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

Rosalind Parkes-Ratanshi, Katie Wakeham, Jonathan Levin, Deodata Namusoke, James Whitworth, Alex Coutinho, Nathan Kenya Mugisha, Heiner Grosskurth, Anatoli Kamali, David G Lalloo, on behalf of the Cryptococcal Trial Team

Summary

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. We studied fluconazole as primary prophylaxis against cryptococcal disease in patients awaiting or starting antiretroviral therapy in Uganda.

Methods In this prospective, double-blind randomised controlled trial, we enrolled HIV-positive adults with CD4 counts less than 200 cells per µL, cryptococcal antigen (CrAg)-negative, naive for antiretroviral therapy, and coming from five local AIDS organisations in Masaka district, Uganda. Enrolment took place between Sept 14, 2004, and Feb 1, 2008. Participants were randomly allocated to placebo or 200 mg fluconazole three times per week (1:1) in blocks of 40. Randomisation was done with ralloc procedure in Stata. Participants were reviewed after 4 weeks and referred for antiretroviral therapy, then seen every 8 weeks. Participants discontinued trial treatment when CD4 counts reached 200 cells per µL (median 197 days). Primary endpoints were invasive cryptococcal disease and all-cause mortality. Secondary endpoints were time to first episode and incidence of oesophageal candidosis, time to first episode and incidence of oropharyngeal or vaginal candidosis, and time to first hospital admission or death. The primary safety endpoint was cessation of trial drug because of transaminase concentrations higher than five times the upper limit of normal (ULN), or other major adverse events. Analyses were done by intention to treat and included all participants enrolled in the trial. Participants and researchers were masked to group assignment. This trial is registered with controlled-trials.com, number ISRCTN 76481529.

Results Of 1519 individuals enrolled, 760 participants received fluconazole and 759 received placebo. 19 developed cryptococcal disease, one in the fluconazole group and 18 in the placebo group (p=0·0001); adjusted HR (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 lower than 200 cells per µL. Fluconazole was effective against cryptococcal disease both before (aHR=11·0 [1·4–85·3]) and after start of antiretroviral therapy (no cases in fluconazole *vs* seven cases on placebo). Seven participants died from cryptococcal disease, none in the fluconazole group. All-cause mortality (n=189) did not differ between the two groups (p=0·46). Fluconazole reduced the time to first episode of oesophageal, and oropharyngeal and vaginal candidosis, as well as the incidence of all candidosis (p<0·0001), but had no effect on hospital admission or death. The frequency of elevated transaminases (>5×ULN) was similar between groups (aHR=0·94 [0·65–1·35]).

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

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Medical Research Council (MRC)/Uganda Virus Research Institute (UVRI) Uganda Research Unit on AIDS, UVRI, Entebbe, Uganda (R Parkes-Ratanshi PhD, K Wakeham MRCP, Prof J Levin PhD, Prof H Grosskurth PhD, A Kamali MSc); **Liverpool School of Tropical Medicine, Liverpool, UK** (R Parkes-Ratanshi, K Wakeham, Prof D G Lalloo FRCP); **Department of Infectious Diseases, Imperial College of Science and Technology, London, UK** (R Parkes-Ratanshi); **University of Witwatersrand, Johannesburg, South Africa** (Prof J Levin); **The AIDS Support Organisation (TASO), Masaka, Uganda** (D Namusoke MA, A Coutinho MPH); **Wellcome Trust, London, UK** (J Whitworth PhD); **Infectious Diseases Institute, Kampala, Uganda** (A Coutinho); **Ministry of Health, Kampala, Uganda** (N K Mugisha MPH); **and London School of Hygiene and Tropical Medicine, London, UK** (Prof H Grosskurth)

Correspondence to: Dr Rosalind Parkes-Ratanshi, C/o MRC Unit on AIDS in Uganda, PO Box 49, Entebbe, Uganda **r.parkes@imperial.ac.uk**

Background

Cryptococcal disease is one of the most common CNS infections in individuals with HIV. Infection is acquired by inhalation of environmental spores or desiccated yeast cells: clinical disease might not occur for months to years after exposure and might be preceded by asymptomatic cryptococcal antigenaemia.[1] The disease is particularly problematic in sub-Saharan Africa, where the incidence in severe immunosuppression can reach 10% yearly and it can cause up to 17% of deaths in individuals with HIV.[1,2] Untreated, the mortality is 100% for those with HIV and, even with optimum treatment, about 30% of individuals die.[3,4] Survivors often have severe disabilities.[5] 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]

Before the availability of antiretroviral therapy in the USA, results from a randomised controlled trial of primary prophylaxis with fluconazole showed a reduction in the incidence of cryptococcal disease but no effect on mortality.[9] Other studies[10–18] suggested benefit from

routine prophylaxis with itraconazole or fluconazole, but most were small and retrospective. A Cochrane review[19] 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 world.[19] Only two studies have been done in developing countries,[20,21] both in Thailand and with less than 10% of participants receiving antiretroviral therapy. Results from one of these studies[20] suggested that fluconazole prophylaxis reduced invasive fungal infections and mortality, but had no effect on cryptococcal events; results from the other[21] showed a reduction in systemic fungal diseases (including cryptococcosis) with itraconazole but no survival advantage.[21]

The incidence of cryptococcal disease has recently declined in industrialised countries, predominantly because of antiretroviral therapy.[22–24] International initiatives led to progress in the provision of antiretroviral therapy in resource-poor countries, but only 30% of those who need this therapy in sub-Saharan Africa are receiving it.[25] Cryptococcal disease remains common in sub-Saharan Africa, both in those who are yet to start and those who are in the first months of antiretroviral therapy.[26,27]

Primary prophylaxis against cryptococcal disease has never been formally tested in Africa or any setting in which effective antiretroviral therapy is available. We aimed to examine the efficacy and safety of fluconazole as primary prophylaxis against cryptococcal disease before initiation and in the first few months of antiretroviral therapy.

Methods

Study design and participants

In this double-blind, randomised, placebo-controlled trial, we recruited participants between Sept 14, 2004, and Feb 1, 2008, from five local HIV/AIDS organisations in Masaka and Kalangala districts, Uganda: 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 Ssese islands in Lake Victoria.

We screened potential participants for eligibility at a dedicated study clinic sited at TASO; from April, 2006, we also screened and enrolled participants at Ministry of Health clinics on the Ssese islands. Adults naive for antiretroviral therapy with laboratory confirmation of HIV infection (Murex HIV-1.2.0, Murex Biotech, Dartford, UK; Vironostika HIV Uni-form II plus O, Biomerieux, Marcy l'Etoile, France; Cambridge Biotech HIV-1 Western blot, Maxim Biomedical In, Rockville, USA) and a CD4 count of less than 200 cells per µL (FACSCount Becton Dickinson, USA) were eligible for the study. We tested for cryptococcal antigen (CrAg; Remel, Lexana, USA; dilution first done to exclude pronase effect) and excluded participants with a serum titre of CrAg higher than 1/8; it was deemed unethical to randomise these patients. Other exclusion criteria were pregnancy or lactation, concentrations of liver transaminases (liver-function test) more than three times the upper limit of normal (ULN), and moribund patients. Participants with oral and vaginal candidosis at screening were treated with topical clotrimazole or nystatin, or if refractory, ketoconazole (200–400 mg daily for 5 days); symptomatic oesophageal candidosis was treated with fluconazole (minimum 14 days) and enrolment delayed for 4 weeks.

Information about 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. Ethics approval was gained from the Uganda National Council for Science and Technology and the Ethics Committees of Uganda Virus Research Institute (UVRI), Uganda, and the Liverpool School of Tropical Medicine, UK. 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 per µL.

Randomisation and masking

An independent statistician prepared a list for randomisation to fluconazole or matching placebo (1:1) in random permuted blocks of size 40. Randomisation 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 from a trial nurse. Blinded samples of trial drug were assessed at the University of Liverpool, UK, for consistency with trial drug labelling. Participants, trial medical staff, data management team, and endpoint committee were masked to group assignment.

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 every 8 weeks 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. Liver-function tests were done every 8 weeks and CD4 counts measured every 16 weeks. A serum sample was stored at every follow-up for subsequent CrAg testing. Women were tested for pregnancy, counselled about avoiding pregnancy, and offered contraception at every appointment. Patients were offered co-trimoxazole (trimethoprim–sulfamethoxazole) prophylaxis (480 mg 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 admitted to hospital 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 radiograph, blood cultures (BACTEC 9120 blood culture system, Becton Dickinson, Franklin Lakes, USA) and lumbar puncture (routine cerebrospinal fluid [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 higher than 1/8 on two occasions, or a CSF positive for CrAg, or *Cryptococcus neoformans* grown from blood or CSF culture.

Participants who developed cryptococcal disease were treated with amphotericin (0·8–1·0 mg/kg daily, intravenously) for 14 days followed by fluconazole 400 mg daily for 8 weeks, and secondary fluconazole prophylaxis (200 mg daily). Oral and vaginal candidosis was diagnosed by culture and treated with topical nystatin or clotrimazole; refractory cases received oral ketoconazole (200–400 mg daily for 5 days). Oesophageal candidosis was diagnosed by dysphagia with a positive oropharyngeal culture, and treated with fluconazole 200 mg daily for 2 weeks; trial drug was suspended during this period.

The trial was designed before the availability of antiretroviral therapy in Uganda. In the first year of the trial, antiretroviral therapy free of charge became widely available and trial protocols were modified. At the initial 4-week follow-up, patients' liver function was tested to exclude side-effects of trial drug, and participants were given a referral letter to their preferred care provider of antiretroviral treatment, which documented CD4 count, full blood count, liver-function tests, and medical problems. Providers of antiretroviral therapy had a minimum client preparation time for initiation of therapy of 6 weeks. Participants entered this pathway at the 4-week point to not delay initiation of antiretroviral therapy. Providers independent of the trial team chose and monitored regimens of antiretroviral therapy (two nucleoside reverse transcriptase inhibitors and one non-nucleoside reverse transcriptase inhibitor).

Participants continued taking the trial drug until the end of the trial (minimum 12 weeks) or until their CD4 count reached 200 cells per µL, at which point they were deemed to be no longer at risk of cryptococcal disease. Trial drug was also stopped if transaminases exceeded five times the ULN, if an adverse event deemed to be related to trial drug occurred, 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 6 months.

Deaths and potential episodes of cryptococcal disease were retrospectively reviewed by an independent endpoint review committee. Those who died outside hospital had a verbal autopsy, and their most recently

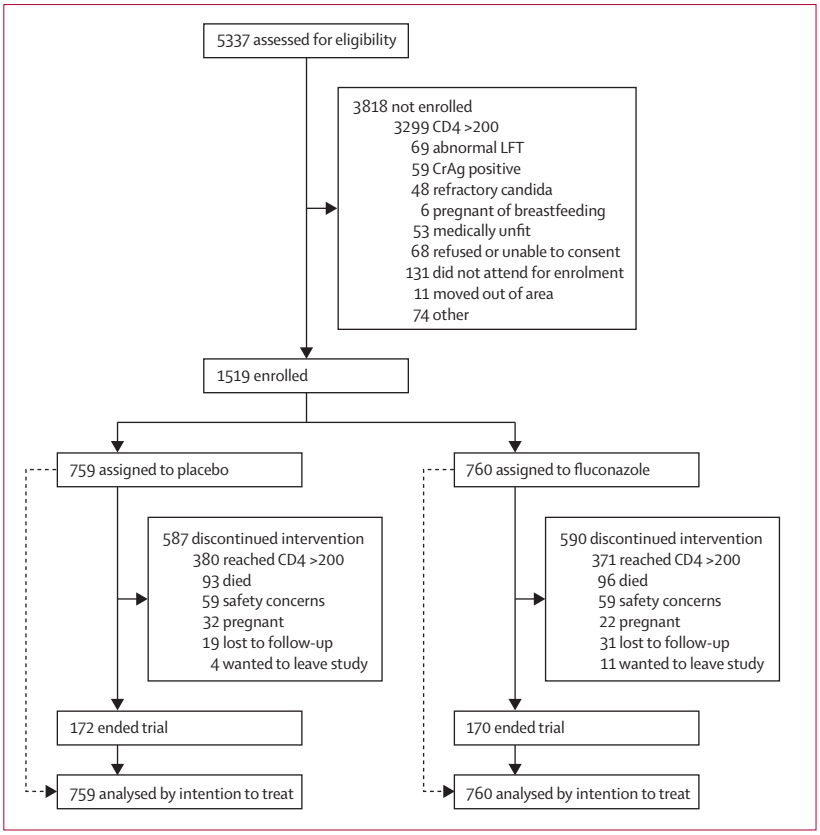

***Figure 1*: Trial profile**

	Placebo (n=759)	Fluconazole (n=760)
Sex		
Male	251 (33%)	286 (38%)
Female	508 (67%)	474 (62%)
Age (years, mean [SD])	35·8 (8·8)	35·9 (9·1)
Age group (years)		
<25	47 (6%)	58 (8%)
25–34	323 (43%)	306 (40%)
35–44	270 (36%)	269 (35%)
>45	119 (16%)	127 (17%)
CD4 count (median (IQR))	112 (48–157)	110 (45–160)
CD4 count (grouped)		
150–199	231 (30%)	237 (31%)
100–149	185 (24%)	168 (22%)
50–99	150 (20%)	152 (20%)
1–49	193 (25%)	203 (27%)
WHO stage		
1	20 (3%)	18 (2%)
2	164 (22%)	175 (23%)
3	524 (69%)	506 (67%)
4	51 (7%)	61 (8%)

Data are n (%) unless otherwise stated. Percentages might not total 100 because of rounding.

***Table 1*: Baseline characteristics**

	Age (years)	Sex	Time to event (days)*	Time on ART (days)	CD4 count (cells per μL)†	Serum CrAg titre (at diagnosis)	Blood culture	CSF CrAg	CSF culture	Died within 4 weeks
1‡	32	M	71	..	14	1/512	*C neoformans*	Positive	*C neoformans*	No
2	31	M	215	78	18	Negative	*C neoformans*	Negative	Negative	Yes
3	36	F	117	56	139	1/32§	Negative	Negative	Negative	No
4	35	M	132	39	30	1/128	Negative	Positive	Negative	No
5	38	M	105	7	124	1/1024	*C neoformans*	Positive	*C neoformans*	No
6	46	M	101	3	31	1/1024	*C neoformans*	Positive	*C neoformans*	No
7	33	F	117	..	14	1/128	*C neoformans*	Negative	Negative	No
8	30	F	27	..	3	1/1024	*C neoformans*	Positive	*C neoformans*	No
9	35	F	62	..	2	1/1024	NA	NA	*C neoformans*	Yes
10	33	M	59	..	78	1/512	Negative	Positive	*C neoformans*	No
11	58	F	85	13	66	1/16§	Negative	Negative	Negative	No
12	52	M	66	..	27	1/512	*C neoformans*	Positive	*C neoformans*	No
13	34	M	185	..	76	1/2048	*C neoformans*	Positive	*C neoformans*	No
14	31	F	6	..	70	1/256	*C neoformans*	Positive	*C neoformans*	No
15	39	M	101	38	7	1/128	Negative	Positive	Negative	No
16	37	M	55	..	26	1/512	*C neoformans*	Positive	*C neoformans*	No
17	32	M	48	..	8	1/512§	Negative	NA	NA	Yes
18	38	F	41	..	8	1/512	*C neoformans*	Positive	*C neoformans*	No
19	28	F	13	..	15	1/8	*C neoformans*	Positive	Negative	No

ART=antiretroviral therapy. CrAg=cryptococcal antigen. CSF=cerebrospinal fluid. NA=not available. M=male. F=female. *Time from trial enrolment to cryptococcal event (second CrAg for those diagnosed on CrAg alone). †CD4 count at study enrolment. ‡On fluconazole. §Diagnosed on serum CrAg alone.

Table 2: **Diagnosis of cryptococcal events**

stored serum was tested for CrAg. The endpoint review committee had access to participants' files, hospital notes, verbal autopsy data, and retrospective CrAg results, but were masked 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 participants). Secondary efficacy endpoints were: time to first episode of oesophageal candidosis, time to first episode of oropharyngeal or vaginal candidosis, time to first hospital admission or death, incidence of candidosis (allowing for multiple episodes), and incidence of hospital admission (allowing for multiple admissions). The primary safety endpoint was cessation of trial drug because of high concentration of transaminases (>5×ULN) or other major adverse event.

Statistical Analysis

The original sample size of 590 participants was based on an annual incidence 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. Because antiretroviral therapy became available in Uganda, the sample size was re-estimated to account for the reduction in cryptococcal incidence in participants who started the therapy. Recruitment of 770 participants in each group (to contribute 530 person-years of observation) was estimated to give 80% power to detect a reduction of 75% in cryptococcal disease at the 5% level (22 cryptococcal events).

We analysed all endpoints by intention to treat including all enrolled participants. Participants were deemed to be at risk of an event until they had the event, stopped taking trial drug because of their CD4 count reaching 200 cells per μL, died, or the trial ended. Those who stopped the trial drug because of an adverse event or pregnancy were deemed at risk until end of trial. Participants lost to follow-up or who withdrew were deemed at risk until the last time seen. We analysed the primary outcomes using survival analysis. We used Kaplan-Meier survival curves to show the time to event in the two treatment groups and a log-rank test to see whether distributions differed between groups. A further log-rank test stratified the exposure time by status of antiretroviral therapy. We fitted Cox regression models with terms for baseline concentrations of CD4 cells (categorised as <50, 50–99, 100–149, or 150–199 cells per μL for mortality, and as <50 or 50–199 cells per μL for cryptococcal events due to small numbers) and for status of antiretroviral therapy as a time-varying covariate. We did a formal test examining a potential interaction of

	Overall							Before antiretroviral therapy					On antiretroviral therapy				
	Placebo		Fluconazole					Placebo		Fluconazole			Placebo		Fluconazole		
	Events	Rate (per 100 PYO)	Events	Rate (per 100 PYO)	Unadjusted log-rank χ^2 (p value)	aHR* (95% CI)	Hetero-geneity log-rank χ^2 (p value)	Events	Rate (per 100 PYO)	Events	Rate (per 100 PYO)	aHR† (95% CI)	Events	Rate (per 100 PYO)	Events	Rate (per 100 PYO)	aHR† (95% CI)
Primary outcomes																	
Cryptococcal disease	18	2·8	1	0·15	15·3 (p=0·0001)	18·7 (2·5–140·7)	Non-estimable	11	5·1	1	0·5	11·0 (1·4–85·3)	7	1·6	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	40·8 (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	2·4	4	0·89	2·9 (0·91–9·3)
First episode of oropharyngeal or vaginal candidosis	159	29·5	24	3·7	116·6 (p<0·0001)	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	0·2 (p=0·67)	1·05 (0·87–1·26)	1·04 (p=0·31)	..	..	..	..	..	..	..	..	..	..
Incidence of hospital admissions	284	43·7	278	42·6	0·1 (p=0·73)	1·0 (0·9–1·2)	0·34 (p=0·56)	..	..	..	..	..	..	..	..	..	..
Incidence of all candidosis	272	41·2	29	4·4	198·6 (p<0·0001)	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)

PYO=person-years of observation. aHR=adjusted HR. *HR adjusted for baseline CD4 group and for before or after starting antiretroviral therapy as time varying covariate. †HR adjusted for baseline CD4 group. ‡Exact CI for rate ratio was used since HR could not be estimated because no cryptococcal events occurred on the fluconazole group after starting antiretroviral therapy.

Table 3: **Efficacy outcomes**

antiretroviral therapy by treatment group for all-cause mortality; too few events occurred to do this analysis for the cryptococcal primary endpoint. We applied a Bonferroni correction to adjust for multiple significance testing and used a 2·5% significance level for the two primary endpoints. We used similar methods to analyse the secondary endpoints. To assess the incidence of any episode of candidosis or hospital admissions, we adapted survival analysis methods to allow for multiple events within participants, as described by Cleves.[28]

Role of the funding source

The sponsors of the study did not have a role in design, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of 5337 participants screened, 759 were randomly assigned to placebo and 760 to fluconazole (figure 1). The baseline characteristics of the two groups were well balanced (table 1). More women than men were enrolled, which is consistent with the usual pattern of HIV-care seeking in Uganda.[29] Participants were at risk in the primary analysis for a median of 30 weeks (IQR 25–53) on placebo and 30 weeks (25–54) on fluconazole. The median total follow-up was 60 weeks (28–123) on placebo and 59 weeks (27–124) on fluconazole. About half of participants stopped trial drug because their CD4 count reached 200 cells per µL (figure 1), a quarter 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 (figure 1). 1298 participants (85%) started antiretroviral therapy (641 given fluconazole and 657 given placebo) at a median time of 11 weeks (IQR 7–17) after enrolment, of whom 1063 (82%) received a regimen containing nevirapine. The median time to antiretroviral therapy was 82 days for the fluconazole group and 87 days for the placebo group.

18 participants given placebo and one given fluconazole developed cryptococcal disease (table 2). The risk of developing the disease was significantly higher in the placebo group than in the fluconazole group (log-rank χ^2=15·3, p=0·0001) (table 3, figure 2). The HR for development of cryptococcal disease on placebo compared with that on fluconazole was 18·7 (95% CI 2·5–140·7), adjusting for baseline CD4 count and whether or not the participant was on antiretroviral therapy. Fluconazole reduced cryptococcal events both before and after start of antiretroviral therapy (table 3). No cryptococcal events occurred in participants who stopped taking trial drug when their CD4 count reached 200 cells per µL. The overall rate of cryptococcal events was higher in the placebo group than in the fluconazole group (table 3). On average, 44·6 patients would require fluconazole prophylaxis to prevent one case of cryptococcal disease.

Of the 19 developing cryptococcal disease, 13 (68%) had a CD4 count of less than 50 cells per µL at the time of diagnosis, four (21%) had a CD4 count of 50–99 cells per µL, and the remaining two (11%) had CD4 counts of 124 cells per µL and 139 cells per µL (table 1). Cryptococcal infection occurred predominantly in patients with WHO stage 3 (12 [63%]) or 4 (four [21%]) at baseline. The number needed to treat was 22·8 for those with a baseline CD4 count of less than 100 cells per µL (two events missed) and 44·1 for baseline WHO stage 3 and 4 together. Positive cryptococcal cultures, including the one in the fluconazole group, were all sensitive to fluconazole.

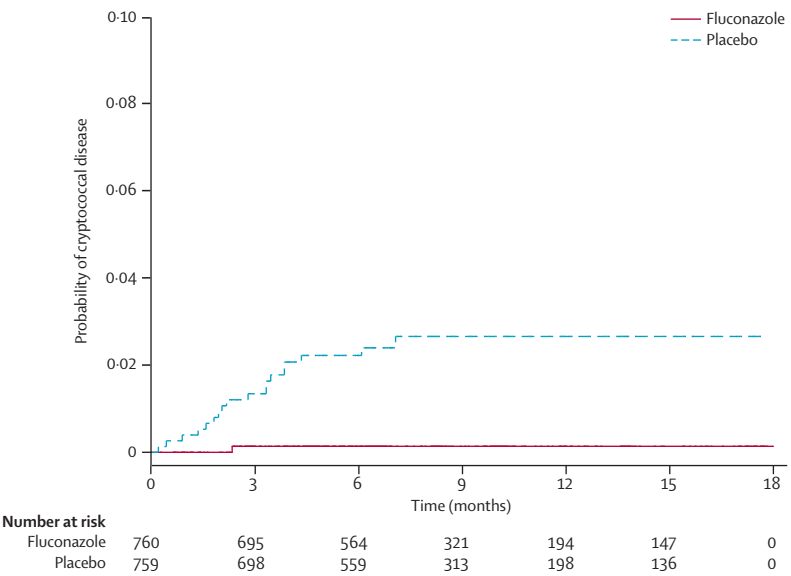

Figure 2: **Incidence of cryptococcal disease by treatment group**

	Placebo (n=759)		Fluconazole (n=760)		Unadjusted log rank (p value)	aHR* (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>5×ULN	57	9·6	58	9·5	0·01 (p=0·93)	1·02 (0·70–1·47)
Due to other adverse event	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 at least one side-effect	136	25·35	123	22·12	0·83 (p=0·36)	1·12 (0·88–1·43)
Other						
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)

aHR=adjusted HR. LFT=liver-function test. ULN=upper limit of normal. *Adjusted for baseline CD4 group and for before or after start of antiretroviral therapy as time varying covariate. †Rates are per 100 person-years of observation. ‡Numbers of patients are indicated in brackets.

Table 4: **Safety, toxicity, and loss to follow-up**

Fluconazole had no effect on survival (table 3). The HR for death in the placebo group versus that in fluconazole group was close to 1, adjusting for baseline CD4 count and ART status as a time dependent covariate (table 3). The endpoint 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 in the placebo group. Nine deaths (five in the placebo group, four in the fluconazole group) occurred after participants had stopped taking the trial drug. Including these deaths in the survival analysis changed the adjusted HR from 0·96 to 0·97.

Fluconazole significantly reduced the incidence for the first episode of all types of candidosis (p<0·0001; table 3). The effect of fluconazole was greater before than after antiretroviral therapy (table 3). Overall, 63 participants developed 74 episodes of oesophageal candidiasis: 66 episodes in 55 participants given placebo and eight episodes in eight participants given fluconazole. The incidence for first occurrence of oesophageal candidosis in the placebo group dropped from 21·3 per 100 person-years of observation before antiretroviral therapy to 2·39 per 100 person-years of observation after the therapy, but remained constant in the fluconazole group (table 3). Fluconazole also reduced oral and vaginal candidosis (p<0·0001) with a stronger effect before therapy (table 3) than after therapy. The effect of fluconazole on oral candidosis alone was also greater before than after antiretroviral therapy, although still significant after initiation of the treatment (data not shown). The effect of fluconazole on vaginal candidosis alone was similar before and after therapy (data not shown). The incidence of hospital admission or death did not differ between the two groups (table 3).

59 participants on placebo and 59 on fluconazole stopped trial drug because of safety concerns (table 4). 115 participants had transaminases more than five-times the ULN (57 in the placebo group, 58 in the fluconazole group) and three had Stevens-Johnson syndrome (two placebo, one fluconazole). The use of nevirapine as antiretroviral therapy did not increase the risk of hepatotoxic effects. In those given nevirapine, 27 (5%) of 522 in the fluconazole group and 34 (6%) of 541 in the placebo group stopped trial drug because of elevated transaminases. 54 women became pregnant; they were reviewed by an independent doctor during pregnancy and infants were reviewed by a paediatrician. There was no evidence of excess miscarriage (seven of 32 in the placebo group, six of 22 in the fluconazole group, p=0·65), stillbirth (none of 13 live births in the placebo group *vs* one of eight in the

fluconazole group, Fishers exact p=0·38), or fluconazole-related abnormalities in live born babies. Severe (grade 4) anaemia did not differ between groups. Mild side-effects attributed by the study physicians to the trial drug (including headache, nausea, and abdominal pain) were experienced by 259 participants (136 given placebo and 123 given fluconazole).

Loss to follow-up and withdrawal events were more frequent in the fluconazole group than in the placebo group (table 4). Withdrawal occurred at a median of 83 days (IQR 26–174) and loss to follow-up at a median of 138 days (IQR 84–195); the timing of antiretroviral therapy or the proportion of participants who started the therapy did not differ between groups. 26 of 50 participants lost to follow-up were subsequently located and known to be alive: a chance imbalance in movement from the trial area (ten of 31 in the fluconazole group, three of 19 in the placebo group) was a major contributor to the difference.

Discussion

This trial showed fluconazole to be highly effective and safe in the prevention of invasive cryptococcal disease with a protective effect that occurred both before the start and in the first months of antiretroviral therapy. The overall degree of protection was much greater than that seen in the only other large randomised trial of azole prophylaxis (panel),[9] but, despite this result, fluconazole prophylaxis had no effect on survival. The incidence of cryptococcal disease and number of cryptococcal events was lower than that predicted when the trial was designed; the rapid roll out of antiretroviral therapy in Uganda was unexpected. Patients were enrolled with CD4 counts of less than 200 cells per µL in the expectation that CD4 counts would drop during the trial. However, most patients started antiretroviral therapy within 3 months of enrolment, which reduced substantially the time at risk of cryptococcal disease; 774 (51%) of 1519 patients never had a CD4 count less than 100 cells per µL. The trial steering group considered this issue during the trial but felt that reduction of the CD4 entry criteria in the middle of the trial was not appropriate. Additionally, randomisation of patients with a positive CrAg at baseline was deemed unethical. The study, therefore, excluded participants with incipient cryptococcal disease or those at highest risk of developing the disease.

The low incidence of cryptococcal disease and low case fatality rate (seven [37%] of 19) due to intensive surveillance and rapid initiation of treatment meant that, although a strong effect was recorded on cryptococcal-specific mortality (none *vs* seven deaths), no effect was noted on all-cause mortality. In fact, only results from one study in Thailand[20] (of 90 patients) have shown a survival advantage in HIV-infected patients from azole prophylaxis (HR 4·3 [95% CI 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 in the placebo group were attributed to cryptococcal disease.[20]

Fluconazole was safe in routine use: the incidence of hepatic enzyme elevation of grade 3 or 4 was similar in the two groups. We found no evidence of hepatoxic effects when fluconazole was given with nevirapine, in keeping with other studies.[32] 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 antiretroviral treatment in each group. Higher rates of cryptococcal disease than those seen in this trial have been described previously in Africa and case-fatality rates reach 60% even when antiretroviral therapy is available.[1–3] Both the proportion of patients accessing the therapy and the speed of its access were unusual in our study. In routine practice in sub-Saharan Africa, where access to antiretroviral therapy is often restricted, or in other continents with limited access, the benefits of fluconazole prophylaxis might be even greater. Cryptococcal events occurred no later than 3 months after initiation of antiretroviral therapy suggesting the need for a restricted duration of prophylaxis once prophylaxis is started. The reduction of oesophageal, oral, and vaginal candidosis is an additional benefit.

The results of this trial have substantial policy implications. Less than half of people needing antiretroviral therapy in sub-Saharan Africa currently access the treatment, and low CD4 counts at presentation are common.[33,34] Initiation of treatment is often delayed by several weeks because of stock-outs.[35,36] Up to 20% of early mortality on antiretroviral therapy is due to cryptococcal disease in sub-Saharan Africa.[1] In this context,

Panel: Research in context

Systematic review

We searched PubMed using combinations of the terms "cryptococcus", "cryptococcal disease", "cryptococcal meningitis", "azole", and "prophylaxis". A Cochrane Review[19] has examined azole primary prophylaxis against cryptococcal disease. The five studies included 1500 patients in total and two small studies (219 patients) from Thailand, but none from Africa. Azole prophylaxis reduced the incidence of cryptococcal disease (relative risk 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 is also the first to include a large proportion of patients commencing antiretroviral therapy. Unlike previous studies, only individuals who were CrAg negative were included in the trial. Fluconazole was highly effective in reducing the risk of cryptococcal disease both before and after initiation of antiretroviral therapy. Recent data[30,31] suggest that there is benefit in CrAg screening and treatment of CrAg positivity before antiretroviral therapy. Results from this study show that fluconazole primary prophylaxis is a complementary strategy that can prevent the development of cryptococcal disease in those waiting for antiretroviral therapy or in those with low CD4 counts in the early stages of the treatment.

fluconazole prophylaxis buys time for the patient to start treatment and protects against cryptococcal disease until immune reconstitution occurs. Fluconazole prophylaxis is therefore of enormous potential benefit for individuals who are unable to access or who are waiting for antiretroviral therapy, or for those negative for CrAg with low CD4 counts (<100 cells per µL) in the early stages of therapy.

Results from studies[30,31] have shown that a positive screening for CrAg predicts a high risk of cryptococcal disease and mortality at the start of antiretroviral therapy. In one study,[31] no-one with a negative CrAg measured shortly before initiation of therapy developed cryptococcal disease; CrAg screening at initiation of therapy may be cost effective.[37] Primary prophylaxis with fluconazole is a complementary strategy. CrAg positivity identifies those at highest risk, but we have shown that patients negative for CrAg might develop cryptococcal disease when there is a delay between CrAg screening and initiation of antiretroviral therapy. Modelling of data from Cambodia[38] suggested that screening was more cost effective than prophylaxis if the CD4 count was higher than 50 cells per µL. We believe that the relative benefit of screening or prophylaxis for those with CD4 counts of more than 100 cells per µL predominantly depends on the delay before initiation of antiretroviral therapy.

Overall, our results provide substantial evidence to support present WHO recommendations that "in areas where cryptococcal disease is common, antifungal prophylaxis with azoles should be considered for severely immunocompromised people with HIV (WHO clinical stage 4 or CD4 <100 cells per µL), whether on antiretroviral therapy or not."[39] However, 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 reduction of candida infections and prevention of cryptococcal disease in patients waiting to access or in the early phase of antiretroviral therapy.

Contributors

RP-R, KW, AK, DGL, and HG participated in the trial design, data analysis, data interpretation, and writing of the report. JL participated in the data analysis, data interpretation, and writing of the report. RP-R and DGL participated in the literature search. JL and RP-R participated in the design of the figures. RP-R and KW participated in the data collection. JW, AC, NKM, and DN participated in the trial design and writing of the report. The Cryptococcal Trial Team included Freddie Kibengo, David Katende, Ivan Namakoola, Abu-Baker Ggayi, Jane Margaret Amony, Christine Nagawa, Christine Musoke, Victor Nanono, Doreen Bamukama, Victoria Nabbona, Jane Muwanga, Stella Nakate, Grace Katooko, Rose Tindebywa, Stephen Nkayivu, Eunice Kajura, Baker Kiyemba, Sebastian Kazibwe, Chadress Kabagenyi, Lubega Dennis, Anita Namagende, Jane Taban, and Mike Mukasa. The data and safety monitoring committee consisted of Andrew Nunn (chair), Silver Bahendeka, and Rod Hay. The trial steering committee consisted of Tim Peto (chair), David Denning, Elly Katabira, and Jonathan Mermin. The endpoint review committee consisted of Alison M Elliott and Martin Nsubuga.

Conflicts of interest

We declare that we have no conflicts of interest.

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