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Primary prophylaxis of cryptococcal disease using fluconazole in HIV positive Ugandan adults - a double blind, randomised, placebo controlled trial

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Abstract

Background—Cryptococcal disease remains an important cause of morbidity and mortality in HIV infected individuals in sub-Saharan Africa, despite the introduction of antiretroviral therapy (ART). Fluconazole has not been previously studied as primary prophylaxis against cryptococcal disease in patients awaiting or starting ART.

Methods—This prospective, double blind randomized controlled trial compared 200mg fluconazole three times per week to identical placebo in Uganda. 1519 ART naïve adults with CD4 counts <200 cells/μL were enrolled. Patients were excluded if baseline cryptococcal antigen (CrAg) was positive. Patients were randomly allocated to placebo or control (1:1) in blocks of 40 without stratification. Participants were seen after 4 weeks and referred for ART, then seen every 8 weeks. Participants discontinued trial treatment when CD4 counts reached 200 (median 197 days). Analyses were carried out on an intention to treat basis and included all participants enrolled in the study. Primary end points were invasive cryptococcal disease and all cause mortality. Secondary

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Conflict of interest

None of the authors declare a conflict of interest

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Data and Safety Monitoring Committee: Andrew Nunn (chair), Silver Bahendeka, Rod Hay.

Trial Steering Committee Tim Peto (chair), David Denning, Elly Katabira, Jonathan Mermin.

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endpoints were first episode and incidence of oesophageal candidiasis, time to first episode and incidence of oro-pharyngeal or vaginal candidiasis, time to first hospital admission or death. The primary safety end point was stopping trial drug due to elevated transaminases above 5x upper limit of normal or other major adverse events. The trial is registered on controlled-trials.com; ISRCTN 76481529

Results—760 participants received fluconazole and 759 received placebo. 19 developed cryptococcal disease, one on fluconazole and 18 on placebo ($P<0.001$); adjusted Hazard Ratio (aHR) 18.7 (95%CI 2.5-140.7). One case of cryptococcal disease could be prevented by treating 44.6 patients with baseline CD4 counts <200 cells/ μ L. Fluconazole was effective both pre-ART (aHR=11.0; 95%CI 1.4-85.3) and after starting ART (no cases on fluconazole vs. 7 cases on placebo after starting ART). Seven participants died from cryptococcal disease, none on fluconazole. All cause mortality ($N=189$) did not differ between the two arms ($P=0.46$). The frequency of elevated transaminases ($>5x$ upper limit) was similar in both arms (aHR=0.94, 95% CI 0.65-1.35).

Conclusions—Fluconazole was safe and effective as primary prophylaxis against cryptococcal disease, both before starting and during early ART. Cryptococcal infection was less common than anticipated because of the rapid commencement of ART and exclusion of those with positive CrAg. In patients with negative CrAg on screening, fluconazole prophylaxis can prevent cryptococcal disease while waiting for ART and in the early weeks of ART, particularly in those with CD4 counts <100 cells/ μ L.

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Keywords

Cryptococcus; primary prophylaxis; Fluconazole

Background

Cryptococcal disease is one of the commonest central nervous system infections in HIV infected individuals. Infection is acquired by the inhalation of environmental spores or desiccated yeast cells: clinical disease may not occur for months to years after exposure and may be preceded by asymptomatic cryptococcal antigenaemia¹. The disease is particularly problematic in sub-Saharan Africa, where the incidence in severe immunosuppression may reach 10% and it may cause up to 17% of deaths in HIV infected individuals^{1, 2}. Untreated, the mortality is 100%, and even with optimal treatment, around 30% of individuals die^{3, 4}. Survivors often have severe disabilities⁵. The gold standard treatment of amphotericin and flucytosine is costly and difficult to administer in resource poor settings^{4, 6}, fluconazole is therefore often used with poorer outcomes^{7, 8}.

Prior to the availability of ART in the USA, a randomised controlled trial (RCT) of primary prophylaxis using fluconazole showed a reduction in the incidence of cryptococcal disease but no effect on mortality⁹. Other Western studies suggested benefit from routine prophylaxis with itraconazole or fluconazole, but most were small and retrospective^{10–18}. A Cochrane review of cryptococcal primary prophylaxis concluded that azoles reduced incidence of cryptococcal disease but that the effect on mortality was unclear, and that

studies were needed in the developing world¹⁹. Only two studies have been done in developing countries, both in Thailand and with less than 10% of participants receiving ART. One suggested that fluconazole prophylaxis reduced invasive fungal infections and mortality but had no effect on cryptococcal events²⁰; the other demonstrated a reduction in systemic fungal diseases (including cryptococcosis) with itraconazole but no survival advantage²¹.

The incidence of cryptococcal disease has recently declined in industrialised countries, predominantly due to ART^{22–24}. International initiatives led to progress in the provision of ART in resource poor countries but only 30% of those who need ART in sub-Saharan Africa are receiving it²⁵. Cryptococcal disease remains common in sub-Saharan Africa, both in those who are yet to start ART or who are in the first months of ART^{26, 27}.

Primary prophylaxis against cryptococcal disease has never been formally tested in Africa or any setting where effective ART is available. We performed a double-blind, randomized, placebo controlled trial to examine the efficacy and safety of fluconazole as primary prophylaxis against cryptococcal disease prior to ART initiation and in the first few months of ART.

Methods

Ethics statement

Information on the trial was provided during group and individual meetings and through leaflets in the local language. Participants gave written or (if illiterate) witnessed (by a person independent of the trial team) verbal consent to screening and enrolment. Ethical approval was gained from the Uganda National Council for Science and Technology and the Ethics Committees of UVRI and the Liverpool School of Tropical Medicine. An independent data monitoring committee monitored accumulating data regularly. At completion of the trial, all participants were offered fluconazole if their CD4 count was still below 200 cells/ μ L.

Study design and population

Participants were recruited from five local HIV/AIDS organisations in Masaka district, Uganda, between November 2004 and January 2008: the AIDS Support Organisation (TASO), Masaka Regional Referral Hospital, Uganda Cares Masaka, Kalangala District Health Services and Kitovu Mobile AIDS Organisation. Participants were from predominantly rural communities, including the Ssesse Islands in Lake Victoria.

Potential participants were screened for eligibility at a dedicated study clinic sited at TASO; from April 2006 participants were also screened and enrolled at Ministry of Health clinics on the Ssesse islands. ART naive adults with laboratory confirmation of HIV infection (Murex HIV-1.2.0, Murex Biotech; HIV Uni-form II plus O, Biomerieux; Cambridge Biotech HIV-1 Western blot) and a CD4 count less than 200 cells/ μ L (FACSCount Becton Dickinson, USA) were eligible for the study. Cryptococcal antigen (CrAg) (Remel, Lexana, USA) testing was done (dilution first performed to exclude pronase effect) and participants with a serum CrAg titre >1:8 on 2 occasions were excluded; it was considered unethical to

randomise these patients. Other exclusion criteria were pregnancy or lactation, liver transaminases (LFT) > 3 x upper limit of normal (ULN), and moribund patients. Participants with oral and vaginal candidiasis at screening were treated with topical clotrimazole or nystatin, or if refractory, ketoconazole (200-400mg daily for 5 days); symptomatic oesophageal candidiasis was treated with fluconazole (minimum 14 days) and enrolment delayed for 4 weeks.

An independent statistician prepared a list for 1:1 randomisation to fluconazole or matching placebo in random permuted blocks of size 40. Randomization was not stratified by site. Trial drug was packaged and labelled by an independent clinician and pharmacist. Participants were allocated to sequential trial numbers on enrolment and received the corresponding sealed trial drug pack. Blinded samples of each batch of trial drug were assessed at the University of Liverpool, UK for consistency with trial drug labelling.

Trial procedures

Eligible and consenting participants were enrolled and received either 200 mg of fluconazole or matching placebo (manufactured by Cipla, India) three times a week. Participants were seen 4 weeks after enrolment and then 8-weekly for follow up. New clinical symptoms or signs were assessed and intercurrent illnesses treated. Pill counts and adherence to trial medication were assessed at routine visits to encourage adherence. LFTs were performed 8-weekly and CD4 counts measured every 16 weeks. A serum sample was stored at each follow-up for subsequent CrAg testing. Women underwent a pregnancy test, were counselled about avoiding pregnancy and offered contraception at every appointment. Patients were offered co-trimoxazole prophylaxis (480mg daily) according to national guidelines. Participants were encouraged to attend the clinic or contact the trial team if they felt unwell between routine visits and if necessary were hospitalised under the care of the trial team. Field workers attempted to contact non-attendees at home if a routine appointment was missed.

Suspected cryptococcal cases were investigated with a serum CrAg test, chest X-ray, blood cultures (BACTEC 9120 blood culture system, Becton Dickinson) and lumbar puncture (routine CSF microscopy, India ink microscopy, glucose analysis, and CSF culture at 37°C for 14 days on Sabouraud dextrose agar). Invasive cryptococcal disease was defined as symptoms of cryptococcal disease with a serum CrAg titre >1:8 on two occasions, or a positive CSF CrAg or *Cryptococcus neoformans* grown from blood or CSF culture.

Participants who developed cryptococcal disease were treated with amphotericin (0.8-1mg/kg/day iv) for 14 days followed by fluconazole 400mg daily for 8 weeks, and secondary fluconazole prophylaxis (200mg daily). Oral and vaginal candidiasis was diagnosed by culture and treated with topical nystatin or clotrimazole; refractory cases received oral ketoconazole (200mg-400mg daily for 5 days). Oesophageal candidiasis was diagnosed by dysphagia with a positive oro-pharyngeal culture, and treated with fluconazole 200mg daily for 2 weeks; trial drug was suspended during this period.

The trial was designed prior to the availability of ART in Uganda. In the first year of the trial free of charge ART became widely available and trial protocols were modified. At the initial

four week follow up, LFTs were performed to exclude side effects of trial medication and participants given a referral letter to their preferred ART care provider documenting CD4 count, full blood count, LFTs and medical problems. ART providers had a minimum six week client preparation time for ART initiation. Participants entered this pathway at the four week point so as not to delay ART initiation. ART regimens (of two nucleoside reverse transcriptase inhibitors and one non-nucleoside reverse transcriptase inhibitor) were chosen and monitored by providers independent of the trial team. .

Participants continued trial medication until the end of the trial (minimum 12 weeks) or until their CD4 count reached 200 cells/ μ L when they were no longer considered to be at risk of cryptococcal disease. Trial drug was also stopped if transaminases exceeded 5x ULN, if there was an adverse event considered to be related to trial drug, if women became pregnant, if participants wanted to leave the trial or if they moved away from study area. Once trial drug was stopped, participants were reviewed at the clinic every six months.

Deaths and potential episodes of cryptococcal disease were retrospectively reviewed by an independent end point review committee (EPRC). A verbal autopsy was performed on those who died outside hospital and the most recently stored serum was tested for CrAg. The (EPRC) had access to participants' files, hospital notes, verbal autopsy data and retrospective CrAg results, but were blind to treatment group.

Endpoints

The two co-primary endpoints were time to first episode of invasive cryptococcal disease and all cause mortality. All cause mortality was redefined as a primary endpoint (previously a secondary endpoint) in place of mortality from cryptococcal disease in 2006, when it became clear that both the number of cryptococcal events and the case fatality rate was lower than anticipated (after enrolment of 796). Secondary efficacy endpoints were: time to first episode of oesophageal candidiasis, time to first episode of oro-pharyngeal or vaginal candidiasis, time to first hospital admission or death, incidence of candidiasis (allowing for multiple episodes) and incidence of hospital admission (allowing for multiple admissions). The primary safety end point was stopping trial drug due to elevated transaminases above 5x ULN or other major adverse event.

Statistical Analysis

The original sample size of 590 participants was based on an annual rate of invasive cryptococcal disease of 10.3% and had 80% power to detect a 75% reduction in the incidence of cryptococcal disease at the 5% significance level. As ART became available in Uganda, the sample size was re-estimated to account for the reduction in cryptococcal incidence in participants who started ART. Recruitment of 770 participants in each arm (to contribute 530 person years of observation (PYO)) was estimated to give 80% power to detect a reduction of 75% in cryptococcal disease at the 5% level (22 cryptococcal events).

Analyses were carried out on an intention to treat basis and included all enrolled participants. Subjects were considered to be at risk of an event until they experienced the event, stopped taking trial drug due to their CD4 count reaching 200, died or the trial ended. Subjects who stopped trial drug due to an adverse event or pregnancy were considered at

risk until trial end. Participants lost to follow-up or who withdrew were considered at risk until the last time seen. Primary outcomes were analysed using survival analysis. Kaplan-Meier survival curves were used to illustrate the time to event in the two treatment arms; a log rank test was used to determine whether distributions differed between arms. A further log-rank test stratified the exposure time by ART status. Cox regression models were fitted with terms for baseline CD4 (categorized as <50, 50-99, 100-149 or 150-199 cells/ μ L for mortality and as <50 or 50-199 cells/ μ L for cryptococcal events (due to small numbers) and for ART status as a time-varying covariate. A formal test examining a potential ART by treatment arm interaction was carried out for all cause mortality; there were too few events to do this for the cryptococcal primary endpoint. A Bonferroni correction was applied to adjust for multiple significance testing and a 2.5% significance level used for the two primary endpoints. Similar methods were used for analysis of secondary outcomes. To assess the incidence of any episode of candidiasis or hospital admissions, survival analysis methods were adapted to allow for multiple events within participants as described by Cleves²⁸

Role of the funding source

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Results

Study Population

5337 participants were screened and 1519 enrolled. 759 participants received placebo and 760 received fluconazole (Figure 1). There were no baseline differences between the groups (Table 1). More women were enrolled than men, consistent with the usual pattern of HIV care seeking in Uganda²⁹.

Participants were at risk in the primary analysis for a median of 30 weeks (IQR 25-53) on placebo and 30 weeks (IQR 25-54) on fluconazole. The median total follow up was 60 weeks (IQR 28-123) on placebo and 59 weeks (IQR 27-124) on fluconazole. 751 (49.4%) participants stopped trial drug because their CD4 count reached 200 cells/ μ L; 407 (26.8%) stopped at the end of the trial; 54 (3.5%) stopped due to pregnancy; 50 (3.3%) were lost to follow-up and 15 (1.0%) withdrew consent. All other participants stopped because of a cryptococcal or safety endpoint. 1298 participants (85.4%) started ART (641 on fluconazole and 657 on placebo) at a median time of 11 weeks (IQR 7-17 weeks) after enrolment, of whom 1063 (81.9%) received a regimen containing nevirapine. The median time to ART was 82 and 87 days for the fluconazole and placebo arms respectively.

Cryptococcal disease

Eighteen participants taking placebo and one taking fluconazole developed cryptococcal disease (table 2). The risk was significantly higher in the placebo than in the fluconazole arm (log-rank $X_2 = 15.27$, $P < 0.001$) (figure 2). The hazard ratio for developing cryptococcal

disease on placebo compared to fluconazole was 18.74 (95% CI 2.5-140.7), adjusting for baseline CD4 count and whether or not the participant was on ART. Fluconazole reduced cryptococcal events both before and after starting ART (table 3). Before ART, there were 11 cryptococcal events on placebo and one event on fluconazole. After starting ART, seven participants on placebo but none on fluconazole developed cryptococcal disease. No cryptococcal events occurred in participants who stopped taking trial drug when their CD4 count reached 200. The overall rate of cryptococcal events was 2.80 per 100 PYO in the placebo arm and 0.15 per 100 PYO in the fluconazole arm (Table 3). On average 44.6 patients would require fluconazole prophylaxis to prevent one case of cryptococcal disease. 13 (68.4%) of those developing cryptococcal disease had a CD4 count <50 cells/ μ L at the time of diagnosis, 4 (21.0%) had a CD4 count between 50-99 cells/ μ L, and the remaining 2 (10.5%) had CD4 counts of 124 and 139 cells/ μ L. Cryptococcal infection occurred predominantly in patients with WHO stage 3 (12 [63.2%]) or 4 (4 [21.0%]) at baseline. The NNT was 22.8 for those with a baseline CD4 count < 100 cells/ μ L (two events missed) and 44.1 for baseline WHO stage 3 and 4 together. Positive cryptococcal cultures, including the one in the fluconazole arm, were all sensitive to fluconazole.

Survival

Fluconazole had no effect on survival; 93 subjects on placebo and 96 on fluconazole died ($P=0.82$ on log rank test). The hazard ratio for death in placebo vs. fluconazole groups was 0.96, adjusting for baseline CD4 count and ART status as a time dependent covariate. The end point review committee judged cryptococcal disease to be the definite cause of death in seven participants and a possible cause of death in one; all were on placebo. 9 deaths (5 placebo, 4 fluconazole) occurred after participants had stopped trial drug. Including these deaths in the survival analysis changed the adjusted hazard ratio from 0.96 to 0.97.

Secondary endpoints

Fluconazole significantly reduced the incidence rate for the first episode of oesophageal candidiasis, oral and vaginal candidiasis ($P<0.001$) (table 3). The effect of fluconazole was greater before ART (Table 3). Overall, 63 participants developed 74 episodes of oesophageal candidiasis; 66 episodes in 55 participants on placebo and 8 episodes in 8 participants on fluconazole. The incidence rate for first occurrence of oesophageal candidiasis in the placebo arm dropped from 21.3 per 100 PYO before ART to 2.39 per 100 PYO after ART, but remained relatively constant in the fluconazole arm (table 3). Fluconazole also reduced oral and vaginal candidiasis ($P<0.001$) with a stronger effect before ART (table 3). The effect of fluconazole on oral candidiasis alone was greater before ART than after ART, although still statistically significant after initiating ART. The effect of fluconazole on vaginal candidiasis alone was similar before and after ART. The incidence of hospital admission or death did not differ between the two arms.

Safety

59 participants on placebo and 59 on fluconazole stopped trial drug because of safety concerns (Table 4). 115 participants had transaminases above 5x ULN (57 placebo, 58 fluconazole) and three had Stevens-Johnson syndrome (2 placebo, 1 fluconazole). Taking nevirapine as ART did not increase the risk of hepatotoxicity. In those on nevirapine, 27/522

(5.2%) and 34/541 (6.3%) taking fluconazole and placebo respectively stopped trial drug due to elevated transaminases. 54 women became pregnant; they were reviewed by an independent physician during pregnancy and infants by a paediatrician. There was no evidence of excess miscarriage (7/32 on placebo, 6/22 on fluconazole, $P=0.65$), stillbirth (0/13 vs. 1/8 live births, Fishers Exact $P=0.38$) or of fluconazole related abnormalities in live born babies. Severe (grade 4) anaemia did not differ between arms. Mild side effects attributed by the study physicians to the trial drug (including headache, nausea, and abdominal pain) were experienced by 259 participants (136 placebo, 123 on fluconazole). The loss to follow up (LTFUP) and withdrawal rates were higher in the fluconazole arm than in the placebo arm (table 4). Withdrawal occurred at a median of 83 days (IQR 26-174) and LTFUP at a median of 138 days (IQR 84-195); there was no difference between the arms in the timing of ART or the proportion who commenced ART. 26/50 LTFUP were subsequently located and known to be alive: a chance imbalance in movement from the trial area (10/31 LTFUP on fluconazole, 3/19 LTFUP on placebo) was a major contributor to the difference.

Discussion

This trial showed fluconazole to be highly effective and safe in preventing invasive cryptococcal disease with a protective effect that occurred both before starting ART and in the first months of ART. The overall degree of protection (aHR 18.7) was much greater than that seen in the only other large RCT of azole prophylaxis⁹, but despite this, fluconazole prophylaxis had no effect on survival. The incidence of cryptococcal disease and number of cryptococcal events was lower than anticipated when the trial was designed; the rapid roll out of ART in Uganda was unexpected. Patients were randomised with CD4 counts below 200 cells/ μ L in the expectation that CD4 counts would drop during the trial. However, most patients started ART within three months of enrolment, reducing the time at risk of cryptococcal disease considerably; 774 (51.0%) patients never had a CD4 count below 100 cells/ μ L. The Trial Steering Group considered this issue during the trial but felt that reducing the CD4 entry criteria in the middle of the trial was not appropriate. In addition, randomising patients with a positive baseline CrAg was considered unethical; the study therefore excluded those with incipient cryptococcal disease or those at highest risk of developing cryptococcal disease.

The low incidence of cryptococcal disease and relatively low case fatality rate (7/19, 37%) due to intensive surveillance and rapid initiation of treatment meant that although there was a strong effect on cryptococcal specific mortality (0 vs. seven deaths), there was no impact on all cause mortality. In fact, only one Thai study (90 patients) has demonstrated a survival advantage in HIV infected patients from azole prophylaxis (HR 4.3, 95% confidence limits (0.9, 19.8) $p=0.065$); although there was a trend towards a reduction in cryptococcal disease with fluconazole, only two of the nine deaths on placebo were attributed to cryptococcal disease²⁰.

Fluconazole was safe in routine use: the incidence of grade 3 or 4 hepatic enzyme elevation was similar in the two arms. We found no evidence of hepatotoxicity when fluconazole was

coadministered with nevirapine, in keeping with other studies³¹. The safety of fluconazole at this prophylactic dose means that it could be used in clinics without laboratory support.

The trial population was representative of a rural sub-Saharan African setting and the findings were robust with similar baseline findings and ART treatment in each arm. Higher rates of cryptococcal disease have been described previously in Africa and case fatality rates reach 60% even when ART is available^{1–3}. Both the proportion of patients accessing ART and the speed of access to ART were unusual in our study. In routine practice in Sub-Saharan Africa where ART access is often more limited or other continents with limited access, the benefits of fluconazole prophylaxis may be even greater. Cryptococcal events occurred no later than three months after ART initiation suggesting the need for a limited duration of prophylaxis once on ART. The reduction of oesophageal, oral and vaginal candidiasis is an additional benefit.

The results of this trial have considerable policy implications. Less than half those needing ART in sub-Saharan Africa currently access ART and low CD4 counts at presentation are common^{32, 33}. Delays of several weeks before ART initiation often occur and are compounded by stock-outs^{34, 35}. Up to 20% of early mortality on ART is due to cryptococcal disease in sub-Saharan Africa¹. In this context, fluconazole prophylaxis “buys time” for the patient to start ART and then protect against cryptococcal disease until immune reconstitution occurs. Fluconazole prophylaxis is therefore of enormous potential benefit for individuals who are a) unable to access ART, b) waiting for ART or c) CrAg negative patients with low CD4 counts (<100 cells/ μ L) in the early stages of ART.

Studies have shown that a positive screening CrAg predicts a high risk of cryptococcal disease and mortality when starting ART^{36 37}. In South Africa, no-one with a negative CrAg measured shortly before ART initiation developed cryptococcal disease³⁷; CrAg screening at ART initiation may be cost effective.³⁸ Fluconazole primary prophylaxis is a complementary strategy. CrAg positivity identifies those at highest risk, but we have shown that CrAg negative patients may develop cryptococcal disease when there is a delay between CrAg screening and ART initiation. Cambodian data modelling suggested that screening was more cost-effective than prophylaxis if the CD4 count was higher than 50 cells/ μ L³⁹ We believe that the relative benefit of screening or prophylaxis for those with CD4 counts <100 predominantly depends upon the delay before ART initiation.

Overall, our results provide substantial evidence to support current WHO recommendations that “where cryptococcal disease is common, antifungal prophylaxis with azoles should be considered for severely immuno-compromised people with HIV (WHO clinical stage 4 or CD4 < 100 cells/ μ L), whether on antiretroviral therapy or not.”⁴⁰, although our data suggest that WHO clinical stage 3 should be included. Fluconazole is a safe, well tolerated intervention that could be given in the community, improving quality of life by reducing candida infections and preventing cryptococcal disease in those waiting to access ART or in the early phase of ART.

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Panel: Research in context

Systematic review. A Cochrane Review has examined azole primary prophylaxis against cryptococcal disease. The five studies comprised 1500 patients in total and included two small studies (219 patients) from Thailand, but none from Africa. Azole prophylaxis reduced the incidence of cryptococcal disease (RR 0.21) but did not affect mortality.

Interpretation. This study is the first to investigate the role of primary prophylaxis in Africa where the burden of cryptococcal disease is greatest and the first to include a large proportion of patients commencing antiretroviral therapy. Unlike previous studies, only those who were CrAg negative were included in the study. Fluconazole was highly effective in reducing the risk of cryptococcal disease both before and after initiation of ART. Recent data suggest that there is benefit in CrAg screening and treatment of CrAg positivity prior to ART. This study shows that fluconazole primary prophylaxis is a complementary strategy that can prevent the development of cryptococcal disease in those waiting for ART or those with low CD4 counts in the early stages of ART.

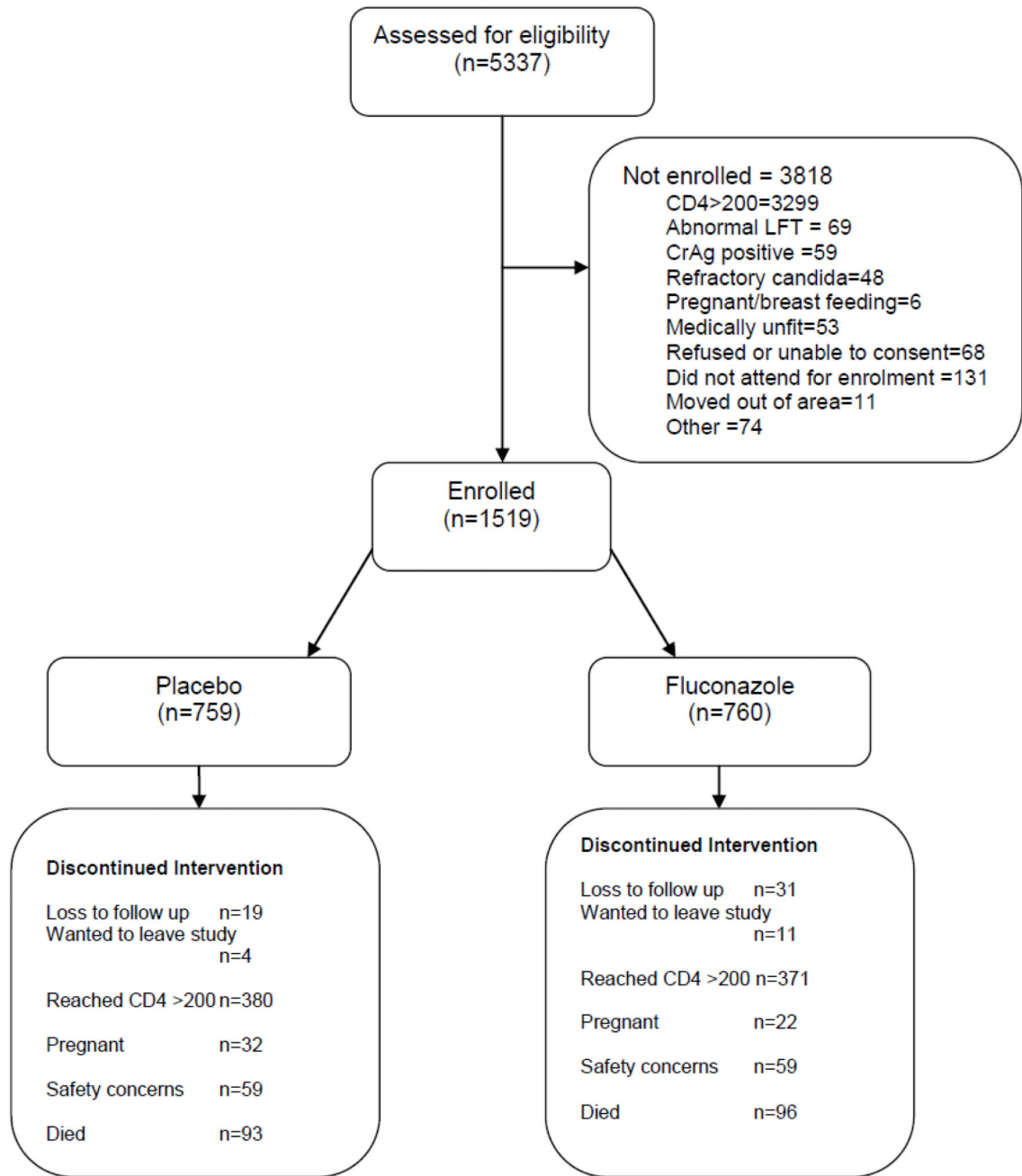


Figure 1. Trial Profile

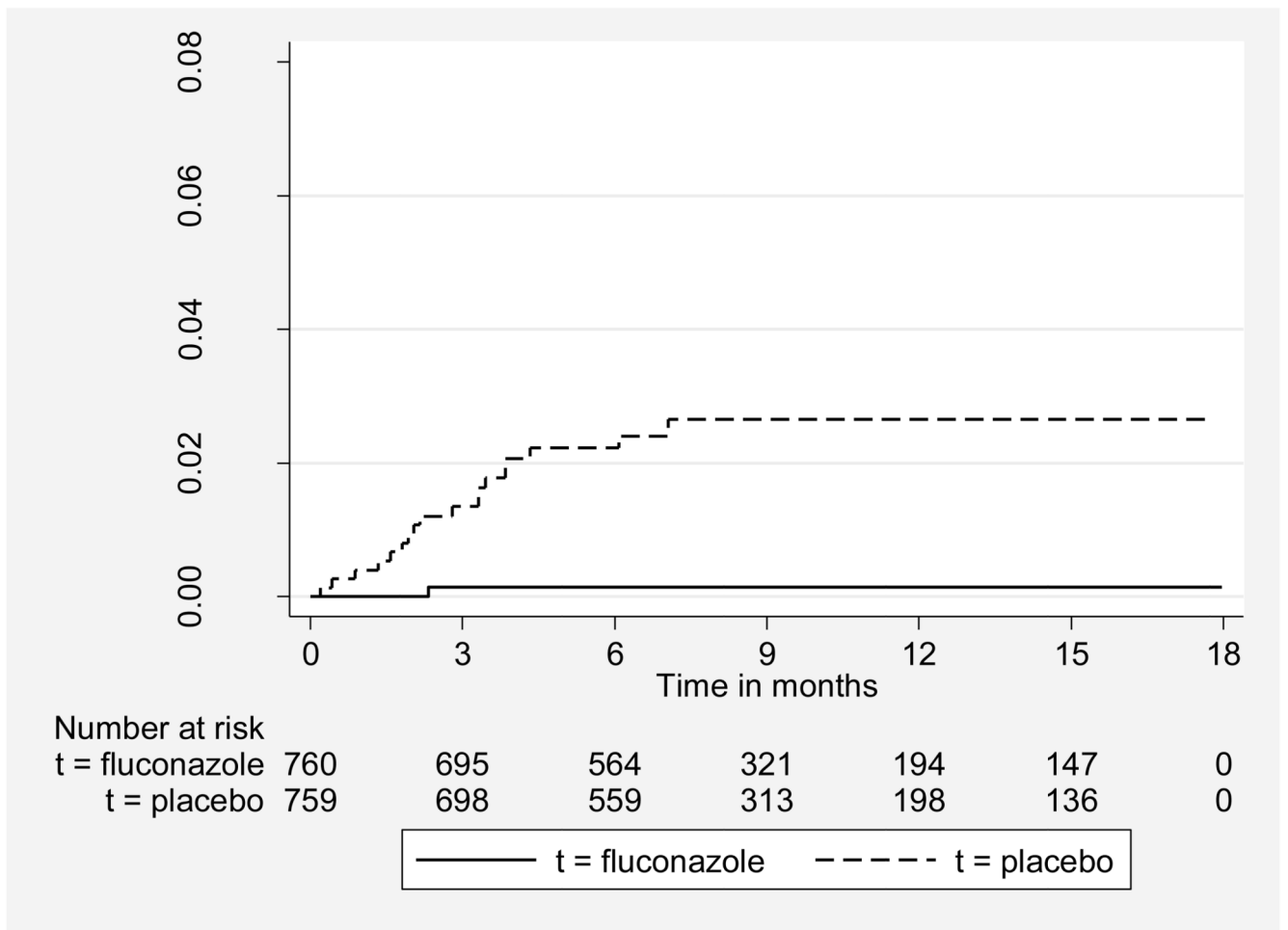


Figure 2. Incidence of cryptococcal disease by treatment arm

Table 1
Baseline Characteristics

Variable		Placebo (n=759)	Fluconazole (n=760)	Overall (n=1519)
Sex	Male n (%)	251 (33.1%)	286 (37.6%)	537 (35.4%)
	Female n (%)	508 (66.9%)	474 (62.4%)	982 (64.6%)
Age (years Mean (s.d.))		35.8 (8.8)	35.9 (9.1)	35.8 (9.0)
Age (grouped)	<25	47 (6.2%)	58 (7.6%)	105 (6.9%)
	25-34	323 (42.6%)	306 (40.3%)	629 (41.4%)
	35-44	270 (35.6%)	269 (35.4%)	539 (35.5%)
	45 +	119 (15.7%)	127 (16.7%)	246 (16.2%)
CD4 count (median (IQR))		112 (48 – 157)	110 (45 – 160)	111 (46 – 159)
CD4 count (grouped)				
	150 – 199	231 (30.4%)	237 (31.2%)	468 (30.8%)
	100 – 149	185 (24.4%)	168 (22.1%)	353 (23.2%)
	50 – 99	150 (19.8%)	152 (20.0%)	302 (19.9%)
	1 - 49	193 (25.4%)	203 (26.7%)	396 (26.1%)
WHO stage	1	20 (2.6%)	18 (2.4%)	38 (2.5%)
	2	164 (21.6%)	175 (23.0%)	339 (22.3%)
	3	524 (69.0%)	506 (66.6%)	1030 (67.8%)
	4	51 (6.7%)	61 (8.0%)	112 (7.4%)

Table 2

Diagnosis of Cryptococcal Events

Age	Sex	Time to Event [§] (days)	Time on ART (days)	CD4 count [‡] (cells/µl)	Serum CrAg titre (at diagnosis)	Blood Culture	CSF CrAg	CSF culture	Died within four weeks
1*	M	71	--	14	1:512	<i>C. neoformans</i>	pos	<i>C. neoformans</i>	N
2	M	215	78	18	neg	<i>C. neoformans</i>	neg	neg	Y
3	F	117	56	139	1:32 [^]	neg	neg	neg	N
4	M	132	39	30	1:128	neg	pos	neg	N
5	M	105	7	124	1:1024	<i>C. neoformans</i>	pos	<i>C. neoformans</i>	N
6	M	101	3	31	1:1024	<i>C. neoformans</i>	pos	<i>C. neoformans</i>	N
7	F	117	--	14	1:128	<i>C. neoformans</i>	neg	neg	N
8	F	27	--	3	1:1024	<i>C. neoformans</i>	pos	<i>C. neoformans</i>	N
9	F	62	--	2	1:1024	N/A	N/A	<i>C. neoformans</i>	Y
10	M	59	--	78	1:512	neg	pos	<i>C. neoformans</i>	N
11	F	85	13	66	1:16 [^]	neg	neg	neg	N
12	M	66	--	27	1:512	<i>C. neoformans</i>	pos	<i>C. neoformans</i>	N
13	M	185	--	76	1:2048	<i>C. neoformans</i>	pos	<i>C. neoformans</i>	N
14	F	6	--	70	1:256	<i>C. neoformans</i>	pos	<i>C. neoformans</i>	N
15	M	101	38	7	1:128	neg	pos	neg	N
16	M	55	--	26	1:512	<i>C. neoformans</i>	pos	<i>C. neoformans</i>	N
17	M	48	--	8	1:512 [^]	neg	N/A	N/A	Y
18	F	41	--	8	1:512	<i>C. neoformans</i>	pos	<i>C. neoformans</i>	N
19	F	13	--	15	1:8	<i>C. neoformans</i>	pos	neg	N

[§]Time from study enrolment to cryptococcal event (second CrAg for those diagnosed on CrAg alone)

[^]Diagnosed on serum CrAg alone

[‡]CD4 count at study enrolment

*On fluconazole

N/A- not available

neg=negative

pos=positive

Table 3

	Overall																
	Placebo					Fluconazole											
	Events	Rate / 100 PYO	Events	Rate / 100 PYO	Unadjusted log rank χ^2 (P-value)	aHR ¹ (95% C.I.)	Heterogeneity LR χ^2 (P-value)	Events	Rate / 100 PYO	aHR ² (95% C.I.)	Events	Rate / 100 PYO	aHR ² (95% C.I.)				
Primary outcomes																	
Cryptococcal disease	18	2.8	1	0.15	15.27 (P=0.001)	18.74 (2.5 – 140.7)	Non-estimable	11	5.14	1	0.48	11.0 (1.4- 85.3)	7	1.63	0	0	∞^* (1.45- ∞)
Deaths on trial drug	93	14.1	96	14.5	0.05 (P=0.82)	0.96 (0.72 – 1.27)	0.55 (P=0.46)										
Secondary outcomes																	
First episode of oesophageal candidiasis	55	8.7	6	0.91	40.76 (P<0.001)	9.4 (4.0-21.8)	5.44 (P=0.02)	45	21.3	2	0.96	22.2 (5.4- 91.7)	10	2.39	4	0.89	2.9 (0.91-9.3)
First episode of oropharyngeal or vaginal candidiasis	159	29.5	24	3.7	116.64 (P<0.001)	7.4 (4.8 - 11.4)	5.40 (P=0.02)	119	61.4	11	5.3	11.4 (6.1- 21.1)	40	11.6	13	3	4.0 (2.1-7.4)
Hospital admission or death	235	44.3	229	42.5	0.18 (P=0.67)	1.05(0.87 - 1.26)	1.04 (P=0.31)										
Incidence of hospital admissions	284	43.7	278	42.6	0.12 (P=0.73)	1.04 (0.88-1.22)	0.34 (P=0.56)										
Incidence of all candidiasis	272	41.2	29	4.4	198.6 (P<0.001)	9.6 (6.5-14.1)	7.10 (P=0.008)	187	85.7	12	5.7	14.8 (8.2- 26.5)	85	19	17	3.8	5.3 (3.1-8.9)

¹ Hazard ratio adjusted for baseline CD4 group and for before or after starting ART as time varying covariate.

² Hazard ratio adjusted for baseline CD4 group

* Exact CI for rate ratio has been used as HR cannot be estimated since there were no cryptococcal events on the fluconazole arm after starting ART.

Table 4
Safety, toxicity and loss to follow up

Outcome		Placebo n=759		Fluconazole n=760		Unadjusted log rank P-value	aHR ^I (95% CI)
		Event	rate	Events	rate		
Withdrawal of trial drug	Due to an adverse event	59	9.9	59	9.6	0.00 (P=0.99)	1.04 (0.72 – 1.49)
	Due to LFT<5ULN	57	9.6	58	9.5	0.01 (P=0.93)	1.02 (0.70-1.47)
	Due to other AE	2	0.33	1	0.16	0.32 (P=0.57)	1.99 (0.18-22.2)
	Pregnancy	32	7.8	22	5.4	1.83 (P=0.18)	1.44 (0.84-2.49)
Serious adverse events	Life threatening	53(49)	8.29	49(42)	7.62	0.16 (P=0.69)	1.02 (0.69-1.51)
	Anaemia (Grade 4)	49	10.0	62	12.8	1.38 (P=0.24)	0.79 (0.54 - 1.15)
	Events resulting in disability	9	1.38	5	0.76	1.17 (P=0.28)	1.88 (0.63-5.65)
Reported side effects	Nausea	38	6.11	35	5.57	0.11 (P=0.74)	1.09 (0.69 – 1.72)
	Headache	9	1.39	10	1.53	0.06 (P=0.81)	0.89 (0.36 – 2.19)
	Abdominal pain	25	3.91	22	3.42	0.21 (P=0.65)	1.13 (0.64 – 2.01)
	Rash	27	4.27	17	2.64	2.36 (P=0.12)	1.59 (0.86 – 2.91)
	Other	49	8.01	46	7.41	0.10 (P=0.75)	1.07 (0.72 – 1.61)
	Participants reporting SEs	136	25.35	123	22.12	0.83 (P=0.36)	1.12 (0.88 – 1.43)
Loss to follow-up		19	3.30	31	5.15	2.43 (P=0.12)	0.60 (0.34 – 1.07)
Withdrawal		4	0.69	11	1.83	3.15 (P=0.076)	0.36 (0.12 – 1.14)

^I Hazard ratio adjusted for baseline CD4 group and for before or after starting ART as time varying covariate.