Unexpected Serious Adverse Events

Unexpected Serious Adverse Events are untoward medical events which fit one or more of the criteria for SAEs above and which are not considered a part of normal clinical progression of disease or an expected drug reaction. Any event which becomes of concern to the investigators or study doctors during the course of the trial may be reported as a USAE.

9.4 Assessment of Adverse Events

Adverse events will be defined according to the Common Terminology Criteria for Adverse Events (CTCAE) definitions: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. New AIDS defining events will be defined according to the revised CDC criteria modified for this trial (see Section 17.10, Appendix 10). In the event that an adverse event is not described within the CTCAE definitions, or is a new AIDS defining event as defined in Appendix 10, the following generic severity grading will be used:

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL)*.

Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL**.

Grade 4 Life-threatening consequences; urgent intervention indicated.

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Details on the grading of specific adverse events can be found in appendix 11. [Note: "Life-threatening" as a severity grade is not necessarily the same as "life-threatening" as a "serious" criterion used to define a serious adverse event. The former is a "potential" threat to life and the latter is an "immediate" threat to life.]

A **laboratory abnormality** only needs to be recorded as a clinical adverse event if it is associated with an intervention. Intervention includes, but is not limited to, discontinuation of a current treatment, dose reduction/delay of a current treatment, or initiation of a specific treatment. In addition, any medically important laboratory abnormality may be reported as an adverse event at the discretion of the investigator. This would include a laboratory result for which there is no intervention but the abnormal value suggests a disease or organ toxicity. Laboratory events will be graded according to CTCAE definitions.

If clinical sequelae are associated with a laboratory abnormality, the diagnosis or medical condition should be reported as the adverse event (e.g., renal failure, haematuria) - not the laboratory abnormality (e.g., elevated creatinine, urine RBC increase).

10 RECORDING AND REPORTING OF ADVERSE EVENTS AND PROTOCOL VIOLATIONS

10.1 Adverse event recording

Grade 3 and 4 adverse events will be **recorded** in the case report form and entered to the study database. Grade 1 & 2 events will not be recorded since in a severe disease such as cryptococcal meningitis the number of low grade adverse events is likely to be high representing a large reporting

burden which may impact upon the quality of recording and reporting of more important grade 3 and 4 adverse events.

10.2 Adverse event reporting

Serious adverse events and serious unexpected adverse events will be **reported** to the Principal Investigator within 7 days of occurrence, or sooner according to local requirements. The Principal Investigator will report all unexpected serious adverse events to the DMEC within 10 days of occurrence. Unexpected serious adverse events will be reported to the responsible ethical committees within 10 days of occurrence or as required by the committee.

10.3 Protocol violations

Protocol violations are events which contradict or omit protocol instructions. Violations which compromise patient safety or the integrity of trial data will be recorded and reported to the responsible Ethics Committees as required by the regulations of each Committee.

11 STATISTICS

11.1 Sample size and power considerations

The trial is powered for the primary endpoint, i.e. overall survival during the 10 week follow-up period.

There are few data on which to estimate the potential effect size of dexamethasone on mortality from cryptococcal meningitis. We have based our estimate of the effect size on data from tuberculous meningitis, which shares clinico-pathological features with cryptococcal meningitis. In a large RCT of dexamethasone to treat tuberculous meningitis in Vietnam, the hazard ratio (HR) for death was 0.69 at 9 months in favour of dexamethasone [25]. Most of this effect occurred during the first 3 months of treatment. The dose of dexamethasone we will test is the same as used in that trial. Additionally, in a study of dexamethasone in bacterial meningitis in Vietnam the HR was 0.43 in patients with microbiologically confirmed disease, and 0.59 in a study based in Europe, again in favour of dexamethasone [34, 35]. These analyses were all based on the intention-to-treat principle (including non-compliant patients) and it seems sensible to expect a similar rate of non-compliance in our study. Therefore, we believe a target hazard ratio of 0.7 in favour of dexamethasone to be reasonable for our trial.

In order to detect such a risk reduction of 30% with 80% power at the two-sided 5% significance level, a total of at least 247 deaths need to be observed. A major aim of this trial is to generate robust evidence

across both continents. To achieve this goal, the sample size calculation assigns the same target number of events (deaths) to each continent. Setting the target number to 130 deaths per continent ensures a sufficiently large total number of deaths and also guarantees a power of 83% to detect a larger treatment effect corresponding to a hazard ratio of 0.6 in subgroup analyses by continent.

The 10 week death rate in patients in Vietnam is 30%, and in Malawi 50% [38]and the target hazard ratio of 0.7 corresponds to absolute risk reductions in mortality from 30% to 22%, or from 50% to 38%. Assuming that these mortality rates are representative for the respective continents, recruitment of 500 patients from Asia and 300 patients from Africa (800 patients in total) is sufficient to observe the target number of deaths. Assuming a 10% loss to follow-up rate, we will need to recruit 880 patients (330 from Africa and 550 from Asia).

11.2 Analysis

11.2.1 Analysis of the primary endpoint and 6-month mortality

The analysis will be based on a stratified Cox proportional hazards model allowing for separate baseline hazards for each continent (Asia or Africa) and treatment allocation as the only covariate. The stratification is based upon the expectation of different mortalities in the control arm by continent but similar (relative) effects of the intervention across continents. The proposed test is essentially equivalent to using a stratified log-rank test to compare the two treatment arms. We prefer to use the Cox model as it automatically provides treatment effect estimates and confidence intervals in addition to the p-value.

In a second stage, overall survival will be modeled using the Cox proportional hazards regression model with stratification by continent and the following covariates (in addition to the treatment group): country, baseline fungal load, Glasgow coma score less than 15, and ARV status at study entry (ARV naïve or experienced).

Potential heterogeneity of the treatment effect will be assessed based on appropriate interaction (likelihood ratio) tests and the following pre-defined sub-grouping variables:

- Continent
- Country
- IDSA indications for steroid treatment at baseline (cryptococcoma with mass effect, acute respiratory distress syndrome or IRIS: yes or no)
- Glasgow coma score <15 (yes or no)
- Naïve to ARVs at study entry versus on ARVS at study entry

All Cox regressions and sub-group analyses will be performed for the primary endpoint (10-week survival) as well as for 6-month survival. Kaplan-Meier plots and explicit survival estimates at 10 weeks and 6 months of follow-up will also be calculated for the full populations and for each continent separately.

11.2.2 Analysis of secondary endpoints

Neurological disability. The disability score at week 10 and month 6 of follow-up is defined as the higher (worse) of the "simple question" and the Rankin score assessed at that time point and will be categorized as good outcome, intermediate disability, severe disability, or death (in case the patient died prior to the respective time point) as previously described [10] (also see appendix 2). The proportion of patient with a good outcome will be compared between the two arms with a logistic regression adjusted for continent (in addition to the treatment arm). Patients lost to follow up will be analyzed according to their last recorded disability status. If the rate of patients lost to follow-up exceeds 10%, we will also perform an alternative analysis based on multiple imputation of missing values.

Rate of CSF sterilisation during the first 2 weeks (based on available data from selected sites only). Fungal decline in the first 14 days will be modeled with a joint model for longitudinal and survival data. The longitudinal part of the model will be a linear mixed effects model with longtitudinal log-CSF quantitative culture fungal counts as the outcome, continent and interaction terms between the treatment groups and the time since enrolment of the measurement as fixed covariates and a random patient-specific intercept and slope. The survival part of the joint model models mortality up to 2 weeks depending on the treatment group, continent and the patient-specific random intercepts and slopes. The survival part acts as a missing data mechanism to allow for potentially informative truncation of quantitative count measurements due to death.
Longitudinal measurements of intracranial pressure during the first 2 weeks will be modeled in the same way.

Adverse events. The frequency of serious and grade 3&4 adverse reactions as well as the frequency of specific adverse events will be summarized (both in terms of the total number of events as well as the number of patients with at least one event). The proportion of patients with at least one such event

(overall and for each specific event separately) will be summarized and (informally) compared between the two treatment groups based on Fisher's exact test**.**

The rate of IRIS and the rate of relapse (defined as antifungal treatment intensification or re-treatment) will be modeled with cause-specific proportional hazards models stratified by continent taking into account the competing risk of prior death. Secondary time-to-event endpoints (time to new AIDS-defining illness or death and time to new neurological event or death) will be analyzed with Kaplan-Meier curves and Cox regression models as described for the primary endpoint above. The proportion of patients with blindness or visual deficit will be compared between the two arms with a logistic regression (as for the proportion of patients with good disability described above).

11.2.3 Analysis populations

The primary analysis population for all analysis is the full analysis population containing all randomized patients except for those mistakenly randomized without cryptococcal meningitis. Patients will be analyzed according to their randomized arm (intention-to-treat). In addition the primary endpoint will be analyzed on the per-protocol population which will exclude the following patients: major protocol violations and those receiving less than 1 week of administration of the randomized study drug for reasons other than death.

12 INTERIM ANALYSIS AND ROLE OF THE DATA MONITORING AND ETHICAL COMMITTEE (DMEC)

An independent DMEC will oversee the trial. Unexpected serious adverse events with treatment allocation blinded will be reported to the DMEC within 10 days of occurrence and followed-up until resolution. The DMEC will perform formal interim analyses after every 50 deaths. According to the sample size calculations, we expect to observe around 247 deaths during the course of the study. Thus, 4-5 planned formal interim analyses after 50, 100, 150, 200, and (possibly) 250 deaths will take place.

At these interim analyses, the DMEC will receive a report including unblinded summaries of mortality, serious adverse events, grade 3&4 adverse events, and estimates of the rate of CSF sterilisation during the first 14 days (from selected sites only) by treatment arm. The report will be prepared by the DMEC statistician and distributed to all DMEC members for review. Based on these data, the committee will make recommendations on the continuation, cessation or amendment of the study. The study statistician will remain blinded throughout the study but will aid in setting-up the code for generating

the interim analysis summaries. The randomization list will be sent to the DMEC statistician directly from the study central pharmacist.

Unless the benefit of adjuvant treatment with dexamethasone is shown "beyond reasonable doubt" at an interim analysis, no formal stopping for efficacy is foreseen. The Haybittle-Peto boundary, requiring $p<0.001$ at interim analysis to consider stopping for efficacy, will be used as guidance. A level of significance of 1% will be used as a guide for stopping the trial early because of a detected harm of dexamethasone. In addition, the DMEC will receive conditional power curves to assess whether it remains realistic that the trial will demonstrate superiority of dexamethasone conditional on the data accrued up to the point of the interim analysis. Importantly, the DMEC recommendations will not be based purely on statistical tables but will also use clinical judgment.

As the dissemination of preliminary summary data could influence the further conduct of the trial and introduce bias, access to interim data and results will be confidential and strictly limited to the involved independent statistician and the monitoring board and results (except for the recommendation) will not be communicated to the outside and/or clinical investigators involved in the trial.

Further reviews will be at the discretion of the DMEC or the request of the Trial Steering Committee. All DMEC reports, replies or decisions will be sent to the Trial Steering Committee and the responsible Research Ethical Committees.

13 ETHICAL CONSIDERATIONS

13.1 Declaration of Helsinki and Good Clinical Practice

The Investigator will ensure that this study is conducted in compliance with the current revision of the Declaration of Helsinki (Seoul 2008) and the Medical Research Council Guidelines on Good Clinical Practice (1998).

13.2 Ethical Review

The Oxford University Tropical Research Ethics Committee (OxTREC) will serve as the ethical committee of reference for this trial. The study protocol and its associated documents will be submitted to the ethical committee of reference and all other ethical committees as required by local regulation in each site and country. The Investigator will submit and, where necessary, obtain approval from the ethical committee of reference and the responsible local committees for all substantial amendments to the original approved documents. Annual reviews of the trial will be conducted by the ethical committee of reference and as required by all other committees.

13.3 Informed consent

The study staff will discuss the study with all potential adult participants or, in the case of a participant who is unable to give informed consent independently, with a representative appropriate within local culture. Study staff will describe the purpose of the study, the study procedures, possible risks/benefits, the rights and responsibilities of participants, and alternatives to enrolment. The participant or representative will be invited to ask questions which will be answered by study staff, and they will be provided with appropriate numbers to contact if they have any questions subsequently. If the participant or representative agrees to participate, they will be asked to sign and date an informed consent form. A copy of the form will be given to them to keep. If required, the participant or representative will be given up to 24 hours to consider the study provided the participant remains eligible for the study.

Participants who were consented by a representative will be approached to consider consent independently if at any time during study participation s/he becomes able to consider consent independently.

In addition to the procedures above, illiterate signatories will have the informed consent form read to them in the presence of a witness who will sign to confirm that the form was read accurately and that the participant or representative agrees to participation. All patient information sheets and Consent/Assent forms will be written in the local language and will use terms that are easily understandable. Clinical care will not be delayed in any case during consideration of consent.

13.4 Risks

This study will use a drug that has been studied thoroughly and its toxicities are well described. Details can be found in the Study Treatment section of this protocol. Patients will be closely monitored for all adverse events and treated as per standard of care. Additional volumes of blood and cerebral spinal fluid will be taken for research tests. These volumes have very little risk of affecting the participant's health. Some phlebotomy may be performed more often than is required by clinical care. This procedure carries the small risk of bruising and infection.

Dexamethasone may be growth suppressing in children and fetuses. Therefore children and pregnant women have been excluded from this trial.

13.5 Benefits

It is unknown if study participants who receive study treatment will benefit. The additional monitoring and follow-up of patients by dedicated study staff may be of benefit to patients treated in resource limited settings. Treatment costs for trial participants will be supported including payment of all study treatment and standard anti-fungal treatment.

Training in laboratory and clinical procedures, research methods and good clinical practice, will be given to all participating centres. Investigators will engage with the HIV/AIDS community in each setting to ensure that trial conduct is cohesive with local patient support services.

13.6 Alternatives to Study Participation

The alternative to participation in this study is routine care by the doctors in the hospital. Patients will be responsible for their own treatment costs as per local norms and hospital policy.

13.7 Confidentiality

Participants will be assured that all information generated in this study will remain confidential. The trial staff will ensure that the participants' anonymity is maintained. Participant's names will be recorded at the time of enrolment to allow for their identification at follow-up visits. However identifiable information will be linked to stored data or samples only by a protected Master List. This list will not be shared outside the study staff. All documents will be stored securely and all reports or samples will be coded without identifying information. Direct access will be granted to authorized representatives from the host institution and the regulatory authorities, if applicable, to permit trial-related monitoring and inspections.

13.8 Withdrawal of Participants from the Study

Each participant has the right to withdraw from the study at any time. All patients who withdraw will be referred for treatment as per routine clinical care. The reason for withdrawal will be recorded in the CRF. Study drug will be managed as detailed in Section 8.7 and 8.9.

13.9 Sample Sharing and Storage

Samples collected will be used for the purpose of this study as stated in the protocol and stored for future use in studies not yet conceived, which may include genetic studies. Consent will be obtained from subjects for genetic testing and for sample storage and/or shipment of specific samples to collaborating institutions for investigations that cannot be performed locally. Any proposed plans to use

samples other than for those investigations detailed in this protocol will be submitted to the relevant ethics committees prior to any testing.

The participants will be identified only by a study specific participant number and/or code in any database. The name and any other identifying detail will NOT be included in any study data electronic file.

13.10 Sponsorship and Insurance

The University of Oxford has appropriate insurance-related arrangements in place in respect of the University's role as research sponsor for this study.

14 DATA

14.1 Data collection and entry

Source documents will be generated during the study by the site study staff at participating institutions. Source documents include all original recordings of observations or notations of clinical activities, and all reports and records necessary for the evaluation and reconstruction of the clinical trial. Source documents include, but are not limited to, the subject's medical records, research case record forms (paper or electronic), laboratory reports, ECG tracings, x-rays, radiologist's reports, subject's diaries and questionnaires, biopsy reports, ultrasound photographs, progress notes, pharmacy records, and any other similar reports or records of procedures performed during the subject's participation in the study.

Access to applicable source documents is required for study purposes. The site investigators are responsible for maintaining any source documentation related to the study. Source documentation should support the data collected on the CRF when the CRF is not the original site of recording. Source documentation must be available for review or audit by the sponsor or designee and any applicable regulatory authorities.

Case Report Forms (CRFs) will be used as a data collection tool. The study team will transfer the information from the source documents onto the CRFs. CRFs may be used as source documents if they are the primary data collection tool for specified data as documented in written standard operating procedures. The site Investigators are responsible for maintaining accurate, complete and up-to-date records. These forms are to be completed on an ongoing basis during the course of the study by authorized individuals.

Corrections to paper CRFs must be initialed and dated by the person making the correction and must not obliterate the original entry. All CRFs should be reviewed by the designated study staff and signed as required with written or electronic signature, as appropriate.

Selected study members (study doctors or nurses) will be trained on how to enter all clinical data as source information from the CRFs and from laboratory source documents into an internet-based computerized data entry system called CliRes hosted by OUCRU Viet Nam. Data entry will occur simultaneously as data are being generated during the trial as soon as possible after the information is generated. Data may be manually entered or scanned and electronically uploaded dependant on available software. Source documents and electronic data will be verified according to the Trial Monitoring Plan.

Following study completion and the main analyses and publication of the study results, the study sub-datasets consisting of the patient data from particular recruiting sites will be available to the investigators from those sites.

14.2 Record Retention

The investigator is responsible for retaining all essential documents listed in the MRC guidelines on Good Clinical Practice. All essential documentation for all study subjects are to be maintained in original paper format by the investigators in a secure storage facility for a minimum of 3 years and as required by local regulations thereafter. All essential documentation will be converted from paper to electronic format (if required) and stored centrally for at least 10 years after the completion of the trial and as required by local regulations thereafter. All stored records are to be kept secure and confidential.

15 MONITORING

The trial will be conducted in compliance with this protocol, Medical Research Council Guidelines of Good Clinical Practice and any applicable regulatory requirement(s).

The study will be adequately monitored by the OUCRU or their designate. Monitors will visit the clinical research site to monitor all aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of adequately

signed informed consent documents for each enrolled subject; 2) to verify the prompt, complete and accurate recording of all monitored data points, and prompt reporting of all SAEs and unexpected SAEs; 3) to compare abstracted information with individual subjects' records and source documents (subjects' charts, case report forms, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); 4) to verify the supply and condition of the study drug and the accurate and secure assignment of randomization code; and 5) to ensure protection of study subjects, investigators' compliance with the protocol, and completeness and accuracy of study records. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements and applicable guidelines are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

16 PUBLICATION

Any publication or presentation during the active phase of the study must have permission from the Investigators. The investigators will define the strategy for publication, resolve any problems of authorship and maintain the quality of publications. All publications will acknowledge the appropriate authors and funding sources according to normal academic practice. The investigators are the custodian of the data and specimens generated from this trial.

17 APPENDICES

17.1 Appendix 1 Dexamethasone Dosing

Period	**Dexamethasone Dose**
Week 1	0.3 mg/kg iv
Week 2	0.2 mg/kg iv
Week 3	0.1 mg/kg po
Week 4	3.0mg total/day po
Week 5	2.0mg total/day po
Week 6	1.0mg total/day po
Week 7 onwards	Stops

17.2 Appendix 2 Outcome and disability grading: "Two simple questions" and Rankin score

The 'Two simple questions'

Does the patient require help from anybody for everyday activities? *(For example eating, drinking, washing, brushing teeth, going to the toilet.)*	Yes/no	Yes= Poor outcome
Has the illness left you with any other problems?	Yes/no	Yes= Intermediate outcome No= Good outcome

The Modified Rankin Scale

Grade	Description
0	No symptoms
1	Minor symptoms not interfering with lifestyle
2	Symptoms that lead to some restriction in lifestyle, but do not interfere with the patients ability to look after themselves
3	Symptoms that restrict lifestyle and prevent totally independent living
4	Symptoms that clearly prevent independent living, although the patient does not need constant care and attention.
5	Totally dependent, requiring constant help day and night.

Grade 0: Good outcome, Grade 1 or 2: Intermediate outcome, Grade 3-5: poor outcome

17.3 Appendix 3 Cryptococcal Quantitative cultures, Cryptococcal Archiving- Standard Operating Procedure

Equipment:

1. Gilson Pipette – PI00 or P200
2. Sterile 200 microL Pipette Tips
3. Small Glass Bijoux containing 0.9mls sterile water (4 per sample)
4. Sabouraud dextrose agar plates (5 per sample)
5. Sterile eppendorfs for storing CSF
6. Nalgene storage boxes
7. Pro-Lab Microbank Beads for Archiving
8. Safety Cabinet (Class II)
9. Sterile loops

Methods

Plate preparation

1. Sabouraud dextrose agar
2. Dry in an incubator (30^{o}C) for 30 minutes prior to use

3. Mark each plate into 2 halves using a permanent marker

4. Label each plate with

a. the trial study number,

b. the date the sample was withdrawn from the patient,

c. the date the sample was processed (this should usually be the same date as the date that the sample was taken)

d. the dilution strength i.e. 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}

If the study number is not yet known use the patient's name and date of birth or age

Bijoux preparation

1. Use small Bijoux

2. dispense 0.9 mls of distilled water in each and fasten the lid tightly

3. Autoclave to sterilize

4. before use ensure that the volume in the bijoux looks correct (i.e. appears to be 0.9mls)

4. When processing a sample label each Bijoux with the Study number and the dilution strength (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4})

Samples

1. Samples should be processed the same day they are taken from the patient, as soon as possible after being drawn

2. Prior to processing samples should be kept fridge cold (5-8^{o}C)

3. The samples should be processed in a class 1 or 2 safety cabinet, since there is an aerosolisation risk because of the vortexing

4. Neat samples and diluted samples should be vortexed before every step - ie before making the next serial dilution and before inoculating the plate.

5. Perform 10 fold serial dilutions 4 times on each sample:

a. Vortex neat CSF

b. Withdraw 100 microL (0.1 ml) using a P 100 or P200 pipette

c. Innoculate the aliquot into 0.9ml sterile water in a small Bijoux

d. Vortex the dilution and repeat 3 times to create 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} dilutions

e. Use a new pipette tip for each stage of the dilution process

f. Immediately inoculate the plates

Plate inoculation

1. Label the plates as above

2. Use a P 100 or P200 pipette set to 100 microL

3. Use a new pipette tip.

4. Start from the most dilute dilution (10^{-4})

5. Vortex the dilution

6. Draw up 100 microL dilution

7. Innoculate one half of the plate with approximately 20 x 5 microL drips from the pipette

8. Vortex the dilution

9. Draw up l00microL and inoculate the other half of the plate in the same way

10. Repeat for all the other dilutions, working from the weakest to the neat CSF.

11. If at any stage you are concerned that the tip may have become contaminated then use a new tip

12. Incubate at 30°C for 1 week

Reading samples

1. Start a new recording sheet for each patient

2. Record the patients study number on each sheet

4. Count the colonies by holding the plate over a dark background

4. Identify the plate that has between 20 and 200 colonies. Use this plate to estimate the fungal burden. If there is no plate with more than 20 colonies, then use the plate with positive growth to estimate the fungal burden.

5. Count the number of colonies on each side of the plate and calculate the mean number of colonies.

6. Determine the fungal burden by multiplying the number of colonies by 10^(n+1), where n is the fold number of dilutions of the plate that is read. Eg, if the plate with between 20 and 200 colonies is the 10^{-2} dilution, and the mean number of colonies is 80, then n = 2 and the fungal burden is 80 X 10^3 = 80 000 CFU/ml.

5. Record plates with confluent growth as 'confluent growth'

Processing CSF

1. After making the plates the remaining CSF should be centrifuged at 3-4000rpm for 15 minutes and the resultant supernatant and pellets decanted to eppendorf's and stored at -20 to -80^{o}C.

2. Label the eppendorfs containing the CSF's with the study numberand the date it was TAKEN FROM the PATIENT - not the date it was processed (although
these should usually be the same). Also mark whether the sample is a pellet (use a capital 'P')
or supernatant (use a capital '**SN'**).
3. If there is insufficient CSF sample to centrifuge, then freeze it as whole CSF (and label it as such).

Archiving samples

1. The first available C. *neoformans* growth for each patient should be archived using Pro lab Microbank beads.
2. Use the neat sample from Day 1 (or a later day of the study if there is no Day 1 sample available).
3. Run the sterile loop through **ALL** the colonies from each side of the neat culture plate (from the quantitative culture assessment) and inoculate the beads as per the manufacturer's instructions. Run the loop through all the colonies rather than using a single colony because *Cryptococcus* populations within patients may be heterogeneous.
4. Repeat for the other side of the plate with a new set of beads (so there are two sets of each sample archived).
5. If there are colonies of different morphology on the same plate then archive these separately, and describe them on the tube label- e.g. patient XXXX, 'smooth colony', and Patient XXXX 'irregular colony'.
6. Label each bead tube with the study number, '*C. neoformans'* and the date that the sample WAS TAKEN FROM THE PATIENT (not the date it was cultured or archived).
Try not to use a plate if it is contaminated with mold - subculture first if there are no mold-free plates available.

Which other samples should be archived?

Most samples will only need the earliest available sample to be archived. But, there are circumstances where other samples may need to be archived.
Please also archive:
Any sample where there is increasing growth throughout the course of treatment Eg if the day 14 sample has a higher colony count than the day 7, please also archive the day 7 and day 14 colonies.

Potential Hazards

1. Blood-borne viruses:

CSF samples may be from patients with HIV or other blood-borne viruses. A green laboratory coat and gloves must be worn at all times when working on specimens. The risk of transmission is low, but all patient samples should be handled within the safety cabinet. All staff must be checked for hepatitis B virus immunity prior to commencing work as per local procedures, and vaccinated as appropriate. In the event of a splash or other injury stop work immediately and follow your local guidelines.

2. *Cryptococcus neoformans* var *grubii* and *Cryptococcus neoformans* var *gattii* are ACDP category 2 organisms. They pose a small risk of infection to workers, treatment is available, and there is no risk of person to person transmission. Category 2 organisms can be worked with on the open bench. However, where there is a risk of aerosolisation, a safety cabinet should be used.

17.4 Appendix 4 Definition of Cryptococcal Meningitis associated immune reconstitution syndrome

Case definition for paradoxical cryptococcal immune reconstitution inflammatory syndrome in HIV patients

Antecedent requirements

- Taking antiretroviral therapy
- Cryptococcal disease diagnosed before ART by positive culture or typical clinical features plus positive India ink staining or antigen detection
- Initial clinical response to antifungal therapy with partial or complete resolution of symptoms or signs, fever, or other lesions, or reduction in CSF cryptococcal antigen concentration or quantitative culture

Clinical criteria

- Event occurs within 12 months of ART initiation, reintroduction, or regimen switching after previous failure
- Clinical disease worsening with one of the following inflammatory manifestations of cryptococcosis:
    - Meningitis
    - Lymphadenopathy
    - Intracranial space-occupying lesion or lesions
    - Multifocal disease
    - Cutaneous or soft-tissue lesions
    - Pneumonitis or pulmonary nodules

Other explanations for clinical deterioration to be excluded

- Non-adherence or suboptimum antifungal therapy, indicated by an increase in quantitative culture or antigen titre, or any positive cryptococcal culture after 3 months of antifungal therapy
- Alternative infection or malignant disease in the affected site
- Failure of ART excluded if possible (eg, failure to achieve ≥1 log10 viral load by 8 weeks of ART)

ART=antiretroviral therapy. CSF=cerebrospinal fluid.

17.5 Appendix 5 WHO clinical staging for HIV/AIDS

Clinical Stage 1
Asymptomatic Persistent generalised lymphadenopathy (PGL) Performance scale 1: asymptomatic, normal activity
Clinical Stage 2
Weight loss, <10% of body weight Minor mucocutaneous manifestations (seborrheic dermatitis, prurigo, fungal nail infections, recurrent oral ulcerations, angular cheilitis) Herpes zoster, within the last 5 years Recurrent upper respiratory tract infections (e.g. bacterial sinusitis) And/or performance scale 2: symptomatic, normal activity.
Clinical stage 3
Weight loss, >10% of body weight Unexplained chronic diarrhoea, > 1 month Unexplained prolonged fever (intermittent or constant), > 1 month Oral candidiasis (thrush) Oral hairy leukoplakia Pulmonary tuberculosis, within the past year. Severe bacterial infections (e.g. pneumonia, pyomyositis) And/or Performance scale 3: bed-ridden, < 50% of the day during the last month
Clinical stage 4
HIV wasting syndrome, as defined by CDC[1]

Pneumocystis carinii pneumonia
Toxoplasmosis of the brain
Cryptosporidiosis with diarrhoea, >1 month
Cryptococcosis, extra pulmonary
Cytomegalovirus (CMV) disease of an organ other than liver, spleen or lymph nodes
Herpes Simplex Virus (HSV) infection, mucocutaneous >1 month, or visceral any duration
Progressive multifocal leukoencephalopathy (PML)
Any disseminated endemic mycosis (e.g. histoplasmosis, coccidioidomycosis)
Candidiasis of the oesophagus, trachea, bronchi or lungs
Atypical mycobacteriosis, disseminated
Non-typhoid Salmonella septicaemia
Extra-pulmonary tuberculosis
Lymphoma
Kaposi's sarcoma (KS)
HIV encephalopathy, as defined by CDC[2]
And/or Performance scale 4: bed-ridden, > 50% of the day during the last month

(Note: Both definitive and presumptive diagnoses are acceptable)

[1] HIV wasting syndrome: weight loss of >10% of body weight, plus either unexplained chronic diarrhoea (>1 month), or chronic weakness and unexplained prolonged fever (>1 month).

[2] HIV encephalopathy: clinical finding of disabling cognitive and/or motor dysfunction interfering with activities of daily living, progressing over weeks to months, in the absence of a concurrent illness or condition other than HIV infection that could explain the findings.

17.6 Appendix 6: Visual assessment

Visual assessment – record the best performance	
Function	**Score**
Normal	1
Blurred	2
Finger counting	3
Movement perception	4
Light perception	5
No Light perception	6

17.7 Appendix 7: Management of raised intracranial pressure

Raised intracranial pressure is a frequent complication of cryptococcal meningitis. The mechanism is not clear, but probably is a combination of impaired CSF drainage, increased CSF production, cerebral oedema and inflammation. Adults have approximately 175 mls of cerebrospinal fluid, and the 24 hour production of CSF is in the order of 550 mls. The normal CSF pressure recorded by lumbar puncture with the patient reclining in the left lateral position is 5-18cm of CSF. There are few data to guide the management of raised intracranial pressure in patients with cryptococcal meningitis, but the recommendations of the IDSA guidelines are that the CSF pressure should be identified at baseline. If the CSF pressure is ≥25 cm of CSF and there are symptoms of increased intracranial pressure during induction therapy, relieve by CSF drainage (by lumbar puncture, reduce the opening pressure by 50% if it is extremely high or to a normal pressure of ≤ 20 cm of CSF). If there is persistent pressure elevation ≥ 25 cm of CSF and symptoms, repeat lumbar puncture daily until the CSF pressure and symptoms have been stabilized for 1-2.

Brain imaging should be considered prior to lumbar puncture in patients with focal neurological signs or profound coma, although its sensitivity for predicting cerebral herniation is poor [4].

17.8 Appendix 8: Antifungal Treatment

All patients will receive amphotericin B deoxycholate 1mg/kg/day for 2 weeks combined with fluconazole 800mg day for the first 2 weeks following randomization. This will be followed by 800mg fluconazole per day for 8 weeks. After this point patients are switched to fluconazole 200mg/day secondary prophylaxis. This continues until the patient has had sustained immune reconstitution (CD4 count >100 cells/uL) secondary to antiretroviral therapy. This is consistent with current IDSA and WHO guidelines for the treatment of cryptococcal meningitis*.

* Perfect JR, *et al*: **Clinical Practice Guidelines for the Management of Cryptococcal Disease:** *Clin Infect Dis* 2010, **50**(3):291-322., and **Rapid Advice: Diagnosis, Prevention and Management of Cryptococcal disease in HIV-infected Adults, Adolescents and Children: World Health Organisation**; 2011

Appendix 9: Amphotericin administration and complications

ADMINISTRATION

Renal impairment occurs in 80% of patients receiving amphotericin, and is reversible provided total dose does not exceed 4 grams. Evidence suggests that sodium depletion increases the chance of amphotericin induced nephrotoxicity. Administration of normal saline prior to amphotericin infusion reduces this risk. This must be followed by a dextrose flush, since amphotericin is incompatible with normal saline.

1. Flush line with 50-100mls dextrose 5%.
2. Administer 1000mls Normal Saline containing 20mmol potassium chloride over 2-4 hours (contra-indications: fluid overload, cirrhosis, heart failure)
3. Flush line with 50-100mls dextrose 5%
4. Administer amphotericin:

Dose:	1mg/kg/day
Infusion solution:	5% Dextrose
Rate of Infusion:	4 Hours

MANAGEMENT OF AMPHOTERICIN-INDUCED RENAL IMPAIRMENT

1. Frequent monitoring in all patients of electrolytes, creatinine and urea.
2. There is no need to reduce the dose of amphotericin unless the creatinine is > 3 X upper limit of normal (ULN).
3. If creatinine exceeds 3 X ULN, discontinue amphotericin for 1 day, then reintroduce at half the previous dose, and gradually increase this dose to the target level over the next 2-3 days, carefully observing renal function.

MANAGEMENT OF AMPHOTERICIN INDUCED HYPOKALAEMIA

Reversible hypokalaemia is common in amphotericin treatment. Potassium levels should be checked twice weekly during the period of amphotericin administration. Hypokalaemia can be treated with oral potassium chloride (1-2 tablets two to three times daily according to response). There is evidence that this can be helped by administering a small daily dose of amiloride (10mg) orally.

MANAGEMENT OF RIGORS

A minority of patients may develop rigors and fevers when starting their infusions with amphotericin. This symptom usually resolves after a few days, but can be helped by prophylactic chlorpheniramine or aspirin.

Anaphylaxis is rare with amphotericin, occurring in less than 1% of patients.

Reference:

SH Khoo, J Bond, DW Denning: Administering amphotericin B – a practical approach. J Antimicrobial Chemotherapy (1994) **33**, 203-21

17.9 Appendix 10: Presumptive and definitive criteria for AIDS defining events

Based on 1993 Revised CDC classification system (MMWR 1992; 41(RR-17): 1-19) and modified for this trial

	Presumptive criteria	**Definitive criteria**
Constitutional disease		
HIV wasting syndrome	Unexplained involuntary weight loss >10% from baseline PLUS persistent diarrhoea with ≥ 2 liquid stools/day for > 1 month OR chronic weakness OR persistent fever > 1 month. Should exclude other causes such as cancer, TB, MAC, cryptosporidiosis or other specific enteritis	
Infections		
Aspergillosis, other invasive	CXR abnormality compatible with aspergillosis PLUS invasive mycelia consistent with Aspergillus on lung biopsy or positive culture of lung tissue or positive culture of sputum	CXR abnormality compatible with aspergillosis PLUS invasive mycelia consistent with Aspergillus on lung biopsy PLUS positive culture of lung tissue or positive culture of sputum
Bartonellosis	Clinical evidence of bacillary angiomatosis or bacillary peliosis PLUS positive silver stain for bacilli from skin lesion or affected organ	Clinical evidence of bacillary angiomatosis or bacillary peliosis PLUS positive culture or PCR for *Bartonella quintana* or *Bartonella henselae*
Candidiasis of bronchi, trachea or lungs	None	Macroscopic appearance at bronchoscopy or histology or cytology (not culture)
Candidiasis, oesophageal	Recent onset retrosternal pain on swallowing PLUS clinical diagnosis or oral candidiasis by cytology (not culture) PLUS clinical response to treatment	Macroscopic appearance at endoscopy or histology or cytology (not culture)
Coccidiodomycosis, disseminated or extrapulmonary	None	Histology or cytology, culture or antigen detection from affected tissue
Cryptococcosis, meningitis or pulmonary	None	Histology or cytology/microscopy, culture or antigen detection from affected tissue
Cryptosporidiosis	None	Persistent diarrhoea > 1 month, histology or microscopy
CMV retinitis	Typical appearance on fundoscopy of discrete patches of retinal whitening, associated with vasculitis, haemorrhage and necrosis, confirmed by opthalmologist	None
CMV end-organ disease	None	Compatible symptoms plus histology or detection of antigen from affected tissue

Appendix 10 Presumptive and definitive criteria for AIDS defining events (cont'd)

Infections	**Presumptive criteria**	**Definitive criteria**
CMV radiculomyelitis	Leg weakness and decreased reflexes or syndrome consistent with cord lesion presenting subacutely over days to weeks. CT/MRI shows no mass lesion. CSF shows >5 WBC with >50% polymorphs and positive CMV PCR, antigen or culture	None
CMV meningoencephalitis	Rapid (days to < 4 weeks) syndrome with progressive delirium, cognitive impairment, ± seizures and fever (often with CMV disease elsewhere) CT/MRI may show periventricular abnormalities.	Rapid (days to < 4 weeks) syndrome with progressive delirium, cognitive impairment, ± seizures and fever (often with CMV disease elsewhere) CT/MRI may show periventricular abnormalities and CSF PCR positive for CMV
HSV mucocutaneous ulceration	None	Persistent ulceration for > 1 month, plus histology or culture or detection of antigen or HSV PCR positive from affected tissue
HSV visceral disease e.g oesophagitis, pneumonitis	None	Symptoms, plus histology or culture or detection of antigen or HSV PCR positive from affected tissue
VZV multidermatomal	≥ 10 typical ulcerated lesons affecting at least 2 non-contiguous dermatomes plus response to an antiviral active against VZV unless resistance is demonstrated	≥ 10 typical ulcrated lesons affecting at least 2 non-contiguous dermatomes plus culture or detection of antigen or VZV PCR positive from affected tissue
Histoplasmosis, disseminated or extrapulmonary	None	Symptoms plus histology or culture or detection of antigen from affected tissues
Isosporiasis	None	Persistent diarrhoea for >1 month, histology or microscopy
Leishmaniasis, visceral	None	Symptoms plus histology
Microsporidiosis	None	Persistent diarrhoea for >1 month, histology or microscopy
MAC, and other atypical mycobacteriosis	Symptoms of fever, fatigue, anaemia or diarrhoea plus acid-fast bacilli seen in stool, blood, body fluid or tissue but not grown on culture and no concurrent diagnosis of TB except pulmonary	Symptoms of fever, fatigue, anaemia or diarrhoea plus culture from stool, blood, body fluid or tissue

Appendix 10 Presumptive and definitive criteria for AIDS defining events (cont'd)

Infections	**Presumptive criteria**	**Definitive criteria**
Tuberculosis, pulmonary	Symptoms of fever, dyspnoea, cough, weight loss, fatigue plus acid-fast bacilli seen in sputum, lavage, or lung tissue, not grown in culture, plus responds to standard TB treatment	Symptoms of fever, dyspnoea, cough, weight loss, fatigue plus positive TB culture or PCR from sputum, bronchial lavage, or lung tissue
Tuberculosis, extrapulmonary	Symptoms, plus acid-fast bacilli seen from affected tissue or blood but not grown in culture, concurrent diagnosis of pulmonary TB or responds to standard TB treatment	Symptoms, plus positive TB culture or PCR from affected tissue
Nocardiosis	Clinical evidence of invasive infection plus microscopic evidence of branching, Gram-positive, weakly acid-fast bacilli from affected tissue	Clinical evidence of invasive infection plus positive culture from blood or affected tissue
Penicillium marneffei disseminated	Characteristic skin lesions plus response to antifungal therapy for penicilliosis (in an endemic area)	Culture from a non-pulmonary site
Pneumocystis pneumonia (PCP)	Symptoms, any CXR appearance and CD4 count < 200, negative bronchoscopy if treated for PCP for > 7 days, no bacterial pathogens in sputum, and responds to PCP treatment	Microscopy or histology
Extra-pulmonary pneumocystis	None	Symptoms plus microscopy or histology
Recurrent bacterial pneumonia	Second pneumonic episode within 1 year, new CXR appearance, symptoms and signs, diagnosed by a doctor	Second pneumonic episode within 1 year, new CXR appearance, detection of a pathogen
Progressive multifocal leucoencephalopathy (PML)	Symptoms and brain scan consistent with PML and no response to treatment for toxoplasmosis	Symptoms and brain scan consistent with PML and positive JC virus PCR in CSF or histology
Rhodococcus equi disease	None	Clinical evidence of invasive infection plus culture of organism from blood or affected tissue
Recurrent *Salmonella* septicaemia	None	Second distinct episode, culture confirmed

Appendix 10: Presumptive and definitive criteria for AIDS defining events (cont'd)

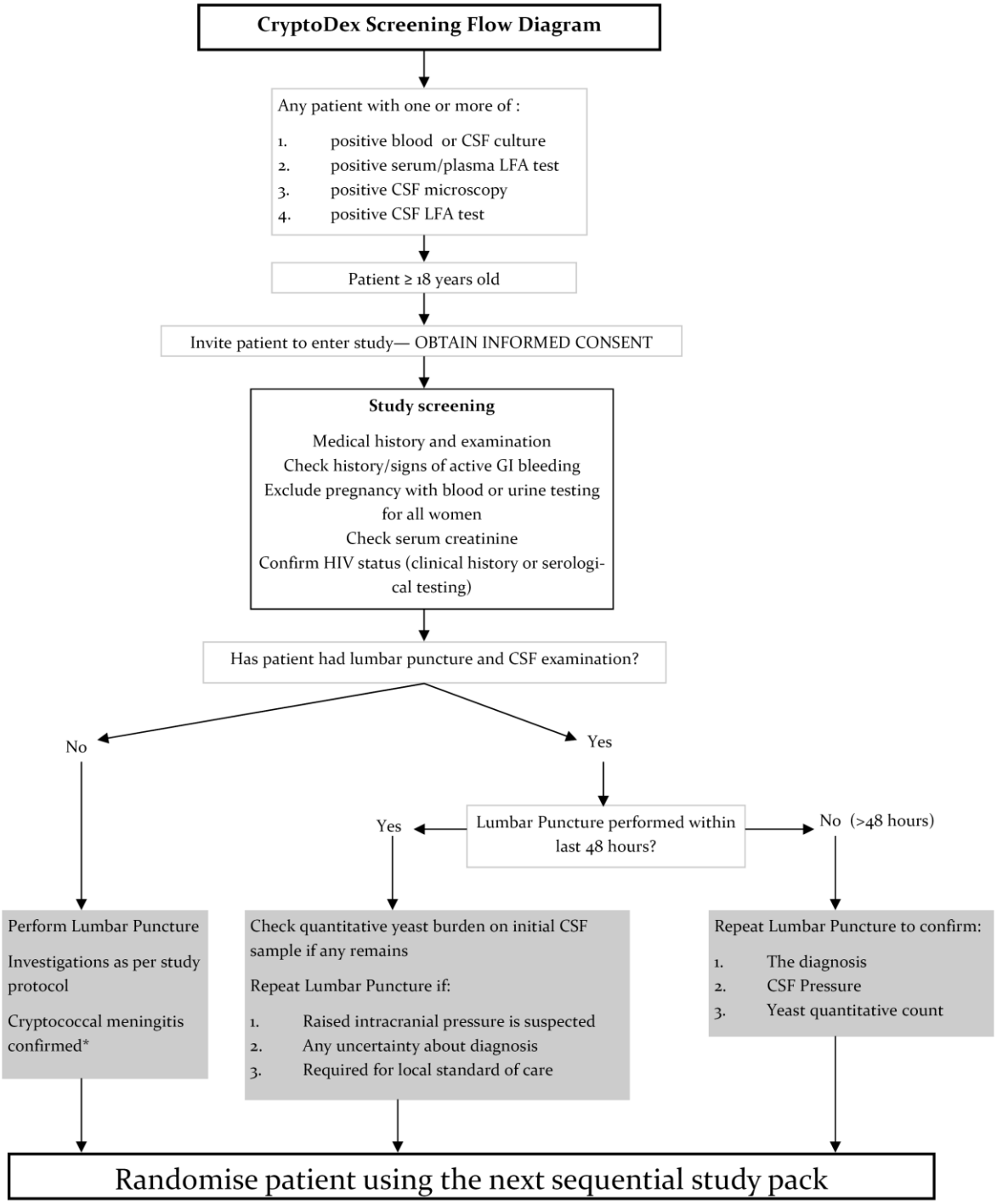
Infections	**Presumptive criteria**	**Definitive criteria**
Cerebral toxoplasmosis	Symptoms of focal intracranial abnormality or decreased consciousness, and brain scan consistent with lesion(s) having mass effect or enhancing with contrast, and either positive toxoplasma serology or response to treatment clinically and by scan	Histology or microscopy
Extra-cerebral toxoplasmosis	None	Symptoms plus histology or microscopy
Neoplasms		
Kaposi's sarcoma (KS)	Typical appearance without resolution. Diagnosis should be made by an experienced HIV clinician	Histology
Cervical carcinoma, invasive	None	Histology
Lymphoma, primary cerebral	Symptoms consistent with lymphoma, at least one lesion with mass effect on brain scan, no response to toxoplasma treatment clinically and by scan	Histology
Lymphoma, non-Hodgkin's B cell	None	Histology
Lymphoma, Hodgkin's	None	Histology
Neurological		
HIV encephalopathy	Cognitive or motor function interfering with usual activity, progressive over weeks or months in the absence of another condition to explain the findings. Should have a brain scan ± CSF examination to exclude other causes.	None
Other		
Indeterminate cerebral lesion (s)	Neurological illness, with evidence for an intracerebral lesion by brain scan, where the differential diagnosis is either cerebral toxoplasmosis. PML, cerebral lymphoma or HIV encephalopathy	

17.10 **Appendix 11: Common Terminology Criteria for Adverse Events**

Hard copies of the CTCAE will be provided for the study staff. Details of the CTCAE criteria can be found at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

17.11 Appendix 12: Screening Study Flow Diagram



Notes
All patients with Cryptococcal meningitis should have HIV confirmed or excluded
All lumbar punctures require verbal or written consent from patients according to site (normal standard of care). Failure to consent to lumbar puncture is a contraindication to lumbar puncture.
Patients may enter the study without lumbar puncture, if it is contraindicated, provided they have positive blood cultures for C. neoformans and they have a clinical syndrome consistent with cryptococcal meningitis.
** If cryptococcal meningitis is not confirmed the patient is not eligible for the study*
Patients who have received more than 7 days of treatment for cryptococcal meningitis are not eligible for the study

17.12 Appendix 13: Sub-studies

Sub-studies may be limited to particular sites. Local ethical approval will be obtained in each country where a sub-study takes place.

17.12.1 Health economics

If effective, the low cost of dexamethasone makes it promising as a cost-effective intervention in low-income settings. To formally assess this, a cost-effectiveness analysis will be conducted in collaboration with the Health Economics Research Centre, University of Oxford. The objective of the analysis will be to estimate the incremental cost-effectiveness ratio (ICER) - expressed as cost per life year gained and cost per Quality Adjusted Life Year (QALY) gained and per Disability Adjusted Life Year (DALY) averted – of dexamethasone treatment compared with standard antifungal therapy for cryptococcal meningitis (CM).

The analysis will collect information during the study on resources used and direct and indirect costs, including health care costs (treatments, medications, consultations, initial and subsequent hospitalisations), and patient/family incurred costs (out of pocket costs, employment). Health care resources used will be obtained primarily from trial case record forms. Unit costs will be obtained in each country from participating centres and national sources. Information on patient employment status and costs will be obtained from patients via a small number of questions administered at recruitment and again at the final follow-up (10 week) visit. The main measures of effectiveness will be 1) life years gained 2) quality adjusted life years gained (QALYs), estimated using the EQ-5D instrument in official English, Vietnamese and Thai languages in its 3-level version, and 3) disability adjusted life years (DALYs) averted, which measures both years of life lost due to premature mortality and years lived with disability. Life years gained will be based on the primary outcome measure of survival at 10 weeks, extrapolated using best estimates of longer term survival in each country for HIV infected adults. QALY and DALY estimates will adjust the life-year estimates taking into account secondary end points including blindness, deafness and other neurological disability which are important sequelae of cryptococcal disease. QALY adjustment will be done using EQ-5D-3L responses, and DALY adjustment using clinical judgment to align recorded morbidity with DALY states. Using both approaches will provide internal validity checks and maximise opportunities to present relevant information to decision makers.

As unit costs, absolute risks, life expectancy and other variables are likely to differ substantially across regions, separate cost-effectiveness estimates will be produced for Asia and Africa; however, a trial-wide estimate of effectiveness will be applied unless there is clear evidence of heterogeneity in effect across regions. All estimates of costs, outcomes and cost-effectiveness will be reported with full recognition of uncertainty, including cost-effectiveness acceptability curve and sensitivity analyses around key parameters including unit costs, long-term life expectancy and disability adjustment.

The economic evaluation is primarily concerned with estimation of cost and outcome differences and cost-effectiveness rather than hypothesis-testing, and a power calculation for a ratio statistic is likely to be highly uncertain, especially as the DALY/QALY measure (the denominator) will itself be a composite of survival and quality/disability adjustment. The proposed size of the study – 880 patients – should permit cost differences of 12% or greater to be reliably detected assuming a coefficient of variance of 60%.

17.12.2 Molecular Epidemiology of *C. neoformans* isolates from Africa and Asia

The multi-centre study offers a golden opportunity to delineate the relationships between African and Asian strains of *C. neoformans* and determine the prevalences of species and varietal forms responsible for disease in each country. Isolates will be typed using the consensus MLST scheme to contribute to the global effort, or other technologies as they become affordable.

17.12.3 B-cell responses in cryptococcal meningitis

Cryptococcus neoformans is ubiquitous, and human exposure is frequent, with high rates of seropositivity in healthy individuals. However, many patients with HIV infection and severe immunosuppression will not develop disease, despite the fact that they are likely to have been exposed at some stage. Currently, other than CD4 count, there are no established risk factors for the development of cryptococcal disease in HIV infected patients. Data are emerging that suggest that defects in innate immunity may represent an additional risk for the development of cryptococcal disease. In particular, defects in the production of naturally occurring IgM (nIgM), and lower levels of IgM-producing B-cells, are implicated and have been detected in a retrospective cohort of patients from the USA. We hypothesize that IgM memory B cell deficiency could portend a risk for cryptococcal disease. We expect to find less IgM memory B

cells and B cell associated gene expression in HIV infected patients with cryptococcal meningitis. In order to test this hypothesis, IgM memory B cell levels (CD27) will be determined in 90 patients randomly selected from 2 sites in this study. CD27 and nIgM levels will be compared between these patients and (1) HIV infected patients with similar CD4 counts but no history of cryptococcal disease, (2) HIV uninfected patients with cryptococcal disease and (3) HIV uninfected patients without cryptococcal disease. Patients from groups (1), (2), and (3) will be recruited from other on-going studies. PBMCs isolated from patients from each group will be stimulated with *C. neoformans in vitro* to enable comparison of transcriptional signatures between patients with and without cryptococcal disease. Linking IgM memory B cell levels to CN-associated signatures will inform the search for diagnostic biomarkers and novel vaccines and therapies for CD. These studies will require 40 mls of EDTA to be drawn from the patient at any point during the 10 week treatment period. A separate informed consent form will be signed by all patients agreeing to participate in this sub-study. This will include consent to ship some of the samples to Albert Einstein College of Medicine, New York City, which has the unique expertise for this work.

17.12.4 Pharmacokinetics/pharmacodynamics.

A sub-study investigating the pharmacokinetics and pharmacodynamics of antifungal therapy in the context of adjunctive treatment with dexamethasone will be nested within this study and based in Vietnam. Other centres interested in this study are welcome to discuss participation with the PI. This will require a separate protocol that will be submitted for approvals separately.

17.12.5 Dexamethasone and adrenal suppression

Background: Iatrogenic administration of exogenous steroids is associated with adrenal suppression, and the sudden cessation of treatment can lead to Addisonian crises which can be life-threatening. For this reason it is common practice to prescribe corticosteroids in tapering (reducing) doses, as described in the CryptoDex study protocol. Tapering the dose is believed to allow recovery of the adrenal cortex during the treatment period. Thus, by the time the exogenous steroid prescription is finished, sufficient recovery of adrenal function will have occurred to allow a resumption of normal endogenous cortisol production, with any risk of an Addisonian crisis averted.

However, there is wide variation in the practice of prescribing steroids and the perceived need for reducing courses. In general, the longer the course of steroids and the higher the dose the more likely the risk of adrenal suppression is considered to be. The variation in practice reflects the lack of hard evidence about the degree of adrenal suppression that occurs with exogenous steroids at particular doses and durations, particularly in severely unwell patients. Furthermore, HIV and AIDS are established risk factors for adrenal insufficiency, with an incidence of between 20-30% in those with AIDS [42, 43], so the CryptoDex study population could have considerable rates of adrenal insufficiency, even in the control arm.

Whether the use of steroids in the doses prescribed in infectious diseases are associated with significant adrenal suppression is not clear. In bacterial meningitis, where the dose is high but the duration short, reducing courses are considered unnecessary [44]. In tuberculous meningitis, the steroids are prescribed with a taper, as will be used in the CryptoDex study [45]. CryptoDex is a blinded randomized placebo controlled trial, so offers an opportunity to describe the effects of dexamethasone on adrenal function in a multi-ethnic population of severely unwell patients.

The short synacthen test (SST) is a widely used and well established diagnostic test to assess adrenal function [46]. Synacthen (tetracosactide) is an analogue of adrenocorticotrophic hormone (ACTH) – the pituitary hormone which controls endogenous cortisol production. The SST measures the response of the patient's adrenal cortex to a dose of Synacthen. The patient's background cortisol level is measured by drawing 3mls of blood at 0900. Then 250mcg of Synacthen is administered intravenously; 3ml samples of blood are taken at thirty minutes and again at sixty minutes to measure the cortisol level after this adrenal stimulation. Adrenal insufficiency is excluded by an incremental rise in cortisol of > 200nmol/L or a peak value > 550 nmol/L at 30 or 60 minutes [46]. The SST is not thought to have any long-lasting physiological effects – in a sample of 64, no patients suffered adverse events [47], the drug's half-life is less than four hours and it is completely eliminated within twenty-four hours [48].

Study Design: The study will be carried out in parallel in two countries, Vietnam and Uganda. We will compare the adrenal responsiveness between patients taking dexamethasone and those taking placebo at 2 weeks and 4 weeks following study randomization using the short Synacthen test. 100 patients will be recruited in both Uganda and Vietnam, giving a sample size of 200. CryptoDex study patients are randomized in small blocks, stratified by site, and this will ensure that roughly equal numbers of patients from each study arm – dexamethasone or placebo – are

recruited into this sub-study. Patients who have agreed to be in the CryptoDex study will be approached for consent to take part in the sub-study. Refusal to consent for the sub-study will have no impact on participation in the CryptoDex trial, or on patient care. Testing will be done in batches in order to contain costs and results will not be analysed until after study unblinding. Only patients enrolled into the CryptoDex study, and who are receiving study drug, will be eligible to enter this sub-study. Patients in whom study drug has been stopped for any reason will not be eligible to enter the study.

Inclusion Criteria

Enrolled within the CryptoDex (04CN) study (fulfill the inclusion and exclusion criteria for that study)

Receiving study drug (dexamethasone or placebo)

Consents to enter the study

Exclusion Criteria

Allergy to Synacthen

Receiving exogenous corticosteroids other than study drug

On oral contraceptive pill (interferes with cortisol assay)

Severe liver disease (interferes with cortisol assay)

Nephrotic syndrome (interferes with cortisol assay)

Primary endpoint

The primary end-point will be the degree of adrenal suppression at 2 and 4 weeks, in each group. The primary comparison will be between the test responses in patients receiving dexamethasone or placebo. Adrenal suppression will be defined conventionally according to the response to the synacthen test at 30 and 60 minutes. Patients in whom the serum cortisol level does not rise ≥ 550nmol/L, or who do not see a rise of greater than 200nmol/L from baseline, will be defined as having clinical adrenal suppression.

Power

This study is descriptive, and since it is not clear how much adrenal suppression there will be at the 2 or 4 week periods, the study size of 200 patients has been chosen for pragmatic reasons.

However, assuming that the risk of adrenal suppression in patients receiving placebo is between 20-30% at both time-points, and that dexamethasone increases this risk, the study will have >85% power to detect an absolute risk increase by 20% or more at either time-point. Similarly, if in truth dexamethasone does not increase the risk of adrenal suppression, the study would have a chance of >85% that the two-sided 95% confidence interval for the absolute risk difference between the two arms would exclude increases in the risk of adrenal suppression due to dexamethasone by 20% or more, i.e. to prove 'non-inferiority' of dexamethasone with a 'non-inferiority margin' of 20%.

Analysis

The study will not be analysed until the trial is complete, since analysis requires unblinding of the treatment allocation. Two-sided 95% confidence intervals for the absolute risk difference of meeting diagnostic criteria for adrenal suppression between patient groups (dexamethasone versus placebo) at each time point (2 weeks and 4 weeks) will be calculated using the method of Agresti and Caffo[49]. The Wilcoxon signed-rank test will be used to compare the absolute responses to the synacthen test at each time point. Secondary analyses will include the change in the proportion of patients with adrenal suppression over time, and comparison of synacthen responsiveness between Vietnam and Uganda.

Risks to the patient

There can be mild discomfort associated with blood-letting. Synacthen has a short biological half-life and the test is safe[44]. The rise in cortisol seen with the short Synacthen test is not considered to have anything other than a short-lived physiological effect.

Equipment:

Peripheral intravenous cannula

3x plain tube for blood collection

3x Saline flush 5ml

Procedure:

The test will be carried out on study days 14 and 28. In order to manage weekend enrollment, the synacthen test can be carried out up to 2 days after each of the scheduled test days and be

eligible for analysis (ie if day 14 is a Saturday, then the test can be performed on any of days 14, 15 or 16).

Baseline (T = 0 minutes): Sample 1

Patient IV access obtained with an indwelling catheter

3mls blood collected in a plain tube and immediately transported on ice to the laboratory. (The sample will be spun at 3000rpm for 5 minutes at 5°C; serum separated and serum stored at -80°C.)

250mcg Synacthen administered as a bolus

Line flushed with 5ml normal saline

T = 30 mins: Sample 2

0.5mls blood drawn from cannula, and discarded

3mls blood collected in a plain tube and immediately transported on ice to the laboratory. (The sample will be spun at 3000rpm for 5 minutes at 5°C; serum separated and stored at -80°C.)

Line flushed with 5ml normal saline

T = 60 mins: Sample 3

0.5mls blood drawn from cannula, and discarded

3mls blood collected in a plain tube and immediately transported on ice to the laboratory. (The sample will be spun at 3000rpm for 5 minutes at 5°C; serum separated and stored at -80°C.)

Line removed

Laboratory procedure:

The sample will be spun at 3000rpm for 5 minutes at 5°C; serum separated and split into two 1ml aliquots, and serum stored at -80°C. In Vietnam samples will be analysed by a commercial provider in Ho Chi Minh City on an automated Abbott platform. Analysis in Uganda will be through the MRC.

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