AN OPEN LABEL RANDOMIZED CONTROLLED TRIAL OF TAMOXIFEN COMBINED WITH AMPHOTERICIN B AND FLUCONAZOLE FOR CRYPTOCOCCAL MENINGITIS

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ABSTRACT

Background: Cryptococcal meningitis has high mortality. Flucytosine is a key treatment but is expensive and rarely available. The anti-cancer agent tamoxifen has synergistic anti-cryptococcal activity with amphotericin in vitro. It is off-patent, cheap, and widely available. We performed a trial to determine its therapeutic potential.

Methods: Open label randomized controlled trial. Participants received standard care - amphotericin combined with fluconazole for the first two weeks - or standard care plus tamoxifen 300mg/day. The primary end point was Early Fungicidal Activity (EFA) - the rate of yeast clearance from cerebrospinal fluid (CSF). Trial registration https://clinicaltrials.gov/ct2/show/NCT03112031.

Results: 50 patients were enrolled, (median age 34 years, 35 male). Tamoxifen had no effect on EFA (-0.48log10 colony-forming units/mL/CSF control arm versus -0.49 tamoxifen arm, difference -0.005log10CFU/ml/day, 95%CI: -0.16, 0.15, P=0.95). Tamoxifen caused QTc prolongation.

Conclusion: High dose tamoxifen does not increase the clearance rate of *Cryptococcus* from CSF. Novel, affordable therapies are needed.

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Key words: Tamoxifen, Fluconazole, amphotericin B, antifungal therapy, cryptococcal meningitis

Introduction

Cryptococcal meningitis is a leading cause of death in HIV-infected patients, with an estimated 223,000 cases in 2014[1]. The vast majority of infections are due to *C. neoformans*, and occur in low-income tropical settings. Current international guidelines recommend initial induction treatment with amphotericin combined with flucytosine, followed by consolidation therapy with fluconazole[2]. This combination delivers the fastest rates of clearance of yeast from cerebrospinal fluid (CSF) and the best survival rates[3,4]. However, even on this gold standard therapy, 30% of patients will die within 10 weeks of diagnosis[3,4]. Adjunctive therapy with corticosteroids, which has proven beneficial in other forms of meningitis, results in worse outcomes[5].

Cryptococcal meningitis can also occur in HIV-uninfected patients, including immunocompetent people and those with other causes of immunosuppression. Survival rates are similar to those seen in HIV-infected patients. There are few data from randomized controlled trials to guide treatment in these circumstances. In Vietnam around 20% of cases of cryptococcal meningitis are in HIV-uninfected patients[6]. Disease is predominantly due to the *C. neoformans* VNIa-5 lineage; *C. gattii* is responsible for around 25% of cases[6-9].

There has been little progress in development of antifungal drugs for cryptococcal meningitis. Amphotericin and flucytosine are each more than 60 years old; the last novel drug class developed was the azoles, introduced 30 years ago. Access to flucytosine is severely restricted by availability and cost, meaning it is rarely used where disease burden is highest. Despite being off-patent, it has been subject to extraordinary price rises in recent years, with a 2 week course now costs around 30,000 USD in the USA[10]. Flucytosine is an unattractive prospect for generic manufacturers, because the location of the majority of patients and the few indications outside cryptococcal disease promise only limited financial returns. These same factors hamper the development of novel treatments for cryptococcal disease, and have

68 driven interest in drug re-purposing[11-13]. Re-purposing can be a solution for neglected diseases provided
69 the new indication accounts for only a minority of total prescriptions, and the *de facto* indications are
70 sufficiently prevalent to ensure availability, price stability and affordability.

71 Tamoxifen, a selective estrogen receptor modulator used to treat breast cancer, has anti-cryptococcal
72 activity, appearing to act synergistically when combined with other antifungals against the type strain *in*
73 *vitro*, and to be fungicidal when combined with fluconazole in the mouse infection model[11,12]. We found it
74 to act synergistically with amphotericin against two-thirds of clinical isolates of *Cryptococcus neoformans*
75 and *C. gattii* from our archive and to have an additive interaction when combined with fluconazole in
76 vitro[14].

77 Tamoxifen is concentrated in brain tissue (10 to 100-fold compared with plasma) and macrophage
78 phagosomes (a site of growth for *Cryptococcus* spp.), is off-patent, cheap (~10US cents/tablet) and widely
79 available[15,16]. Therefore, it is a promising candidate for the treatment of cryptococcal meningitis.
80 Pharmacokinetic data suggest that doses 5 to 10-fold that used in breast cancer (typically 30mg/day)
81 should deliver plasma concentrations of tamoxifen greater than the Minimum Inhibitory Concentration
82 90 (MIC90 16ug/mL) of Vietnamese clinical isolates[15]. Such doses have been used, and well-tolerated, in
83 small cell lung cancer, desmoid tumours, and prostate cancer. These illnesses have comparable or better
84 1 year survival rates than cryptococcal meningitis[17]. While generally well-tolerated, acute side effects that
85 could be detrimental from short-course treatment include QT prolongation of the cardiac
86 de/repolarisation cycle, although the risk of life-threatening arrhythmias appears to be low[18].

87 In Vietnam induction treatment for cryptococcal meningitis consists of amphotericin combined with
88 fluconazole, consistent with WHO recommendations where flucytosine is unavailable[2]. However, this
89 combination is less effective than amphotericin with flucytosine, resulting in slower rates of fungal
90 clearance and worse survival rates[3,4]. The relationship between the rate of fungal clearance from CSF and

survival is generally robust; improving the potency of antifungal therapy is likely to be an effective way to reduce deaths[3-5]. The rate of clearance of yeast from CSF associated with an antifungal treatment (the early fungicidal activity, EFA) is a sensitive measure able to detect differences between treatment regimens likely to be associated with survival benefits with far fewer patients than studies powered to survival itself[19]. Small studies powered to this endpoint can serve to filter treatment regimens that can be taken forward in larger trials[19,20]. We performed an open-label randomised controlled trial to determine whether combining tamoxifen with amphotericin B and fluconazole results in enhanced EFA in HIV infected and uninfected patients with cryptococcal meningitis, and to generate safety data as a prelude to a larger trial powered to mortality [17].

Methods

Study design and participants

The study design is described in detail in the published protocol[17]. In brief, we enrolled 50 patients in two hospitals in Ho Chi Minh City – the Hospital for Tropical Diseases and Cho Ray Hospital. Eligible adult patients (≥18 years of age) had a clinical syndrome consistent with cryptococcal meningitis and one or more of: (1) positive cerebrospinal fluid (CSF) India ink; (2) *C. neoformans* cultured from CSF or blood; (3) positive cryptococcal antigen Lateral Flow Antigen Test (LFA) in CSF. All patients were tested for HIV infection in accordance with standard of care. We excluded patients who were pregnant, had a history of thromboembolic disease, had received more than 4 days of anti-cryptococcal antifungal therapy, had any other indication for tamoxifen, had renal failure, or a rate-corrected (Framingham formula) QT interval >500ms. Written informed consent was obtained from all patients or their representatives.

Interventions

Patients were randomized to receive either standard of care induction antifungal therapy or standard of care plus tamoxifen. Standard of care antifungal therapy consisted of intravenous amphotericin B deoxycholate 1mg/kg/day (Amphotret, Bharat Serums and Vaccines, India) combined with oral fluconazole 800mg/day (Zolmed, Glomed Pharmaceuticals, Vietnam) for the first 14 days following randomisation. Tamoxifen (Nolvadex, AstraZeneca UK Ltd) 300mg/day was given orally. Amphotericin was infused over 4 hours after prehydration with normal saline and potassium supplementation[21]. Fluconazole and tamoxifen were administered simultaneously. All medication was directly observed while the patient was in hospital; all participants were in-patients for at least the first 14 days of the study.

Following induction therapy all patients received fluconazole 800mg once daily for 8 weeks. HIV-infected patients received daily pneumocystis prophylaxis with trimethoprim– sulfamethoxazole. Antiretroviral therapy was instituted 5-6 weeks after diagnosis via the national treatment programme.

Randomisation

Randomization was in a ratio of 1:1, in blocks of 4 or 6, stratified by HIV serostatus (rapid test) and treating centre. The computer generated randomization list was password protected and stored on a secure server to which only the study pharmacist had access. Enrolment logs specific to each centre were used to assign patients to the next available sequential number and corresponding sealed treatment pack.

Outcome Measures

The primary outcome was Early Fungicidal Activity (EFA), defined as the rate of decline in culturable yeast from CSF over the first 2 weeks following randomization.

Secondary outcomes included survival until 10 weeks after randomization, disability at 10 weeks, frequency of grade 3, 4 or serious adverse events, immune reconstitution inflammatory syndrome (IRIS), QTc prolongation, visual deficit at 10 weeks, and time to new neurological events. Adverse events were defined according to the Common Terminology Criteria for Adverse Events (CTCAE) and categorized according to the Medical Dictionary for Regulary Activities system organ class. We categorized prolonged QTc intervals using this classification as normal (<450ms for males, <460ms for females), mildly prolonged (grade 1 or 2, ≥450ms for males or ≥460 for females but ≤500ms) and grade 3 or 4 (>500ms). Disability at 10 weeks was categorised as good, intermediate, poor, or death, as described previously[3,5].

Monitoring and laboratory investigations

Lumbar puncture was performed on study entry, days 3, 7 and 14 following randomization, and more frequently if indicated. Fungal burden was determined as previously described[3]. Twelve-lead electrocardiograms were recorded twice daily (10 seconds at 50mm/sec), immediately before and 2 hours after administration of tamoxifen during the first 14 days, and on days 21 and 28. The QT interval was manually determined by measuring the interval in 3 limb and 3 chest leads, to calculate the median. The median QT interval was corrected (QTc) for rate using the Framingham formula[20]. Calmodulin inhibitors such as tamoxifen have previously been suggested to inhibit CD4 cell apoptosis in HIV infected patients[22]. CD4 counts were measured at baseline and at study week 10. The full laboratory investigation schedule is detailed in the published protocol[17]. Outpatient assessments with medication review were performed weekly until 4 weeks and at the completion of 6 and 10 weeks; more frequent review occurred if clinically indicated. Adherence following hospital discharge was assessed using pill counts. *Cryptococcus* isolates were typed using URA5-RFLP and underwent (microbroth) antifungal susceptibility testing as per CLSI guidelines[23,24]. Previously tested clinical isolates were included as controls.

Sample size

Sample size considerations were based on two separate simulation experiments using data from our previously published trials in cryptococcal meningitis[3,5]. The estimated power was based upon 10,000 repetitions of each experiment. The full methodology is available within the published protocol[17]. Based on these simulations, enrolling 25 subjects per treatment group provided 80% and 90% power to detect a difference in EFA of -0.11 or -0.13 log10 colony-forming units/ml/day, respectively. This size of effect has previously been associated with survival benefit[3,5].

Statistical analysis

For the primary outcome, all recorded longitudinal quantitative fungal count measurements up to day 17 following randomization (allowing for some delays in the day 14 sampling) were included in the analysis. EFA, defined as the decline in fungal count (slope), was modeled based on a joint model consisting of a survival model and a linear mixed effects model with longitudinal log10 CSF quantitative culture fungal counts as the outcome. In the linear mixed effect model, we modeled the treatment groups and the time since enrolment and their interaction as fixed covariates. We used random patient-specific intercepts and slopes. The model was implemented in a Bayesian framework using Rstan. It allows appropriate handling of detection limits with longitudinal measurements and also allows adjustment for informative dropout due to early death within the first 17 days following randomization[25,26].

For the secondary outcomes, overall survival was visualized using Kaplan-Meier curves for each treatment arm and the comparison between them was based on the Kaplan Meier estimates of 10 week mortality. The percentage of individuals with disabilities at 10 weeks and with adverse events of grade 3 or 4 were compared using the chi-squared test; if the expected value of any cell was less than one then Fisher's

exact test was used[27]. We presented the median (IQR) of the difference in CD4 counts over 10 weeks and compared their distributions using the Mann-Whitney-Wilcoxon rank sum test. We compared the trend in QTc over the period of study drug administration (i.e. the first 14 days) between the two treatment arms using a linear mixed effect model which allowed for different non-linear trends between the pre-dose and post-dose measurements. We then used the output of the fitted linear mixed effect model to compute the differences in QTc between treatment arms by study day, separately for pre-dose and 2 hours post-dose measurements. Further details of the analytical approach are available in the the Supplementary Appendix in the Statistical Analysis Plan.

Ethics and study oversight

The study protocol was approved by the Ethical Review Committees of the Hospital for Tropical Diseases, Cho Ray Hospital, and the Vietnamese Ministry of Health, and by the Oxford University Tropical Research Ethics Committee. A trial steering committee with 2 independent members oversaw the running of the trial, and an independent data and safety monitoring committee oversaw trial safety. The first safety analysis was performed after the first 20 patients had reached the primary endpoint. The funding bodies and drug manufacturers played no role in the study design, implementation, analysis, or manuscript preparation. All the authors made the decision to submit the manuscript for publication and vouch for the accuracy and completeness of the data and analyses presented. The trial was registered at https://clinicaltrials.gov/ct2/show/NCT03112031.

Results

Trial recruitment

The study recruited between October 2017 and May 2018. We screened 70 patients, enrolling 50 (40 HIV infected; 10 HIV uninfected) with 24 assigned to the intervention arm and 26 assigned to the control

arm. Reasons for exclusion are shown in the study flow diagram (see Figure 1). One patient who was assigned to the intervention arm did not receive tamoxifen because of severe transaminitis.

Baseline characteristics

The baseline characteristics of the patients were broadly balanced between treatment groups. There were slightly more patients with normal Glasgow coma scores in the control group than in the intervention group (24 of 26 versus 19 of 24, see Table 1).

Primary outcome

There was no detectable difference in the early fungicidal activity (EFA) of the two treatment regimens (see Figure 2A). In the intention-to-treat analysis, the rates of fungal decline per day were -0.48 and -0.49 $\log_{10}$ colony-forming units (CFU)/ml/day in the control and tamoxifen groups respectively (difference -0.005 $\log_{10}$ CFU/ml/day, 95%CI: -0.16, 0.15); p-value = 0.95, see Table 2). There was no detectable difference in EFA in the per-protocol population analysis, or by HIV infection status (see Table 2).

Secondary endpoints

The secondary outcomes in terms of mortality, disabilities, and change in CD4 count are summarized in Table 3. Death occured in 8 of 24 patients in the tamoxifen group and 7 of 26 in the control group (Kaplan-Meier mortality estimates 34% and 27% respectively, risk difference 6.5%; 95% confidence interval [CI], -19.2% to 32.1%; P=0.62 Figure 2B). Fewer patients in the tamoxifen arm were classified as having a good outcome at 10 weeks compared with the control arm (9% versus 36%). We found no

difference in change in CD4 counts in HIV patients by study arm over the 10 week period of follow-up (see Table 3).

The number of patients having grade 3 or 4 adverse events were similar between treatment arms (see Table 4), with the exception of QTc prolongation events. Eight patients had grade 3 or 4 QTc prolongation events in the tamoxifen arm, compared with one in the control arm (p=0.02). The trend and difference in QTc intervals over the first 2 weeks of treatment are shown in Figure 3**Error! Reference source not found.**. Tamoxifen resulted in QTc prolongation over the two week treatment period (p<0.001).

Three patients in the tamoxifen arm had grade 3 or 4 ventricular extra-systole events compared with none in the control arm (p=0.21). A 33 year old male patient who had received tamoxifen suffered a cardiorespiratory arrest following a convulsion on day 21 of the study. He had no history of pre-existing cardiac disease. His ECG on admission had been normal with a QTc of 409 ms, and when performed routinely on the morning of day 21 showed mild sinus bradycardia (57 beats/minute) and a QTc interval of 477ms. The arrest was not associated with ventricular arrhythmia although he had had grade 3 prolongation of QTc during the first 14 days of the study, which had resolved following tamoxifen interruption.

Microbiology and susceptiblity testing

All HIV infected patients, and 7 HIV uninfected patients, had meningitis due to *Cryptococcus neoformans* molecular group VNI. Three HIV uninfected patients had disease due to *Cryptococcus gattii* (VGI). All isolates underwent susceptibility testing. The MIC90 of amphotericin B and fluconazole were 2mg/L and 4mg/L respectively. The MIC90 of tamoxifen was 8mg/L. We estimated the presence of drug interactions by calculating the fractional inhibitory concentration index (FICI) for each isolate. This was ≤0.5 (suggestive of a possible synergistic interaction) for tamoxifen combined with amphotericin in 6 isolates (12%), and for tamoxifen combined with fluconazole in 2 isolates (4%).

Discussion

We wanted to determine whether tamoxifen could be repurposed as an affordable treatment for cryptococcal meningitis. Our study was powered to detect an increase in the rate of yeast clearance of at least -0.11 log10 CFU/ml/day when tamoxifen was added to standard of care therapy. Differences of this order of magnitude are associated with improved survival in patients in low income settings [3-5]. Despite having previously shown that tamoxifen had activity in vitro against historical clinical isolates of *C. neoformans*, we found its addition had no impact on EFA. Therefore we do not believe that proceeding to a larger trial, powered to survival, is justified.

It is not clear why tamoxifen did not provide benefit in our patients. The susceptibilities of the *Cryptococcus* isolates from this study to tamoxifen, fluconazole and amphotericin, were similar to those of isolates from our previous clinical trials [14,28]. However, in contrast with our previous findings we found evidence of synergy when tamoxifen was combined with amphotericin in only 12% (95CI 5%, 24%) of isolates from the trial. This compares with the rate in archived isolates of 67% (95CI 47%, 81%)[14]. Synergy has been suggested as an explanation for the superiority of the amphotericin-flucytosine combination which has delivered improved yeast clearance and survival in a number of trials[29]. In this study, we lack sufficient numbers of isolates where tamoxifen-amphotericin synergy is seen to be able to determine whether synergy per se influences EFA.

A second potential explanation is that we may have failed to attain sufficient concentrations of tamoxifen in our patients. We chose a dose of 300mg/day, based upon the MIC90 of tamoxifen against our historical isolates (16 mg/L) and the expected plasma concentrations this would achieve. Given that tamoxifen is concentrated in the brain (10 to 100-fold), and in macrophage phagosomes, we consider it unlikely that we did not reach drug concentrations greater than the MIC90 at the disease site, although it is possible that absorption of orally administered drug was impaired in our patients.

The rates of adverse events in our study were similar between patients receiving tamoxifen and those in the control arm. Our study was powered to detect a difference in the rate of clearance of yeast from CSF and therefore may have lacked power to detect differences in rates of rarer adverse events. However, there was greater prolongation of the QTc interval in patients on tamoxifen. The mechanism through which tamoxifen causes QT interval prolongation in humans is unknown. In animals there is evidence that the block is multi-channel, due to both inhibition of the I_{KR} and I_{Ca} channels[30-32]. Such multi-channel block is considered to confer a reduced risk of life-threatening arrhythmias compared with drugs that block single ion channels. While we did not have any cases of ventricular tachycardia in our study, there was an episode of cardiac arrest in the tamoxifen arm. There are multiple potential causes of cardiac arrest in patients with cryptococcal meningitis, including intracranial pathology and electrolyte disturbances. The cardiac arrest in our study occurred on day 21, one week after administration of tamoxifen had finished. However, given tamoxifen's half-life of 5 to 7 days, and the doses used, it is possible that this event was related. Fluconazole is also a recognised cause of QT prolongation. Here, the mechanism is believed to be through modulation of the I_{kr} current of the cardiac depolarisation cycle[33]. However, we found little evidence of significant QT prolongation in patients in the control arm of our study, and in fact the acute effect of administration of fluconazole was shortening of the QTc interval.

Our experience with tamoxifen is similar to that reported with the anti-depressant drug sertraline. Sertraline has in vitro fungicidal activity against *Cryptococcus neoformans* and a synergistic effect when combined with fluconazole. Results from a pilot dose-finding study of adjunctive sertraline for cryptococcal meningitis suggested it was a safe and potentially effective treatment, although no contemporaneous controls were enrolled in the trial[34]. Subsequently a large randomised controlled trial powered to mortality was stopped due to futility having enrolled 460 patients[35]. There was no difference in survival or EFA between the standard therapy or sertraline boosted treatment arms. Of note, a small randomized placebo controled trial from Mexico, published after the phase 3 trial had begun, found no

difference in EFA when sertraline was added to amphotericin and fluconazole, although only 12 patients were enrolled and formal statistical testing was not performed[36]. However, it lends further support for the screening of antifungal treatments in small scale studies using this endpoint.

Other drugs suggested as repurposing candidates for cryptococcal meningitis include the calcium antagonists, such as nifedipine and its sister drugs, used to treat hypertension, and flubendazole, an antihelminthic[37]. Flubendazole is perhaps the most promising of these, appearing to be more potent in vitro than fluconazole, and active against *Cryptococcus* isolates across a range of fluconazole susceptibilities. It crosses the blood brain barrier in mice, but data are lacking regarding humans[38]. While nifedipine crosses the blood brain barrier, it seems unlikley that normal doses and oral administration would reach the plasma levels needed to inhibit *Cryptococcus* growth. However, given our experiences with tamoxifen, and those of others with sertraline, we would caution that better laboratory screening methods than those currently in use are needed to identify potential new treatments for cryptococcal meningitis.

In the mean time, improving access to flucytosine remains a key goal. Progress has been made through efforst to increase generic manufacture through the the Unitaid- Clinton Health Access Initiative for Advanced HIV Disease Initiative's partnership with the Global Fund and the President's Emergency Plan for AIDS Relief. This has resulted in price reductions allowing 2 week treatment courses to be procured for around $100 in some locations.

Conclusion

Despite apparent *in vitro* anti-cryptococcal effect including synergy when combined with amphotericin, tamoxifen does not increase the rate of clearance of yeast from cerebrospinal fluid in HIV infected and uninfected patients with cryptococcal meningitis; it is unlikely to result in clinical benefit. Small scale phase 2 trials such as the one presented here should precede the evaluation of potentially

repurposable drugs in clinical endpoint studies. However, the failure of both tamoxifen and sertraline in recent studies underlines the importance of developing novel, specifically anti-cryptococcal drugs. This will require the support of government and charitable bodies to ensure treatments remain affordable.

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Competing Interests

None to declare.

Data Access

The original de-identified clinical data underlying the study are available by emailing the OUCRU Data Access Committee at DAC@oucru.org or ekestelyn@oucru.org (Head of the Clinical Trials Unit and Data Access Committee Chair). The review procedures (the data sharing policy and the data request form) are available on the OUCRU website at http://www.oucru.org/data-sharing/

The statistical code is freely available at https://doi.org/10.5287/bodleian:XmeOzdR8z

Table 1. Clinical and investigation characteristics of patients at study entry

Characteristic	Total	Tamoxifen	Total	Control
	N	N (%) or IQR[¥]	N	N (%) or IQR[¥]
Male sex	24	17 (71)	26	18 (69)
Median age in years	24	35 (31, 39)	26	32 (25, 35)
History of intravenous drug use	24	3 (13)	26	3/26 (12)
HIV infection	24	19 (83)	26	21/26 (81)
Current antiretroviral-therapy use				
None	24	18 (75)	26	22 (84)
≤3 months duration	24	4 (17)	26	2 (8)
>3 months duration	24	2 (8)	26	2 (8)
Median duration of illness — days	24	14 (10, 25)	26	12 (7, 28)
Symptoms				
Headache	24	24 (100)	26	26 (100)
Fever	24	22 (92)	26	23 (88)
Neck stiffness	22	20 (91)	26	21 (81)
Seizures	24	2 (8)	26	3 (12)
Abnormal visual acuity	22	6 (27)	26	4 (15)
Papilledema	21	2 (10)	25	1 (4)
Glasgow Coma Scale score	24		26	
15		19 (79)		24 (92)
11–14		5 (21)		2 (8)
<11		0 (0)		0 (0)
Cranial nerve palsy				
None	24	19 (79)	26	23 (88)
Cranial nerve VI	24	4 (17)	26	1 (4)
Other cranial nerve	24	1 (4)	26	3 (11)
Investigations				
Median CSF opening pressure — cm of CSF	19	26.5 (18, 37)	23	24.5 (16, 47)
Median CSF white-cell count in HIV infected patients — cells/mm3	18	38.5 (7, 52)	20	27 (10, 55)
Median CSF white-cell count in HIV uninfected patients — cells/mm3	5	122 (64, 187)	5	94 (45, 117)
Median CSF glucose — mmol/liter	24	2.47 (1.70, 3.14)	25	2.31 (1.44, 2.76)

Median blood glucose — mmol/liter	24	5.86 (4.92, 6.84)	26	6.21 (5.11, 7.81)
Median CSF: blood glucose ratio	24	0.40 (0.24, 0.53)	25	0.37 (0.16, 0.45)
Median CSF fungal count — log10 CFU/ml	24	4.60 (3.90, 5.17)	26	5.16 (3.17, 5.87)
Median CD4 count in HIV infected patients — cells/mm3	17	20 (8, 49)	21	17 (9, 45)
Median CD4 count in HIV uninfected patients — cells/mm3	5	376 (348, 382)	5	504 (305, 968)
Median creatinine — mg/dl	24	0.82 (0.66, 1.05)	26	0.78 (0.66, 0.98)
QTc interval — ms	24	395.03 (377.55, 410.45)	26	401.20 (374.76, 420.06)

[¥] Median, interquartile range (IQR) for continuous data and N (%) for categorical data

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Table 2. Primary outcome: Early Fungicidal Activity over the first 2 weeks following randomization (log10 colony forming units (CFU)/ml/day).

	Treatment Arm					
Analysis populations	**Total**	**Tamoxifen**	**Total**	**Standard of Care**	**Difference in change**	**p-value[ǂ]**
	N	**Change/day (95% CI[✐])**	**N**	**Change/day (95% CI[✐])**	**(95% CI[✐])**	
Intention-to-treat	24	-0.49 (-0.62, -0.37)	26	-0.48 (-0.61, -0.37)	-0.005 (-0.16, 0.15)	0.95
Per-protocol	23	-0.48 (-0.61, -0.36)	25	-0.48 (-0.61, -0.37)	0.004 (-0.17, 0.17)	0.96
HIV infected patients	19	-0.49 (-0.65, -0.37)	21	-0.42 (-0.55, -0.31)	-0.072 (-0.25, 0.10)	0.41
HIV uninfected patients	5	-0.42 (-0.74, -0.21)	5	-0.57 (-0.93, -0.33)	0.16 (-0.18, 0.55)	0.37

[✐] 95% CI corresponds to Bayesian 95% credible intervals

[ǂ] p-value refers to crude "Wald-type" tests of the mean estimate divided by its standard deviation of the Monte Carlo Markov chain sampling of coefficients derived from the joint model.

Table 3. Secondary outcomes: Death, Disability and Change in CD4 count

Death by 10 weeks	**Tamoxifen N/total (%)**	**Control N/total (%)**	**Risk difference % (95%CI)**	**p- value[ǂ]**
Intention-to-treat population	8/24 (34)	7/26 (27)	6.47 (-19.15, 32.09)	0.62
Per-protocol population	7/23 (31)	6/25 (24)	6.50 (-18.90, 31.89)	0.62
HIV infected patients	7/19 (37)	6/21 (29)	8.39 (-20.99, 37.77)	0.58
HIV uninfected patients	1/5 (20)	1/5 (20)	0.00 (-49.58, 49.58)	1.00
Disability at 10 weeks				0.14
Good	2/23 (9)	9/25 (36)		
Intermediate	7/23 (30)	6/25 (24)		
Severe disability	6/23 (26)	3/25 (12)		
Death	8/23 (35)	7/25 (28)		
Disability at 10 weeks in HIV infected patients				0.05
Good	2/18 (11)	8/20 (40)		
Intermediate	5/18 (28)	6/20 (30)		
Severe disability	4/18 (22)	0/20 (0)		
Death	7/18 (39)	6/20 (30)		
Disability at 10 weeks in HIV uninfected patients				0.68
Good	0/5 (0)	1/5 (20)		
Intermediate	2/5(40)	0/5 (0)		
Severe disability	2/5 (40)	3/5 (60)		
Death	1/5 (20)	1/5 (20)		
Change in CD4 count over 10 weeks (cells/uL)	**Median Change (IQR) (N)**	**Median Change (IQR) (N)**		
HIV infected patients	50.0 (5.00, 142.5) (10)	40.0 (7.0, 76.0) (13)		0.5

	HIV uninfected patients	393.5 (211.3, 613.8) (4)	-257.5 (-413.7, -171.0) (4)	0.02

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359 ǂp-values not corrected for multiple testing.

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Table 4. Grade 3 or 4 Adverse Events by 10 weeks

Event	Tamoxifen (N=24)	Control (N= 26)	p-value[ǂ]
Number of patients with Grade 3 or 4 adverse events (%)			
Any adverse event	24 (100)	26 (100)	1.0
New neurological events	9 (38)	7 (27)	0.62
New AIDS-defining illness (HIV patients only)	3 (16)	5 (24)	0.58
New cardiac events	9 (38)	4 (15)	0.145
Supraventricular tachycardia	1 (4)	0 (0)	0.48
Ventricular extrasystoles	3 (13)	0 (0)	0.21
Right Bundle Branch Block	0 (0)	1 (4)	1.00
QTc prolongation	8 (33)	1 (4)	0.02
Myocardial infarction	0 (0)	1 (4)	1.00
Cardiac arrest	1 (4)	0 (0)	0.48
Other cardiac adverse events	1 (4)	1 (4)	1.0
Laboratory abnormalities			
Anemia	18 (75)	18 (69)	0.89
Leukopenia	2 (8)	2 (8)	1.0
Thrombocytopenia	2 (8)	4 (15)	0.74
Elevated aminotransferase	2 (8)	4 (15)	0.74
Raised Creatinine	3 (13)	6 (23)	0.55
Hyperkalemia	2 (8)	6 (23)	0.48
Hypokalemia	17 (71)	20 (77)	0.87
Hyponatremia	18 (75)	23 (88)	0.39

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362 [ǂ]p-values were not corrected for multiple testing.

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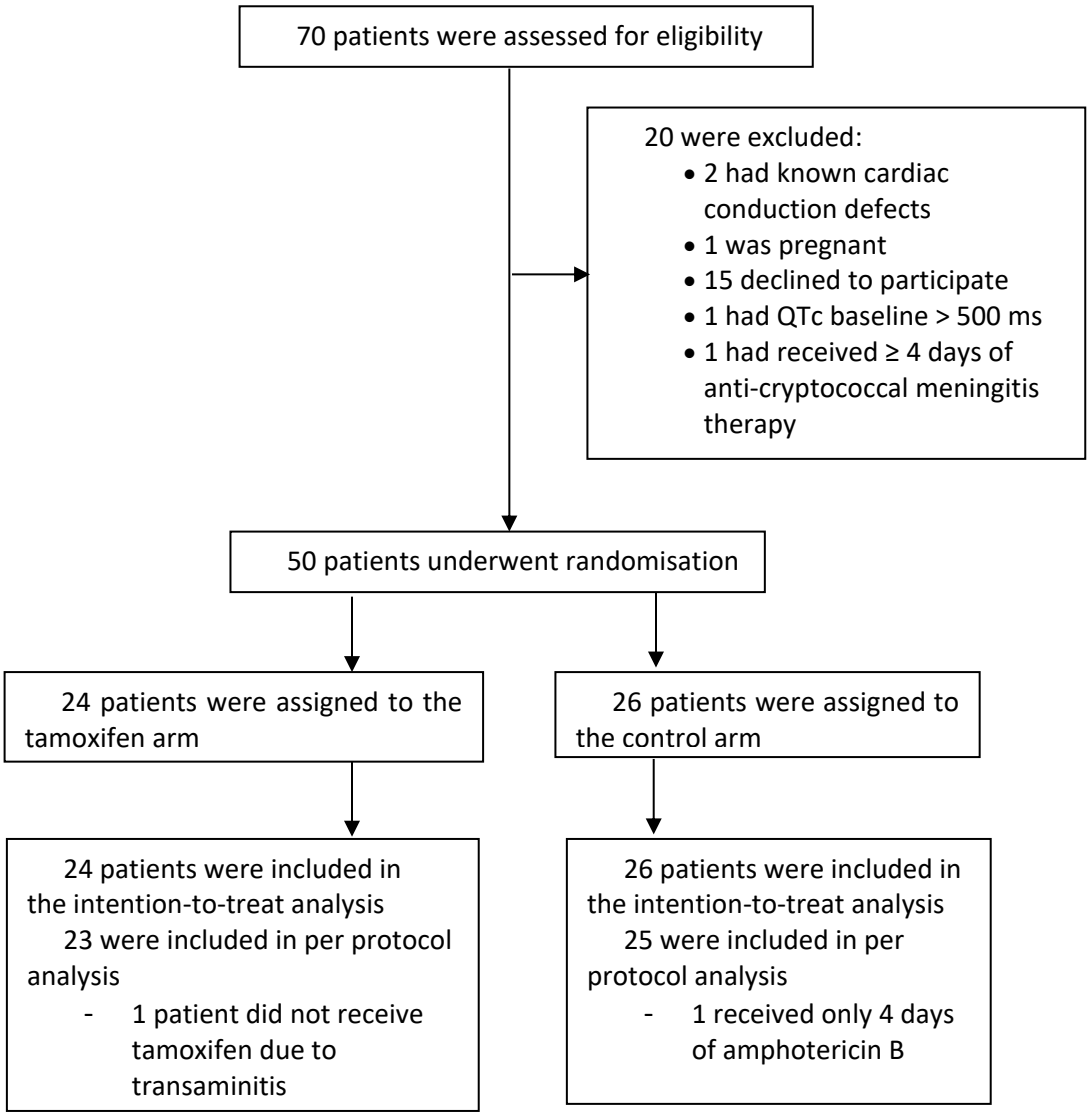
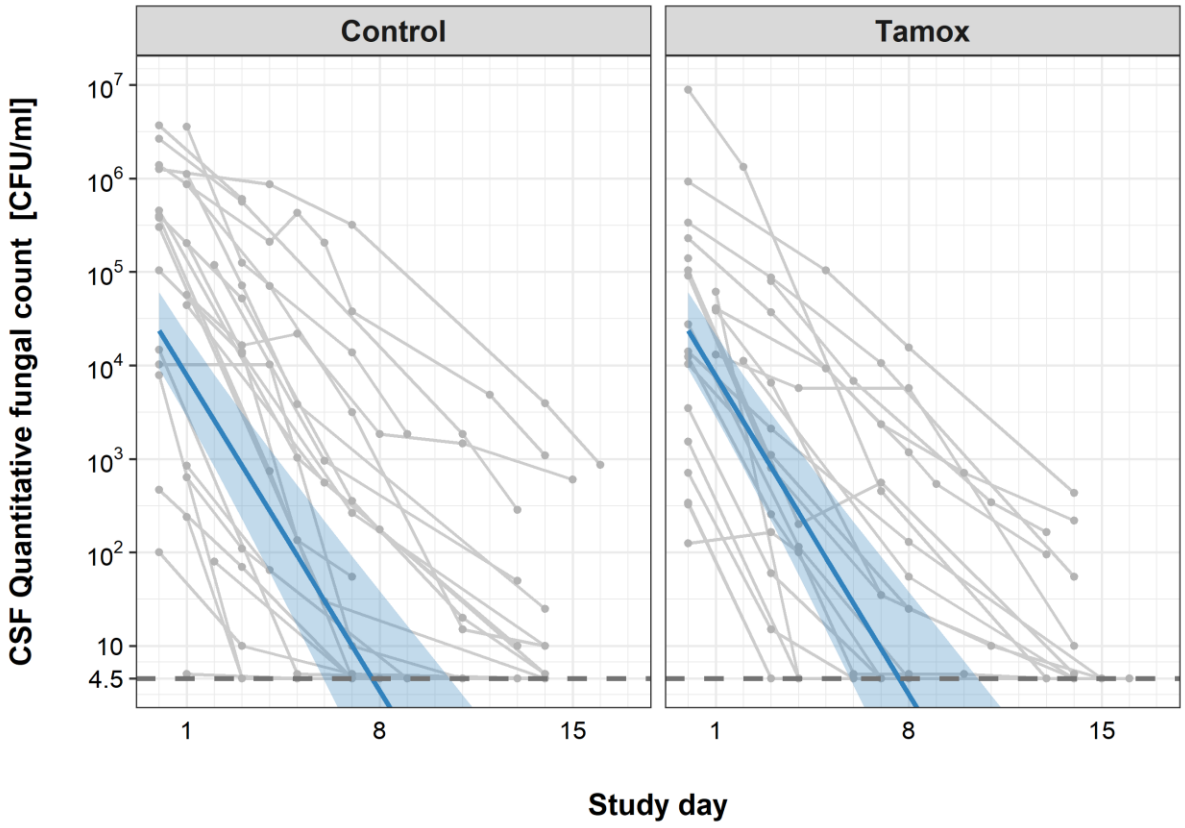
Figure 1. Trial flow chart: Enrollment, Randomization and Follow-up



371 **Figure 2.**

A. Decline in fungal count in CSF as measured in colony-forming units (CFU) per milliliter over the first 2 weeks of treatment by treatment arm. Data from individual patients are shown in grey lines. Bold blue lines show estimated mean with 95% credible intervals (shaded band) of CSF fungal counts based on the joint model described in the statistical analysis. The rate of decline was -0.49 log10CFU/ml/day in patients receiving tamoxifen versus -0.48 log10CFU/ml/day in control patients. The horizontal dashed lines represent the value of detection limit (4.5 CFU/ml). The fitted line crosses the horizontal dashed lines of the detection limit value after day 8 because 25% and 75% of patients had fungal counts under the detection limit at day 8 and 15, respectively.



B. Kaplan-Meier survival cures for each study arm over the 10 week study period. 7 death events occurred

in the control arm versus 8 in the tamoxifen intervention arm by 10 weeks (estimated risk 27% versus 34%, absolute risk difference = 6.5% (95% Confidence Interval -19.2% to 32.1%, p = 0.62).

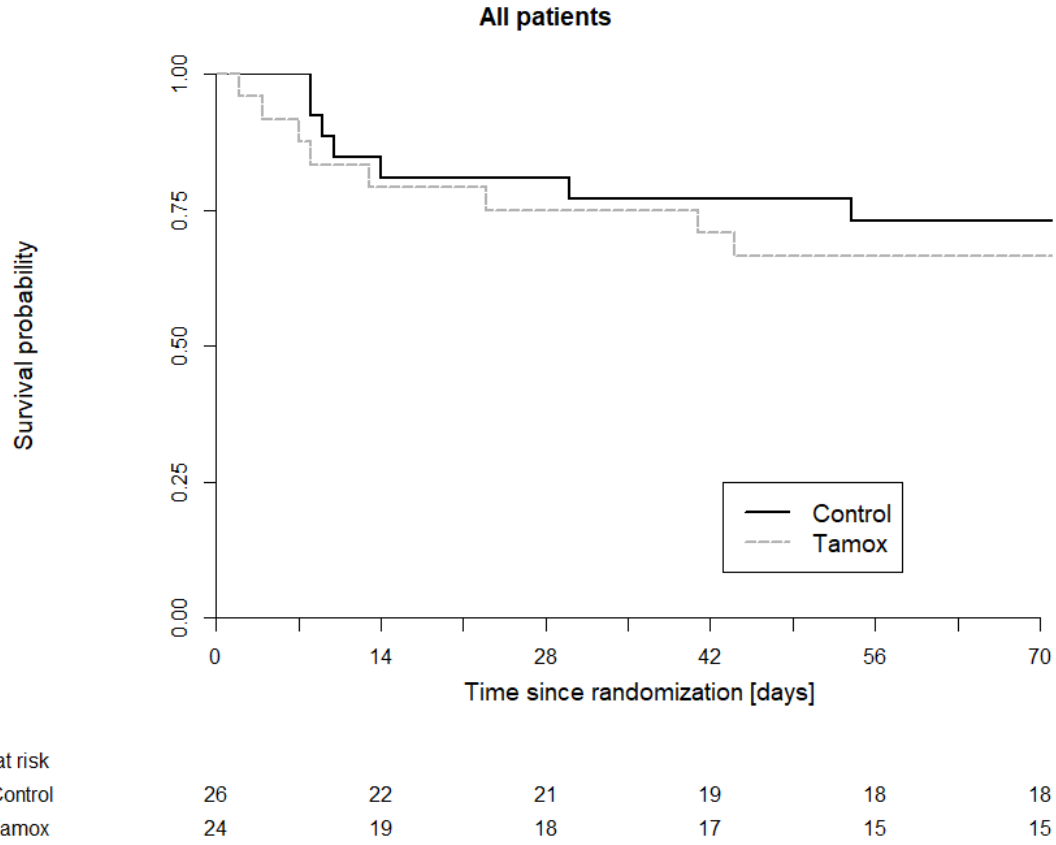


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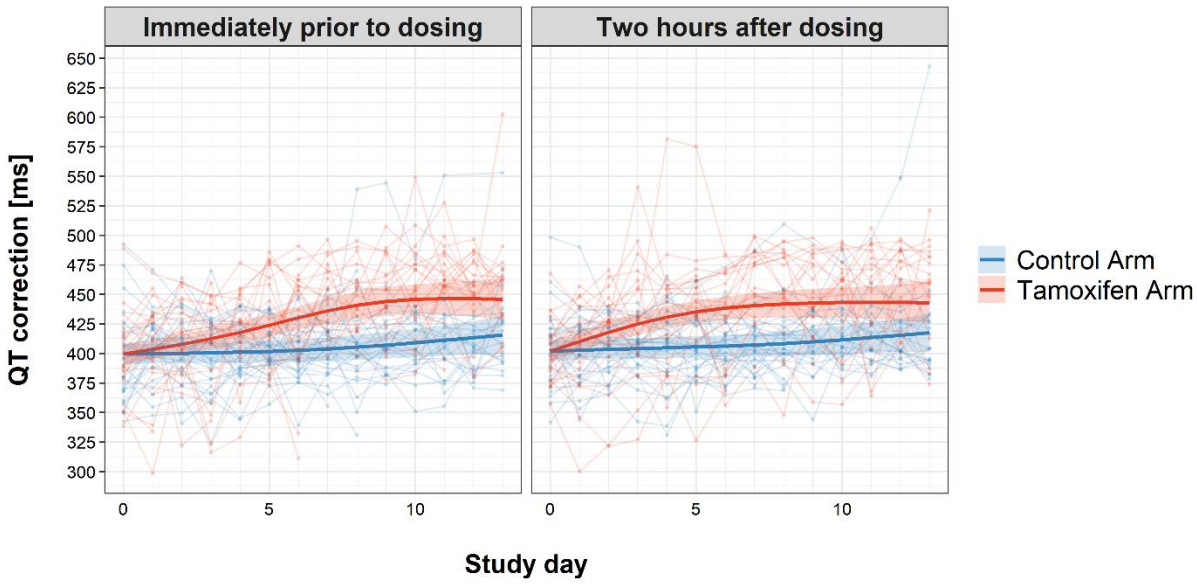
Figure 3. Change in QTc interval over the first 2 weeks of treatment by study arm. Faint lines display change in individual patient QTcs; bold lines display the estimated mean and and shaded bands the 95% Confidence Intervals; blue = control arm, red = tamoxifen arm. The maximum median difference in the QTc intervals between study arms immediately prior to drug administration was 37.07ms (95% CI: 21.09, 53.04) and occurred on day 9 of the study. The largest difference in median QTc 2 hours post-drug administration was 33.44ms (95% CI: 18.67, 48.21) and occurred on day 8 of the study. Additional details regarding change in QTc are provided in the Supplementary Appendix.



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SUPPLEMENTARY APPENDIX

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

Supplement to:

A randomized open label trial of tamoxifen combined with amphotericin B and fluconazole for cryptococcal meningitis

Supplementary Material

Section 1 Statistical Analysis Plan

Purpose

This document details the planned analyses and endpoint derivations for the randomized open label trial of tamoxifen combined with amphotericin B and fluconazole for cryptococcal meningitis (NCT03112031) as outlined in the study protocol. It focuses on the analysis for the main clinical trial outcomes and does not include analysis for any subsidiary studies.

Statistical software

Data derivations will be performed with the statistical software SAS v9.4 (SAS Institute, Cary, North Carolina, US). All statistical analyses will be performed with the statistical software R version R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria)[1].

Interim analyses and early stopping of the trial

Interim analyses for this trial will be conducted by an independent data and safety monitoring board with statistical expertise (Chair: Tim Peto) after the first 20 cases have reached the primary endpoint (completed the first 2 weeks of treatment following randomization or died), as detailed in the study protocol.

Raw data will be transferred from the study statistician (Nhat Le Thanh Hoang) to the Data Safety and Monitoring Board (DSMB) chair and statistician (Tim Peto) in csv format (csv data can be viewed in Excel and imported to any statistical software) together with R code to generate all summary tables.

The trial is not blinded with placebo; however, the clinician investigators of the trial will not be informed of the interim analyses results, but only the decision as to whether to continue the trial or not, and whether any additional safety reporting is needed.

Based on this information, the DSMB chair and statistician will generate the output tables and distribute the interim report amongst the Data Monitoring and Ethics Committee (DMEC) members.

Analysis populations

Intention-to treat population (ITT)

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 not receiving any study treatment will still be included in the ITT. Patients will be analyzed according to their randomized arm (intention-to-treat).

Per-protocol population

The primary endpoint will also 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.

Derivation rules for the definition of study populations

The following will be considered as "major protocol violations":

- Pregnancy
- Less than 1 week of amphotericin B antifungal therapy after randomization for reasons other than death (interpreted in the same way as for the study drug, see below). Amphotericin B antifungal therapy is recorded on the antifungal drug (AFDR) form.
- Less than 1 week of administration of tamoxifen study drug for reasons other than death: To allow that study drug is stopped up to 3 days prior to death, this will be interpreted as receiving <7 days of study drug for those who did not die within the first 9 days and as receiving less than [day of death]-3 doses of study drug for those who died earlier (i.e. <6 doses for patients who die on day 9, <5 doses for patients who die on day 8, …, no study drug at all for patients who die on days 1-4) .

Baseline characteristics

Baseline characteristics will be summarized as median (interquartile-range (IQR)) for continuous data and n (%) for categorical data. The amount of missing data for each baseline characteristic will also be displayed.

Formal comparisons of baseline characteristics between study arms are discouraged by most statisticians (see e.g. Senn SS (2008): Statistical Issues in Drug Development, 2nd Edition, Wiley [p. 98f]) but mandated by some journals. To satisfy all potential publishers, we will calculate p-values (based on the Wilcoxon rank sum test for continuous and Fisher's exact test categorical data) but will only report them if mandated by the journal.

Baseline/date of randomization is defined as the date of the first dose of study treatment (AMPHOTERICINBDATESTART where DAY=1 in dataset AFDR). If a subject did not receive any study treatment at all, baseline will be defined as the date of the baseline (history and examination) assessment (BASE.ASSDTC).

The following baseline characteristics will be summarized by treatment arm [with derivation rules in brackets]:

BASE: Baseline – History and Examination

All recorded variables in the BASE form with the following modifications:

- Free text specifications will not be summarized.
- If dates are given (e.g. date of birth, prior HIV diagnosis, or prior to cryptococcal meningitis), the time from that date to baseline will be summarized rather than the date.
- For fluconazole prophylaxis: only yes/no, not the duration will be summarized
- For any antifungal treatment for THIS CURRENT diagnosis of cryptococcal meningitis BEFORE randomization: Only the given antifungals (yes/no), whether it was fluconazole monotherapy (yes/no) and the maximum recorded days on any prior antifungal treatment will be reported.

- Other Opportunistic Infection Prophylaxis up to this admission: Only the given drugs (co-trimoxazole, isoniazid, and/or other) will be summarized.
- Glasgow coma score (GCS) will also be summarized as a categorical variable with values ≤10, 11-14, and 15.
- For visual acuity, the worst result of both eyes will also be summarized.
- Cranial nerve palsies (CNP) will be summarized as "CNP 6" [CNPLeft6 or CNPRight6 ticked], "Other CNP" [at least one CNP other than CNP 6 ticked], "None" [CNPnone ticked] or "Unable to assess" [CNPUnableAssess ticked].

HEMA (Laboratory investigations – Hematology), CHEMIS (Laboratory investigations – Biochemistry), MICRO (Microbiology) and HIVFU (CD4 and CD8 count)

Baseline results for all values (with proper unit conversion) will be recorded. If no values are available before or at enrolment, values up to one day post enrolment will be used as baseline values for hematology, chemistry, and values up to 14 days post enrolment will be imputed as baseline values for CD4 and CD8. (The latest CD4 value recorded on the Base form will also be included in this derivation as long as it did not occur >3 months (91 days) prior to enrolment.) For chemistry, blood glucose values recorded on the lumbar puncture form will also be included in the derivation.

For microbiology tests, the baseline test result will be summarized as "positive" if at least one positive test result was recorded up to 3 days post enrolment, and "negative" if at least one negative and no positive test result was recorded.

LP (Lumbar puncture)

Baseline results for the following values (with proper unit conversion, if necessary) will be recorded: Opening and closing pressure, WCC, % of lymph, % of neut, % of mono, % of eosin, protein, csf

glucose, csf/blood glucose ratio), and yeast quantitative count. If no values are available at or before enrolment, values up to 1day post enrolment will be used as baseline values. For the calculation of the csf/blood glucose ratio, missing blood glucose values on the lumbar puncture form will be imputed with the blood glucose value recorded on the chemistry form if that value is from the same day as the csf glucose value.

Test results for microbiology cerebrospinal fluid (CSF) tests, the baseline test result will be summarized as "positive" if at least one positive test result was recorded up to 3 days post enrolment, and "negative" if at least one negative and no positive test result was recorded.

IMAGING (XRAY and BRAINSCAN)

The number of patients with a chest Xray, a brain magnetic resonance imaging (MRI), or a brain computerized tomography (CT) scan at baseline (allowing -7/+2 days) and the respective numbers of abnormal findings for each imaging method will be summarized.

Electrocardiogram (ECG) findings

Baseline ECG findings will be presented according to treatment group in terms of heart rate, corrected QT interval (median, IQR, proportion > 500ms). The QT corrected (QTc) will be classified as "normal" (<450ms for males, <460ms for females), mildly prolonged (≥450ms for males or ≥460 for females but ≤500ms) and prolonged (>500ms).

Baseline QTc category values will be summarized by treatment arm. Frequency of omitting doses during treatment if QTc remains >500ms will be also summarized by treatment arm.

Planned analyses

Baseline table for all variables as detailed above for the ITT population will be presented by treatment group.

Primary endpoint – Rate of CSF sterilization during the first 2 weeks

All recorded longitudinal quantitative fungal count measurements up to day 17 (allowing for some delays in the day 14 measurements) will be included in the analysis. Early Fungicidal Activity (EFA) defined as fungal decline (slope) will be modeled based on a linear mixed effects model with longitudinal log10-CSF quantitative culture fungal counts as the outcome, the treatment groups and the time since enrolment, with their interaction, as fixed covariates and random patient-specific intercepts and slopes. The lowest measurable quantitative count is 5 colony-forming units (CFU)/ml and values below the detection limit (which correspond to recorded values of 0) will be treated as <4.5 CFU/ml, i.e. non-detectable measurements will be treated as left-censored longitudinal observations. If a patient who misses day 1 measurement completely at random, we will exclude this patient from the analysis; otherwise we keep this patient in the analysis. Based on this model, EFA will be compared between the two treatment arms in all patients (ITT), in the PP population, and subgroups defined by HIV status (uninfected; infected) and baseline fungal burden (<5 log10 CFU/ml; ≥ 5 log10 CFU/ml). For the ITT population, the comparison between two arms will also be adjusted for study site and HIV status. Correction for multiple testing (Hochberg procedure as implemented in R function multtest:: mt.rawp2adjp) of all the p-values from the tests for difference effects on EFA between two treatment arms or interaction tests between treatment and subgroups will be provided.

The model will be implemented with the R package Rstan version 2.19.2 which allows to appropriately handle detection limits for longitudinal measurements and also to adjust for the selection bias due to early death in the first 14 days. Stan code will be provided in the appendix. Reported "95% confidence intervals" correspond to Bayesian 95% credible intervals and the reported "p-values" refer to crude "Wald-type" tests of the mean estimate divided by its standard deviation. In case Monte Carlo Markov chain diagnostics plots of the fitted stan models indicate failure of

the algorithm we will report results from a mixed model with a detection limit (but ignoring truncation by death) instead and this will be implemented with the R package lmec version 1.0.

Secondary Endpoints-Survival until 10 weeks after randomization

Derivation of overall survival until 10 weeks after randomization

- Definition of time to death: [date of death or censoring] - [date of randomization] + 1
- Definition event indicator: = 1 if patient died = 0 otherwise
- [Date of randomization]: date of the baseline which is derived in Baseline characteristic section
- [Date of death]:
- Final status is death (FINAL.FINALSTT =2) and the corresponding date of death is FINAL.DEATHDATE2.
- [Date of censoring]:
    - If a final status form is available for the patient (which should be the case for every patient at completion of the study) then the date of censoring is defined as the date of study completion (FINAL.FINALDATE1) or, if the patient did not complete the study, the date of last contact (FINAL.LASTDATE3).
    - If the patient is still under follow-up, i.e. no final status form is available, the date of censoring is defined as the last recorded date of an inpatient or outpatient assessment, the week 10, a GCS, hematology, or blood chemistry date, or a study drug administration date.

Planned analysis

- Overall survival will be visualized using Kaplan-Meier curves by treatment arm and displayed with separated panel for each HIV status. The analysis will be based on a Cox proportional hazards regression model with HIV status as stratum variable and treatment arm is the only

covariate. We will test for proportional hazards of the treatment effect by means of Schoenfeld residuals (as implemented in R function survival::cox.zph).

- If we have enough event (at least 30 events in total), survival will be modeled with a multivariable Cox regression model including the following covariates in addition to the treatment group: baseline log10-fungal load (modeled linearly), Glasgow coma score less than 15 (yes or no), interaction between HIV infection status and treatment, and Anti Retrovirus (ARV) treatment status at study entry (naïve or experienced).

Subgroup analyses: The following subgroups are pre-defined

- Per protocol analysis – yes
- HIV serostatus (infected, uninfected)
- Quantitative fungal count at enrolment (<10^5 cells/ml, ≥10^5 cells/ml CSF)

Potential heterogeneity of the treatment effect across sub-groups will be tested using likelihood ratio tests for an interaction term between treatment and the grouping variable. All the p-values from these interaction tests and the test of the treatment effect in survival of the ITT population will be corrected for multiple testing (Hochberg procedure as implemented in R function multtest:: mt.rawp2adjp) due to the small sample size of the first interim analysis n=20, we won't perform subgroup analysis and only do it in the final analysis.

Secondary Endpoints-Disability at 10 weeks

Derivation: The disability score assessed at week 10 is composed of two sub-scores:

- The "two simple questions" score [ACTHELP and ISPROBLEM in datasets WEEK10]:
    - If answer to the first question= yes; outcome is classified as 'severe disability'
    - If answer to the second question = yes; outcome is classified as 'intermediate'
    - If answer to both questions = no; outcome is classified as 'good'
- The modified Rankin score: [LB30 in datasets WEEK10]

- If Rankin score=1; outcome will be classified as 'good'
- If Rankin score =2 or=3; outcome will be classified as 'intermediate'
- If Rankin score =4, =5 or=6; outcome will be classified as 'severe disability'

[Note that the Rankin scale is coded as taking values from 1-6 on the database, i.e. +1 compared to the levels 0-5 according to the published study protocol.]

The worst disability outcome from either questionnaire ("two simple questions" or Rankin score) will be used for analysis. Disability will be defined as "death" if the patient died before the scheduled time point.

Planned analysis

The ordinal 10-week score ("good"> "intermediate"> "severe"> "death") will be compared between the two arms with a proportional odds logistic regression model depending on the treatment arm and HIV infection status. The result will be summarized as a cumulative odds ratio with corresponding 95% confidence interval and p-value. Patients lost to follow up will be analyzed according to their last recorded disability status. If the fraction of patients lost to follow-up exceeds 10%, we will also perform an alternative analysis based on multiple imputation of missing values. See section Treatment of missing values (multiple imputation).

Secondary endpoint – Clinical adverse events and new laboratory adverse abnormalities

Derivation: Adverse events (AE) are all events recorded on the NEW CARDIAC ADVERSE EVENT (NCAE), NEW NEUROLOGICAL EVENT (NNE), NEW AIDS DEFINING ILLNESS (NADI), or OTHER ADVERSE EVENT (OAE) forms. All grade 3&4 AE are collected and will be considered as serious adverse events (SAE); grade 1&2 AE are only collected for NCAE, NNE, and NADI events but not OAE.

New laboratory abnormalities are defined as any worsening of a lab value to grade 3 or 4 (including changes from grade 3 to 4) compared to the subject's previous lab value. In addition, to be

conservative, if a subject's baseline lab missing value, the worst post-enrolment lab value will be considered a new lab abnormality if it is of grade 3 or 4. A grading table for laboratory abnormalities is provided in the Appendix.

Planned analysis

- Summary of all reported AE – overall (separate summaries by type only and by type and subtype will be produced)
- Summary of all grade 3&4 AE – overall and by HIV status
- Summary of grade 3&4 AE with onset within the first 2 weeks by type
- Summary of grade 3&4 AE with onset during weeks 3-4 by type
- Summary of grade 3&4 AE with onset during weeks 5-10 by type
- Summary of total number of grade 3&4 AE per patient
- Summary of new laboratory abnormalities

All the summaries will report the frequency of specific adverse events 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 presented and (informally) compared between the two treatment groups based on Fisher's exact test.

Secondary endpoint - QT prolongation

The QTc will be classified as "normal" (<450ms for males, <460ms for females), mildly prolonged (≥450ms for males or ≥460 for females but ≤500ms) and prolonged (>500ms). All recorded QTc intervals in the first 14 days of treatment and day 21, day 28 following randomization will be included in the analysis. All the measurement after 14 days will be considered as pre-dose measurement and all the QTc measurement values at the time of omitting dose during treatment will be considered as missing values. The main summary measure is the number of patients who has prolonged QTc within the first 14 days and the number of events of QTC

prolongation per patient within the first 14 days per arm. The test for the different effect of treatment arm on QTC prolongation will be based on a linear mixed effect model to the QTc data in which will allow for different trends over the pre-dose and post-dose measurements. The linear mixed effect model was implemented with the R package "lmec". In details, we model the relation between time and QTc in a flexible way using restricted cubic splines. We include an interaction term between the treatment arm and both time variables. A random patient-specific intercept and slope is included to account for the heterogeneity of individuals. The model can be written as follows:

$$\begin{aligned} &\mathrm{Y_i}(\mathrm{t}, \mathrm{post_i}, \mathrm{treatment_i}) \\ &\quad = \alpha + \mathrm{a_i} + (\mathrm{ns}(\mathrm{t}, \mathrm{df} = 3) + \mathrm{ns}(\mathrm{t}, \mathrm{df} = 3) * \mathrm{treatment_i}) * \mathrm{I}(\mathrm{post_i} = 0) \\ &\quad + (\mathrm{ns}(\mathrm{t}, \mathrm{df} = 3) + \mathrm{ns}(\mathrm{t}, \mathrm{df} = 3) * \mathrm{treatment_i}) * \mathrm{I}(\mathrm{post_i} == 1) + \mathrm{b_i} * \mathrm{t} + \epsilon(\mathrm{t}), \end{aligned}$$

where,

- $\mathrm{Y_i}(\mathrm{t}, \mathrm{post_i}, \mathrm{treatment_i})$: QTc measurement at day t ($\mathrm{t} = 0, \ldots, 14, $), of the pre and 2 hours post-dose measurement in treatment group $\mathrm{treatment_i}$ of patient i,
- $\mathrm{ns}(\mathrm{t}, \mathrm{df} = 3)$: natural spline function of time with 3 degree of freedom,
- $\mathrm{post} = 0$ if for pre-dose measurement and =1 for post-dose measurement,
- $\mathrm{treatment}$ =0 for control arm and =1 for Tamoxifen arm,
- $\mathrm{a_i}$ and $\mathrm{b_i}$ are random intercept and random slope of the mixed model,
- $\epsilon(\mathrm{t})$ is the measurement error.

Based on this model, longitudinal of QTc measurements will be compared between treatment arms over the first 14 days of treatment following randomization. In addition, we then used the output of the fitted linear mixed effect model to compute the differences in QTc between treatment arm by study day, separately for pre-dose and 2 hours post-dose measurements, based on the delta method[2].

Analysis of other secondary outcomes

Secondary endpoint – Rate of Immune reconstitution inflammatory syndrome (IRIS) until 10 weeks

Derivation: The derived endpoint will be the competing risks endpoint of the time to first IRIS or death defined as:

Time to event = [date of first IRIS event or death or censoring] - [date of randomization] + 1

Event type:

- 0: "censored": if patient is censored (no IRIS events or death recorded)
  1: "IRIS": if patient had an IRIS event (any adverse event recorded as IRIS)
  2: "prior death": if patient died without prior IRIS

Planned analysis

The rate of IRIS will be modeled with cause-specific proportional hazards models with treatment as the only covariate and stratification by HIV infection status, taking into account the competing risk of prior death. Non-parametric estimates of the cumulative incidence functions for the two competing events (IRIS/relapse and prior death) will also be calculated and displayed by treatment arm and tested using Gray log-rank test for sub-distribution hazard.

Secondary endpoint – Rate of Cryptococcal Meningitis Relapse in the 10 weeks after randomization

As for the endpoint "Rate of IRIS until 10 weeks" (see above) will be analyzed.

Secondary endpoint – Visual deficit at 10 weeks.

The visual acuity at 10 weeks is recorded on a 6-point scale and will be summarized by treatment arm for each eye separately, and overall where "overall" is defined as the worst recorded acuity of either eye. The odds of having "normal acuity" (amongst all surviving patients with a visual assessment) will be informally compared between the treatment arms with a logistic regression model adjusted for HIV status.

Secondary endpoint – Time to new neurologic event or death until 10 weeks

Derivation: The derived endpoint will be the competing risks endpoint of the time to first new neurological event or death defined as:

Time to event = [date of first neurological event or death or censoring] - [date of randomization] + 1

Event type:

- 0/ "censored": if patient is censored (no neurological event or death recorded)
  1/ "NNE": if patient had a new neurological event (defined below)
  2/ "prior death": if patient died without a prior new neurological event

Neurological events are defined as any grade 3 or 4 new neurological events or any fall in GCS ≥2 points, for ≥ 48hrs (which will also be programmed separately based on recorded longitudinal GCS).

Planned analysis: The time to the first new neurological event or death until 10 weeks will be analyzed in the same way as overall survival.

Secondary endpoint – Longitudinal measurements of intracranial pressure during the first 2 weeks

This endpoint will be modeled using a mixed effect model as described for the primary outcome

Secondary endpoint – the change of CD4 cell counts over 10 weeks of survived patients

The change of CD4 cell counts over 10 weeks of survived patients will be summarized and compared using the Kruskal-Wallis rank sum test, separately for HIV status.

Other exploratory analyses

Will be performed as appropriate.

Treatment of missing values (multiple imputation)

Multiple imputation will be performed if do this is the amount of missing values is large. Multiple imputation by chained equations as implemented in the R package "mice" will be used to deal with missing covariate values for all the Cox regression analysis and the proportional odds logistic regression analysis for disability at 10 weeks. Specifically, 20 imputed sets will be generated and the dataset for multiple imputation will include the following variables:

- Baseline variables: continent, country, age, sex, GCS, on ARV at study entry (no/ yes but ≤ 3 months/ yes, > 3 MONTHS), CD4 cell count
- CSF measurements: opening pressure and yeast quant counts at baseline [both log-transformed]
- Outcomes: overall survival until 10 weeks after randomization, Rankin score at 10 weeks (using method polr in mice package).
- Time-to-event outcomes (i.e. overall survival) will be included as the cumulative (cause-specific) baseline hazard at the observed event or censoring time and an event indicator as recommended by White and Royston (Statist. Med. 2009; 28:1982–1998).

Additional planned auxiliary analyses

Summary of time to ARV initiation:

- Categorized outcome: On ARVs at study entry/ARVs started after study entry/No ARVs documented.
- Median (IQR) time to ARV initiation in those who started ARV after study entry.
- Details for subjects with no ARVs documented: Subject died within <42 days without ARV/ subject died after >=42 days without ARV/ subject alive but no ARVs documented.

Number of chest X-rays, CT scans and MRI performed after baseline and proportion with an abnormal result.

Summary whether study drug was terminated before 2 weeks (for reasons other than death) by treatment group – based on tick-box on final status form. Summary of the number of days of tamoxifen treatment after enrolment

Appendix

Grading of laboratory abnormalities

Laboratory tests	**Grade 3**	**Grade 4**

Hematology		
Hemoglobin	6.5 –7.9g/dl	<6.5 g/dl
White cell count	1.0 - 1.9 K/μl or g/L	<1.0 K/μl or g/L
Neutrophils	NEU % xWBC=NEU K/μl :0.5 – 1.0 K/μl	NEU % xWBC=NEU K/μl <0.5 K/μl
Platelets	25 – 50 K/μl or g/L	<25 K/μl or g/L
Biochemistry		
Sodium - HYPONATRAEMIA	120-130 mmol/l	<120 mmol/l
Sodium - HYPERNATRAEMIA	155 – 160 mmol/l	>160 mmol/l
Potassium	2.5 – 3.0 mmol/l	<2.5 mmol/l
Potassium	6.0 – 7.0 mmol/l	>7.0 mmol/l
Hypocalcemia	1.5-1.75 mmol/l	<1.5 mmol/l
Hypercalcemia	3.1-3.4 mmol/l	>3.4 mmol/l
Hypomagnesemia	0.3-0.4 mmol/l	<0.3 mmol/l
Hypermagnesemia	1.23-3.3 mmol/l	> 3.3 mmol/l
Blood glucose	1.7 – 2.2 mmol/l or 30-40 mg/dl 13.9-27.8 mmol/l or 250-500 mg/dl	<1.7 mmol/l or < 30 mg/dl >27.8 mmol/l or >500 mg/dl
Creatinine	>3X BASELINE OR 3-6 X ULN	>6X ULN
Aspartate aminotransferase (AST)	>5-20-X ULN	>20X ULN
Alanine aminotransferase (ALT)	>5-20-X ULN	>20X ULN

ULN for Creatinine: 1.36 mg/dL (males), 1.13 mg/dL (females)

ULN for AST/ALT: 40 IU

General conventions and mock-up tables/templates

General conventions

- All patients randomized up to the time-point of the interim analysis database snapshot will be included in the tables following an intention-to-treat principle. Tables will contain actual (placebo, tamoxifen) and not masked treatment names.
- Statistical tests will report raw p-values and confidence intervals without any adjustment for multiplicity. As described in the protocol stopping for harm of Tamoxifen will be considered if a safety issue emerges which is sufficiently large, in the judgement of the DSMB, to suggest that continued exposure of patients to Tamoxifen is unethical. Early stopping for efficacy of Tamoxifen is not foreseen as this is a pilot study.
- As a guidance for stopping early for harm of tamoxifen, the DSMB should consider the following information:
    - A p-value <0.01 in the direction of harm at an interim analysis.
    - Clear evidence of harm of tamoxifen in terms of safety or morbidity in the absence of any evidence of a survival benefit due to tamoxifen.

Below are mock-up tables/templates of all information that is planned to be provided to the DMEC.

Table 1: Summary of patient characteristics at study entry

Characteristic	Tamoxifen (N=XXX)		Standard treatment (N=XXX)	
	n	Summary statistic	n	Summary statistic

Age (years)	XXX	XX (XX, XX)	XXX	XX (XX, XX)
Sex – male	XX	XX (XX%)	XXX	XX (XX%)
Glasgow Coma Score - 15 - 11 to 14 - 10 or lower	XXX	 XX (XX%) XX (XX%) XX (XX%)	XXX	 XX (XX%) XX (XX%) XX (XX%)
Baseline quantitative fungal count (log10-CFU/ml) QTc (ms) Proportion with QTc>500ms	XXX XX XX	XX (XX, XX) XX XX	XXX XX XX	XX (XX, XX) XX XX

n refers to the number of patients included in the summary statistic, the summary statistic is the number (%) of patients with the characteristic for categorical data and median (IQR) for continuous data.

Note: Table 1 will be generated for all patients, and for patients according to HIV infection status.

Table 2: Summary of the primary outcome: EFA over the 1st 2 weeks after randomization

Population	Tamoxifen(N=XXX)	Placebo (N=XXX)	Estimated change (95% CI) in log10 CFU/mL of

	n	Summary statistic	n	Summary statistic	CSF per day
All patients (ITT)	XXX	X.XX (X.XX, X.XX)	XXX	X.XX (X.XX, X.XX)	X.XX (X.XX-X.XX); p=X.XX
HIV Infected Patients	XXX	X.XX (X.XX, X.XX)	XXX	X.XX (X.XX, X.XX)	X.XX (X.XX-X.XX); p=X.XX
HIV Uninfected Patients	XXX	X.XX (X.XX, X.XX)	XXX	X.XX (X.XX, X.XX)	X.XX (X.XXX.XX); p=X.XX

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373 *Table 3: Summary of key secondary outcome: survival outcome.*

Population	No. of deaths		Hazard ratio (95% CI)	p-value	p-value for heterogeneity *
	Tamoxifen	Placebo			
All patients (ITT)	XX/XX	XX/XX	X.XX (X.XX, X.XX)	X.XX	

Per protocol populations	XX/XX	XX/XX	X.XX (X.XX, X.XX)	X.XX	
HIV status- Infected - Uninfected	XX/XX XX/XX	XX/XX XX/XX	X.XX (X.XX, X.XX) X.XX (X.XX, X.XX)	X.XX X.XX	X.XX
Baseline quantitative fungal count - <5 log10 CFU/ml - ≥5 log10 CFU/ml	XX/XX XX/XX	XX/XX XX/XX	X.XX (X.XX, X.XX) X.XX (X.XX, X.XX)	X.XX X.XX	X.XX

***** Hazard ratios and p-values are based on stratified Cox proportional hazards models allowing for separate baseline hazards according to HIV serostatus, except for subgroup analysis of HIV status, model without stratification will be used.

Figure 1: Kaplan-Meier curves of overall survival by treatment arm.

Standard Kaplan-Meier curves for the two treatment groups with numbers at risk at the bottom will be displayed. The time axis of the Kaplan-Meier curves will extend to the maximum follow-up duration of 70 days.

Note: Figure 1 will be generated for all patients only.

Figure 2: Kaplan-Meier curves of overall survival by treatment arm and stratified HIV status.

Table 4: Summary of other secondary outcomes.

Outcome	Tamoxifen (N=XXX)	Placebo (N=XXX)	Estimate (95% CI); p-value

	n	Summary statistic	n	Summary statistic	
Disability at 10 weeks - Good - Intermediate - Severe disability - Death	XX	 XX (XX%) XX (XX%) XX (XX%) XX (XX%)	XX	 XX (XX%) XX (XX%) XX (XX%) XX (XX%)	OR of status "good": X.XX (X.XX-X.XX); p=X.XX
Change in QTc	XX	X.XX(X.XX-X.XX)	XX	X.XX(X.XX-X.XX)	Difference in estimated change X.XX (X.XX-X.XX); p=X.XX
AUC QTc of the first 2 weeks		XX(XX%)		XX(XX%)	Difference in estimated change X.XX (X.XX-X.XX); p=X.XX
IRIS	XX	XX (XX%)	XX	XX (XX%)	Cause-specifc HR of IRIS event: X.XX (X.XX-X.XX); p=X.XX

Visual deficit at 10 weeks (in survivors) - Normal - Blurred - Finger counting - Movement detection - Light perception - No light perception	XX	 XX (XX%) XX (XX%) XX (XX%) XX (XX%) XX (XX%) XX (XX%)	XX	 XX (XX%) XX (XX%) XX (XX%) XX (XX%) XX (XX%) XX (XX%)	OR for normal vision: X.XX (X.XX-X.XX); p=X.XX
New neurological event or death	XX	XX (XX%)	XX	XX (XX%)	Cause-specifc HR of new neurological event: X.XX (X.XX-X.XX); p=X.XX
Relapses	XX	XX (XX%)	XX	XX (XX%)	Cause-specifc HR of relapse event: X.XX (X.XX-X.XX); p=X.XX
Intracranial pressure	XX	X.XX(X.XX-X.XX)	XX	X.XX(X.XX-X.XX)	Difference in estimated slope X.XX (X.XX-X.XX);

					p=X.XX
CD4 cell count	XX	X.XX(X.XX-X.XX)	XX	X.XX(X.XX-X.XX)	Difference in estimated change from baseline X.XX (X.XX-X.XX); p=X.XX

n refers to the number of patients included in the analysis of each outcome. Relapse is defined as need for antifungal treatment intensification or readmission for treatment of cryptococcal disease (as in the protocol). All analyses were done as outlined in the protocol.

Note: Table 3 will be generated for all patients and by HIV infection status.

Table 5: Summary of clinical grade 3&4 adverse events.

Characteristic	Tamoxifen (N=XX)		Placebo (N=XX)		Comparison (p-value)
	n.pt	n.ae	n.pt	n.ae	
Any adverse event	XX (XX%)	XX (XX%)	XX (XX%)	XX (XX%)	X.XX
New Cardiac Event	XX (XX%)	XX (XX%)	XX (XX%)	XX (XX%)	X.XX
Neurological event	XX (XX%)	XX (XX%)	XX (XX%)	XX (XX%)	X.XX
New AIDS defining illness	XX (XX%)	XX (XX%)	XX (XX%)	XX (XX%)	X.XX
… (other collected AE) …	XX (XX%)	XX (XX%)	XX (XX%)	XX (XX%)	X.XX

n.pt refers to the number of patients with at least one event, n.ae to the total number of adverse event episodes. Comparison between the two groups based on the number of patients with at least one adverse event and Fisher's exact test.

Note: Table 5 will be generated for all patients. AIDS defining illness will only be defined for patients who are HIV seropositive.

Table 6: Summary of laboratory grade 3&4 adverse events.

Table 7: Summary of serious adverse events.

Table 8: Summary of unexpected serious adverse events.

Note: Tables 6-8 will have the same layout as table 5.

Stan code of the joint model

```
data{
 //for longitudinal data
 int<lower=1> N_long; // no. rows
 int<lower=0,upper=1> y2_censInd[N_long]; // under detection limit index
 int<lower=1> I; // no. patient
```

```
414     int<lower=1,upper=I> patid[N_long]; //patid  index for random effect
415
416     vector [N_long]time_scale; // time_scale censoring data
417     real log10fc_obs[N_long];// log10fc observing data
418     real <upper=log10(5)>C;
419     int<lower=1> p_X;
420     int<lower=1> p_X_intercept;
421     int<lower=1> p_X_slope;
422     vector[p_X]mean_X;
423     vector<lower=0>[p_X]sd_X;
424     matrix[N_long,p_X] X_long_scale;//treatment on EFA
425
426     // for survival data
427     matrix[I,p_X_intercept] X_intercept_unscale;//treatment on EFA
428     matrix[I,p_X_slope] X_slope_unscale;//treatment on EFA; .~trt.group only
429     int<lower=0> p_Z;
430     matrix<lower=0>[I,p_Z] Z; //treatment on survival
431     real<lower=0,upper=71>ttdeath[I];
432     int<lower=0> n_time_interval;
433     vector<lower=0,upper=71>[n_time_interval+1]time_spec;
434     vector<lower=0,upper=1>[I] death;
435     matrix<lower=0>[I,n_time_interval]zeros;
436     int<lower=1,upper=n_time_interval>index_interval[I];
437   }
```

```
438
439     parameters{
440       //for longitudinal data
441       vector[p_X] fix_eff_scale;//fixed effects  for intercept
442       real<lower=0> sigma; // SD of error measurement
443       cholesky_factor_corr[2] L_Omega; // prior correlation
444       vector<lower=0,upper=pi()/2>[2] tau_unif;
445       //vector<lower=0>[2] tau; // prior scale
446       matrix[2,I] z;
447       // for survival data
448       vector[p_Z] beta;//fixed effects for survival
449       real<lower=0>lambda[n_time_interval]; // piecewise hazard rate
450       real eta;// trajectory parameter
451       real<lower=0> sigma_lambda;
452     }
453
454     transformed parameters {
455       // for longitudinal data
456       vector[p_X_slope] b_fix_unscale;//original fixed effects for slope
457       vector[p_X_intercept] a_fix_unscale;//original fixed effects  for intercept
458       vector[N_long]fit_X_long_scale;
459       vector[I] a_unscale;// estimated intercept
460       vector[I] b_unscale;// estimated slope
461
```

```
462     matrix[I,2] U_raw; // patient random effects
463     matrix[I,2] U_raw_unscale; // patient random effects
464     // for survival data
465     vector[I] beta_hat;
466     real test;
467     vector<lower=0>[2] tau; // prior scale for random effects
468     for (k in 1:2) tau[k] = 2.5 * tan(tau_unif[k]);
469
470     test=sum((fix_eff_scale[2:p_X].*mean_X[2:p_X])./sd_X[2:p_X]);
471     a_fix_unscale[1]=fix_eff_scale[1]-sum((fix_eff_scale[2:p_X].*mean_X[2:p_X])./sd_X[2:p_X]);
472     a_fix_unscale[2:p_X_intercept]=fix_eff_scale[2:p_X_intercept]./sd_X[2:p_X_intercept];
473     b_fix_unscale=(fix_eff_scale[(p_X_intercept+1):p_X])./sd_X[(p_X_intercept+1):p_X];
474
475     U_raw = (diag_pre_multiply(tau,L_Omega) * z)';
476     for(i in 1:I){
477     U_raw_unscale[i,1]= U_raw[i,1]-(U_raw[i,2])*mean_X[(p_X_intercept+1)]/sd_X[(p_X_intercept+1)];
478     }
479     U_raw_unscale[,2] = U_raw[,2]/sd_X[(p_X_intercept+1)];
480     // compute individual intercept
481     fit_X_long_scale=X_long_scale*fix_eff_scale;
482     a_unscale=X_intercept_unscale*a_fix_unscale+U_raw_unscale[,1];// contains only random effects.
483     // // compute individual  slope
484     for( i in 1:I){
485     b_unscale[i]=X_slope_unscale[i,]*b_fix_unscale+U_raw_unscale[i,2];
```

```
486       }
487       //for survival data
488       beta_hat=Z*beta;
489     }
490
491     model{
492     vector[N_long] mu;
493     vector[I] H;//cummulative hazard function;
494     vector[I] LL;//log density function
495     matrix[I,n_time_interval] integral_ht;// cummulative hazard function
496     // Likelihood for longitudinal component
497     // Set all priors for all parameters of longitudinal component
498     fix_eff_scale~normal(0,10);
499     to_vector(z) ~ normal(0,1);
500     tau_unif ~ uniform(0,pi()/2);
501     L_Omega ~ lkj_corr_cholesky(2);
502
503     for (k in 1:N_long){
504       mu[k]=fit_X_long_scale[k]+U_raw[patid[k],1]+(U_raw[patid[k],2])*time_scale[k];
505       if(y2_censInd[k]==0){
506         log10fc_obs[k] ~ normal(mu[k],sigma);
507       }else{
508         target +=normal_lcdf(C|mu[k],sigma);
509       }
```

```
510     }
511
512     //Likelihood for survival component
513     //Compute the cumulative hazard function from 0 to ttdeath.
514     integral_ht=zeros;
515     for( i in 1:I){
516     integral_ht[i,index_interval[i]]                                                    =
517             lambda[index_interval[i]]*exp(eta*a_unscale[i]+beta_hat[i])*(exp((eta*b_unscale[i])*ttdeath[i])
518             -exp((eta*b_unscale[i])*time_spec[index_interval[i]]))/(eta*b_unscale[i]);
519     for(j in 1:(index_interval[i]-1)){
520     integral_ht[i,j]  =  lambda[j]*exp(eta*a_unscale[i]+beta_hat[i])*(exp((eta*b_unscale[i])*time_spec[j+1])-
521             exp((eta*b_unscale[i])*time_spec[j]))/(eta*b_unscale[i]);
522     }
523     //Integrated hazard for individual i from 0 to survival time t.surv[i]
524     H[i]=sum(integral_ht[i,]);// cummulative hazard function
525     //Survival function
526     LL[i]=(log(lambda[index_interval[i]])+eta*(a_unscale[i]+b_unscale[i]*ttdeath[i])+beta_hat[i])*death[i]-
527             H[i];
528     }
529     target += sum(LL);
530
531     //Set all priors for all parameters of survival component
532     beta ~ normal(0,10);
533     if(n_time_interval>2){
```

```
534     lambda[1] ~ lognormal(0,5);
535     }else{
536       lambda[1] ~ normal(0,5);
537     }
538
539     for(i in 2:n_time_interval){
540       lambda[i]~lognormal(lambda[i-1],sigma_lambda);
541     }
542     sigma_lambda~cauchy(0,2.5);
543     eta ~ normal(0,10);
544     }
545     generated quantities {
546     vector[p_X_slope] b_fix_unscale_true;
547       b_fix_unscale_true[1]=b_fix_unscale[1];
548       for(i in 2:p_X_slope){
549         b_fix_unscale_true[i]=b_fix_unscale[1]+b_fix_unscale[i];
550
```

Section 2 Data Monitoring Committee charter

Data Monitoring Committee (DMC) Overview

1. Trial Description and Study Design

Trial number: **28CN**

Trial design: **A randomized trial of Tamoxifen combined with amphotericin B and fluconazole for cryptococcal meningitis**

Trial sponsor: **University of Oxford**

Number of patients: **50**

Names of sites:

#	Country	City	Name of site	Site number
1	Viet Nam	Ho Chi Minh	Hospital for Tropical Diseases	03
2	Viet Nam	Ho Chi Minh	Cho Ray Hospital	11

Principal Investigators: **Dr Jeremy Day, Dr Nguyen Le Nhu Tung, Dr Le Quoc Hung.**

2. DMC Terms of Reference

This independent DMC has been convened to assess the progress of a clinical study, the safety data and provide recommendations to the sponsor. The members of the DMC serve in an individual capacity and provide their expertise and recommendations. The DMC will review cumulative study data to evaluate safety, study conduct, and data integrity of the study. This charter will outline the roles and responsibilities and serve as the standard operating procedure (SOP) for the DMC

1. To consider the data from interim analyses, information from the investigators and relevant information from other sources

2. In the light of 1, and ensuring that ethical considerations are of prime importance, to report (following each DMC meeting or special meeting if required) to the study sponsor and to recommend on the continuation of the trial
3. To determine if additional interim analyses of trial data should be undertaken
4. To consider any requests for release of interim trial data and to recommend on the advisability of this

3. DMC Membership

This charter will be agreed by all DMC members Composition of membership will be:

Chairperson: Professor Tim Peto (Professor of Medicine, Consultant Physician in Infectious Diseases, General Physician)

Independent members: Dr Matt Scarborough (Consultant, Infectious Diseases and General Medicine, OUH NHS trust), Dr Nguyen Duc Bang (Infectious Disease physician, Pham Ngoc Thach Hospital, Ho Chi Minh City, Viet Nam)

Acronyms

CTU – Clinical Trials Unit (of OUCRU-VN)

DMC – Data Monitoring Committee

OUCRU-VN – Oxford University Clinical Research Unit – Viet Nam

PI – Principal Investigator

TMG – Trial Management Group

Introduction

The purpose of this charter is to define the roles and responsibilities of the Data Monitoring Committee (DMC), delineate qualifications of the membership, describe the purpose and timing of meetings, provide the procedures for ensuring confidentiality and proper communication, and outline the content of the reports.

The DMC will function in accordance with the ICH guidelines for Good Clinical Practice and the approved trial protocol.

The DMC administration will be coordinated by the OUCRU-VN Clinical Trials Unit. All significant communications, meetings and reports will be made in writing, communicated to all relevant parties and maintained with the Trial Master File.

Definitions

The following definitions apply to this protocol:

(S)AE

TABLE	DEFINITION
Adverse Event (AE)	Any untoward medical occurrence in a participant or clinical trial subject to whom an investigational medicinal product has been administered including occurrences that are not necessarily caused by or related to that product.
Grade 3 or 4 Adverse Event:	Any untoward medical occurrence of severity defined as grade 3 or 4 by the Common Terminology Criteria for Adverse Events from National Cancer Institute (CTCAE) http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm
Adverse Reaction (AR)	Any untoward and unintended response to an investigational medicinal product related to any dose administered.
Unexpected Adverse Reaction (UAR)	An adverse reaction, the nature or severity of which is not consistent with the information about the investigational
	medicinal product in question set out in the Summary of Product

	Characteristics (SPC) for that product.
Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR) or Suspected Unexpected Serious Adverse Reaction (SUSAR)	Respectively any adverse event, adverse reaction or unexpected adverse reaction that: Results in death Is life-threatening* Requires hospitalization or prolongation of existing hospitalization** Results in persistent or significant disability or incapacity Consists of a congenital anomaly or birth defect Is another important medical condition***

*The term life-threatening in the definition of a serious event refers to an event in which the participant is at risk of death at the time of the event; it does not refer to an event that hypothetically might cause death if it were more severe, for example, a silent myocardial infarction.

**Hospitalization is defined as an in-participant admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation. Hospitalizations for a pre-existing condition (including elective procedures that have not worsened) do not constitute an SAE.

*** Medical judgement should be exercised in deciding whether an AE or AR is serious in other situations. The following should also be considered serious: important AEs or ARs that are not immediately life-threatening or do not result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above; for example, a secondary malignancy, an allergic bronchospasm requiring intensive emergency treatment, seizures or blood dyscrasias that do not result in hospitalization or development of drug dependency.

Ethical Committee of Reference: the lead ethical committee to which all safety reporting and DSMB reports are issued. In the case of this trial, the ethical committee of reference is the Oxford Tropical Research Ethics Committee.

Roles and Responsibilities

DMC Roles and Responsibilities

1. ***This DMC will***

- Receive, review and feedback when necessary on USAEs reported in detail within 2 weeks of occurrence and followed until resolution
- Meet periodically (see DMC Meetings) to review summary tables of serious adverse events (SAEs), grade 3 & 4 AEs. The DMC may request additional data as required including aggregate and individual subject data related to safety, data integrity and overall conduct of the trial.
- Provide recommendations to continue, modify or terminate the trial depending upon these analyses.
- Communicate other recommendations or concerns as appropriate including requests for additional reviews based on regular reporting and USAE reporting.
- Comply with and operate according to the procedures described in this charter.
- Maintain documentation and records of all activities as described below (see DMC Chairman, DMC Meetings, DMC Reports).
- DMC members will have the ability to review unmasked clinical data (this will only be discussed during closed sessions).
- Consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study.
- What about review the conduct of the study including protocol violations?

2. DMC Chairman will

- Be responsible to archive the interim analysis reports and documentation of rationale for decisions made by the Committee during closed sessions. These will be provided to the Principal Investigator upon completion of the trial.

3. DSMB Statistician will

- Generate the analysis tables and distribute the interim report amongst the DSMB members as described below (see section “Creation of interim analysis reports” below).

Principal Investigator Roles and Responsibilities

The PI will directly or through delegation:

- Assure the proper conduct of the study including collection of accurate and timely data.
- Compile and report USAEs as described below.
- Promptly report potential safety concern(s) to the DMC.
- Communicate with regulatory authorities, ethical committees and investigators, in a manner that maintains patient safety and integrity of the data.

DMC Participation

Membership will be selected by the Principal Investigator and approved by the trial Sponsor. If a DMC member is unable to continue participation on the board, the reason will be documented and a replacement will be selected by the Principal Investigator with the agreement of the other DMC members and endorsement of the Sponsor.

DMC members will declare any existing or potential conflicts of interest to the Principal Investigator who will report to the Sponsor. Conflicts of interest will be reduced to the greatest extent that is consistent with assembling an independent and highly competent DMC. Any questions or concerns that arise regarding conflicts of interest will be addressed by the DMC Chair and the Sponsor if necessary. In the case of the Chair having a conflict, by the Sponsor.

A conflict of interest exists or potentially exists when a member has a personal, professional or financial interest which could unduly influence the member's position with respect to the trial or trial related issues. A conflict of interest should also be addressed if an interest could result in the member's objectivity being questioned by others.

DMC Meetings

1. Projected Schedule of Meetings

Correspondence with the DMC will be initiated by the OUCRU Clinical Trials Unit prior to any subject enrollment in the trial in order for the members to review the charter, to discuss the protocol, agree to the safety reporting procedures, to establish a meeting schedule and to review the study modification and/or termination guidelines. Subsequent interim review meetings will be held to review and discuss interim study data according to the schedule below. Additional meetings may be scheduled at the request of the DMC Chairman or the Sponsor. If scheduled meetings are more than 12 months apart, the DMC Chairman may consider an additional interim review.

Timeline	*Data Review by*	*Type of Data*
Before study initiation	Entire DMC	Study protocol, safety concerns, DMC Charter and associated procedures/reports
After 6 and 12 months of recruitment and yearly thereafter	Entire DMC	Enrolment summary Tables of grade 3 & 4 AEs and SAEs, SARs and SUSARs. Any other requested data

2. Meeting Format

DMC meetings will generally be conducted by teleconference and coordinated by the OUCRU-VN CTU. A quorum, defined as a minimum of 2 members (including the Chairman) will be required to hold a DMC meeting. Any member of the DMC may be absent during the meeting provided data tables are circulated in advance and the member has opportunity to forward any concerns to the Chairman before the meeting. Decisions of the DMC should be made by unanimous consensus. However, if this is not possible, majority vote will decide. When appropriate, DMC review sessions may be held by email exchange in lieu of a meeting.

3. Open and Closed Sessions

Sessions may be open (attended by representatives of the sponsor and study team) or closed (attended only by DMC members) at the direction of the DMC. A report based on each DMC meeting will be organized by the Chairman and submitted to the Sponsor. This report will include a recommendation to:

- Continue the trial without modification
- Continue the trial with modification
- Stop the trial due to safety concerns
- Stop the trial for another reason

Reports will be circulated to all DMC members for their approval before being issued.

4. Creation & conduct of interim analysis reports

The study statistician will generate the code (in the statistical software R) to generate all tables outlined in the Interim Analysis Plan. The intention is to analyze safety outcomes only to prevent stopping the study when important secondary outcomes including antibiotic use may not yet be clear.

Prior to each interim analysis, raw data will be transferred from the study statistician to the DMC statistician together with R code to generate all summary tables. Based on this information, the

DMC statistician generates the tables and distribute the interim report amongst the DMC members.

Interim analysis plan

All planned analyses will be described in detail in a full Statistical Analysis Plan. This section summarizes the main issues.

1. Analysis populations

The primary analysis population for all analyses is the full analysis population containing all randomized patients. Patients will be analyzed according to their randomized arm (intention-to-treat). In addition, the primary end point will be analyzed in the per-protocol population, which will exclude the following patients: patients with a final diagnosis other than TBM, major protocol violations and those receiving less than 1 week of administration of the randomized study drug for reasons other than death.

Of note, this trial includes a nested trial which randomizes participants who develop drug-induced liver injury to one of three management strategies as a sub-study (see Section 12.1 in the protocol for an outline). For the primary analyses of the main trial this second randomization will be ignored and the estimated dexamethasone treatment effect can thus be interpreted as an average effect across these three management strategies. We believe that this is justified because only approximately 100 (19%) subjects are expected to be enrolled in the nested trial with roughly similar numbers from both arms, because the efficacy of the different management strategies is unlikely to depend on whether the patient receive dexamethasone or not as it tests a very different intervention, and because the anticipated effect of the management strategy on survival is relatively small. However, in a supplementary analysis, we will also compare the primary endpoint between the treatment policies “dexamethasone treatment plus standard of care management of drug-related liver injury” vs. “placebo

732 treatment plus standard of care management of drug-related liver injury" using an inverse
733 probability weighting based analytical framework.

734 ***2. Analysis of primary endpoint***

735 The primary endpoint of this trial is overall survival, i.e. time from randomization to death, during 12
736 months of follow-up. Overall survival will be analyzed with a Cox proportional hazards
737 regression model with treatment as the only covariate and stratification by TBM MRC severity
738 grade at enrolment (I, II, or III) and country (Vietnam or Indonesia). The primary effect measure
739 is the resulting hazard ratio comparing dexamethasone vs. placebo with a corresponding two-
740 sided 95% confidence interval and p-value. The significance level of the associated two-sided
741 test will be set to 5%. Kaplan-Meier plots and explicit survival estimates at 3, 6, 9, and 12
742 months of follow-up will also be calculated for the full populations and in the subgroups defined
743 by TBM disease severity and country separately.

744 The proportional hazards assumption will be formally tested based on scaled Schoenfeld residuals and
745 visually assessed by a plot of the scaled Schoenfeld residuals versus transformed time. In case of
746 a significant test, a formal comparison of the absolute risk of death at 12 months between the
747 two groups will also be performed (using a Wald-type test based on Kaplan-Meier estimates at
748 12 months and associated standard errors using Greenwood's formula).

749 The homogeneity of the treatment effect on overall survival across subgroups will be assessed by
750 subgroup analyses and formal tests of interaction between treatment and the following
751 grouping variables: TBM MRC severity grade at enrolment (I, II, or III), country (Vietnam or
752 Indonesia), drug resistance pattern (MDR-TB or rifampicin mono-resistance, isoniazid resistant
753 non-MDR, no or other resistance), ART status at enrolment (ART naïve, ≤3 months of ART, >3
754 months of ART), and CD4 cell count at enrolment (≤100 vs >100 cells/mm3).

To obtain an adjusted treatment effect estimate and to assess the effect of other covariates on survival, the primary endpoint will also be modeled using a multivariable Cox proportional hazards regression model including the following covariates (in addition to the treatment group): TBM MRC severity grade at enrolment, country, drug resistance pattern, ART status and CD4 cell count at enrolment. Multiple imputation will be used to handle missing covariates.

3. Analysis of secondary efficacy endpoints

Neurological disability (as assessed by the ordinal modified Rankin scale) at 12 months will be compared between the two arms with a proportional odds logistic regression model with the treatment assignment as the main covariate and adjustment for TBM MRC severity grade, and country. The result will be summarized as a cumulative odds ratio with corresponding 95% confidence interval and p-value. Patients with a missing 12-month disability assessment will be excluded from the main analysis but an alternative analysis based on multiple imputation (including disability assessments at earlier time points in the imputation model) will also be performed.

Secondary time-to-event endpoints (time to neurological event or death, time to new AIDS event or death) will be analyzed in the same way as the primary endpoint. The number of IRIS and HIV-associated malignancy events in each group will be summarized and the event rate calculated in each arm. Comparisons of the rates between the treatment arms will be based on a cause-specific proportional hazards model of the time to the first IRIS event (or HIV-associated malignancy, respectively) or death with treatment as the only covariate.

4. Analysis of adverse event

The number of patients with any adverse events and specific events, respectively, will be summarized and informally compared between the two treatment arms based on Fisher's exact test. The total number of adverse event episodes per patient will also be summarized and informally compared based on a quasi- Poisson regression model with treatment as the only covariate.

The following subgroups of adverse events will also be separately summarized: grade 3&4 adverse events; serious adverse events; serious adverse events possibly, probably, or definitely related to the study drug; adverse events leading to TB treatment or ARV interruptions. Grade 3&4 laboratory abnormalities will be summarized in the same way as clinical adverse events.

5. Baseline descriptive analyses

Baseline characteristics will be summarized as median (lower and upper quartiles) for continuous data and frequency (percentage) for categorical data. The amount of missing data for each baseline characteristic will also be displayed.

Study Review Criteria, Stopping Rules and Guidelines

1. Safety Analyses

The primary safety endpoint is survival. In addition to the primary safety endpoint, the DMC will consider grade 3 & 4 adverse events, serious adverse events and unexpected or events concerning to the Investigators at the time points defined above.

2. Consideration of External Data

The DMC will also consider data from other studies or external sources during its deliberations, if available, as these results may have an impact on the status of the patients and design of the current study.

DMC Reports

1. Monitoring for Safety

The primary charge of the DMC is to monitor patient safety during the study. Formal DMC safety reviews will occur as specified above (see DMC Meetings).

Safety reporting to regulatory and ethical committees will be in accordance with the requirements of each committee and the study protocol.

2. Content of DMC Reports at Formal Interim Analyses

The detailed content of the interim analysis report will be outlined in a separate document, the Interim Analysis Plan.

3. Monitoring for Study Conduct

The DMC will be updated at each scheduled meeting on study enrolment and major operational issues.

4. DMC Communication of Findings and Recommendations

Following each meeting and within 2 weeks of the meeting the chairman will send findings and recommendations of the DMC in writing to the Sponsor. The report should include the date of the meeting, participants, data reviewed by the Committee and a recommendation to continue the trial with/without modification or to stop the trial on a specified basis. The report may include minutes of relevant non- confidential discussion points and any requests for clarification of further information.

These findings and recommendations can result from both the open and closed sessions of the DMC. If these findings include serious and potentially consequential recommendations that require immediate action, the chairman will promptly notify the Principal Investigator and sponsor.

5. Response to DMC Findings and Recommendations

The Sponsor will review and respond to the DMC recommendations. If the DMC recommends continuation of the study without modification, no formal response will be required. If the recommendations request action, such as a recommendation for termination of the study or modification of the protocol, the Sponsor or Principal Investigator will provide a response stating whether the recommendations will be followed and the plan for addressing the issues.

Upon receipt, the DMC will consider the response and will attempt to resolve relevant issues, resulting in a final decision.

The Principal Investigator will disseminate all DMC reports to the relevant ethical committees according to the reporting requirements of that committee.

DMC Study Closeout

This study may be terminated based on safety issues or DMC monitoring guidelines. A final study report will be issued to the DMC who may recommend continuing action items to the Sponsor based upon the report.

Confidentiality

All data provided to the DMC and all deliberations of the DMC will be privileged and confidential. The DMC will agree to use this information to accomplish the responsibilities of the DMC and will not use it for other purposes without written consent from the Sponsor. No communication of the deliberations or recommendations of the DMC, either written or oral, will occur except as required for the DMC to fulfill its responsibilities. Individual DMC members must not have direct communication regarding the study outside the DMC (including, but not limited to the investigators, IRB/EC, regulatory agencies, or sponsor) except as authorized by the DMC.

Amendments to the DMC Charter

This DMC charter can be amended as needed during the course of the study. All amendments will be documented with sequential revision dates, and will be recorded in the report from the DMC meetings. Each revision will be reviewed and agreed upon by the DMC, the Principal Investigator and the Sponsor. All versions of the charter will be archived in the Trial Master File.

Archiving of DMC Activities and Related Documents

All DMC documentation and records will be retained in the Trial Master File in accordance with local and international regulatory requirements.

Agreement of DSMB Members

Signatures below confirm the agreement of all DSMB members to the contents of this charter and the confidentiality statement above.

Name: Professor Tim Peto Date: Signature:

Name: Dr Matt Scarborough Date: Signature:

Name: Dr Nguyen Duc Bang Date: Signature:

Agreement of Sponsor

Signatures below confirm the agreement of the Sponsor with the contents of this charter.

Name: Evelyne Kestelyn Date: Signature:

Section 3 The difference in QTc between two study arms over the first 2 weeks of study drug administration

Table

Study day	Difference of QTC between study arms before drug using (95% CI)	Difference of QTC between study arms 2 hours after drug using (95% CI)
0	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)
1	3.73 (-0.29, 7.74)	7.44 (3.45, 11.44)
2	7.63 (0.09, 15.18)	14.51 (6.99, 22.02)
3	11.91 (1.75, 22.06)	20.8 (10.69, 30.92)
4	16.73 (5.2, 28.25)	25.96 (14.47, 37.45)
5	22.13 (10.28, 33.97)	29.68 (17.85, 41.5)
6	27.55 (15.32, 39.78)	32.05 (19.82, 44.27)
7	32.29 (18.96, 45.62)	33.24 (19.92, 46.57)
8	35.63 (20.85, 50.42)	33.44 (18.67, 48.21)
9	37.07 (21.09, 53.04)	32.82 (16.86, 48.77)
10	36.82 (19.81, 53.83)	31.54 (14.55, 48.53)
11	35.32 (16.92, 53.72)	29.77 (11.39, 48.15)
12	32.97 (12.41, 53.54)	27.67 (7.13, 48.21)
13	30.21 (6.65, 53.77)	25.41 (1.89, 48.93)

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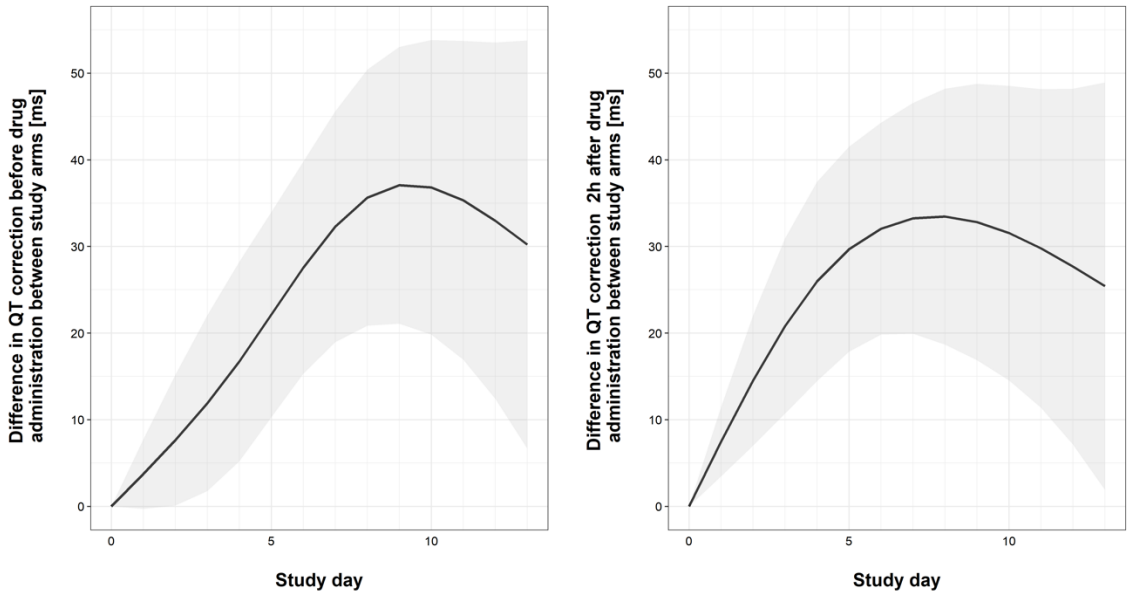
868

869 **Figure** The bold lines and the shaded bands represent the estimated mean difference with 95%
870 Confidence Interval of QTc between two study arms. The output of the fitted linear mixed effect
871 model computes the differences in QTc between study arms by study day, separately for pre-
872 dose and 2 hours post-dose measurements.

873



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Section 4 Adverse events by type and subtype

Adverse events (AEs)	Tamoxifen (N=24)	Control (N=26)	Comparison (p-value) ǂ
Number of patients with adverse events of any grade (%)			
All AEs combined	24 (100%)	26 (100%)	1
IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME	0 (0%)	1 (3.85%)	1
NEW AIDS DEFINING ILLNESS	7 (29.17%)	10 (38.46%)	0.693
Meningitis tuberculosis	1 (4.17%)	1 (3.85%)	1
Other AIDS events	1 (4.17%)	3 (11.54%)	0.661
Other extrapulmonary tuberculosis	1 (4.17%)	0 (0%)	0.48
Pneumocystis jiroveci pneumonia	3 (12.5%)	6 (23.08%)	0.546
Cerebral toxoplasmosis	2 (8.33%)	0 (0%)	0.225
Pulmonary tuberculosis	2 (8.33%)	1 (3.85%)	0.943
NEW CARDIAC ADVERSE EVENT	23 (95.83%)	24 (92.31%)	1
QRS axis abnormal (New axis deviation)	3 (12.5%)	1 (3.85%)	0.545
Supraventricular tachycardia	1 (4.17%)	0 (0%)	0.48
Ventricular extrasystoles	8 (33.33%)	0 (0%)	0.005
Bundle branch block right	0 (0%)	1 (3.85%)	1
Electrocardiogram QT prolonged	18 (75%)	8 (30.77%)	0.004
Atrioventricular block first degree	2 (8.33%)	2 (7.69%)	1
Myocardial infarction	0 (0%)	1 (3.85%)	1
Sinus tachycardia	13 (54.17%)	15 (57.69%)	1
Cardiac arrest	1 (4.17%)	0 (0%)	0.48

Other cardiac adverse event	18 (75%)	13 (50%)	0.127
Sinus bradycardia	3 (12.5%)	3 (11.54%)	1
NEW NEUROLOGICAL EVENT	11 (45.83%)	12 (46.15%)	1
Brain herniation (coning)	0 (0%)	1 (3.85%)	1
Cranial nerve paralysis	1 (4.17%)	1 (3.85%)	1
Depressed level of consciousness (fall in GCS >=2 points for >=48 hours)	7 (29.17%)	7 (26.92%)	1
Headache	1 (4.17%)	0 (0%)	0.48
Hemiplegia/paresis	1 (4.17%)	0 (0%)	0.48
Seizure (fit)	3 (12.5%)	5 (19.23%)	0.793
Other neurological event	2 (8.33%)	5 (19.23%)	0.483
OTHER ADVERSE EVENT	24 (100%)	26 (100%)	1
Hypersensitivity (Allergic reaction)	3 (12.5%)	2 (7.69%)	0.925
Anemia	18 (75%)	18 (69.23%)	0.89
Diarrhea	3 (12.5%)	2 (7.69%)	0.925
Hypertension	0 (0%)	2 (7.69%)	0.491
Hypotension	2 (8.33%)	3 (11.54%)	1
Jaundice	2 (8.33%)	0 (0%)	0.225
Hypokalemia	17 (70.83%)	17 (65.38%)	0.913
Acute Kidney Injury	0 (0%)	3 (11.54%)	0.263
Pleural effusion	0 (0%)	1 (3.85%)	1
Pneumonitis	5 (20.83%)	9 (34.62%)	0.442
Upper gastrointestinal hemorrhage	0 (0%)	1 (3.85%)	1
Vomit	5 (20.83%)	3 (11.54%)	0.61
Other adverse event	20 (83.33%)	22 (84.62%)	1

[ŧ] p-values were not corrected for multiple testing.

Section 5 Results of drug interactions from two-dimensional chequerboard testing of tamoxifen in combination with either amphotericin, fluconazole.

Antifungal combination	Proportion (%) of isolates where particular drug interactions was observed[£]		
	Synergy **FICI ≤ 0.5**	**No interaction** **0.5 < FICI ≤ 4**	**Antagonism** **FICI > 4**
C. neoformans			
Tamoxifen + amphotericin	11 (5/47)	89 (42/47)	0 (0/47)
Tamoxifen + fluconazole	4 (2/47)	96 (45/47)	0 (0/47)
C. gattii			
Tamoxifen + amphotericin	33 (1/3)	67 (2/3)	0 (0/3)
Tamoxifen + fluconazole	0 (0/3)	100 (3/3)	0 (0/3)

[£] Numbers in brackets: Numerators are the numbers of strains where interaction was observed; denominators are the numbers of isolates tested.