Outcomes of flucytosine-containing combination treatment for cryptococcal meningitis in a South African national access programme: a cross-sectional observational study

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Summary

Background Although flucytosine is a key component of WHO-recommended induction treatment for HIV-associated cryptococcal meningitis, this antifungal agent is not widely available in low-income and middle-income countries due to limited production and cost. In 2018, a national flucytosine access programme was initiated in South Africa. We aimed to determine the effectiveness of flucytosine-containing induction regimens in routine care to motivate for the urgent registration of flucytosine and its inclusion in treatment guidelines.

Methods In this cross-sectional study, we compared outcomes of adults aged 18 years and older with incident laboratory-confirmed cryptococcal meningitis treated with or without flucytosine-containing regimens at 19 sentinel hospitals in South Africa. A case of cryptococcosis was defined as illness in an adult with: (1) positive cerebrospinal fluid (CSF) India ink microscopy; (2) a positive CSF cryptococcal antigen test; or (3) culture of *Cryptococcus neoformans* or *Cryptococcus gattii* from CSF or any other specimen. We excluded patients without a case report form, those with an unknown or negative HIV serology result, those with a recurrent episode, and those who did not receive antifungal treatment in hospital. We assessed cumulative in-hospital mortality at 14 days and 30 days and calculated the overall crude in-hospital case-fatality ratio. We used random-effects logistic regression to examine the association between treatment group and in-hospital mortality.

Findings From July 1, 2018, to March 31, 2020, 10 668 individuals were diagnosed with laboratory-confirmed cryptococcal meningitis, 7787 cases diagnosed at non-enhanced surveillance sites and 567 cases from eight enhanced surveillance sites with no access to flucytosine were excluded. Of 2314 adults with a first episode of cryptococcosis diagnosed at 19 facilities with access to flucytosine, 1996 had a case report form and of these, 1539 received induction antifungal treatment and were confirmed HIV-seropositive first-episode cases. Of 1539 patients who received antifungal therapy, 596 (38·7%) individuals received a flucytosine-containing regimen and 943 (61·3%) received another regimen. The median age was 36 years (IQR 32–43) and 906 (58·9%) participants were male and 633 (41·1%) were female. The crude in-hospital case-fatality ratio was 23·9% (95% CI 20·0–27·0; 143 of 596) in those treated with flucytosine-containing regimens and 37·2% (95% CI 34·0–40·0; 351 of 943) in those treated with other regimens. Patients admitted to non-academic hospitals (adjusted odds ratio [aOR] 1·95 [95% CI 1·53–2·48]; p<0·0001) and those who were antiretroviral treatment-experienced (aOR 1·30 [1·02–1·67]; p=0·033) were more likely to receive flucytosine. After adjusting for relevant confounders, flucytosine treatment was associated with a 53% reduction in mortality (aOR 0·47 [95% CI 0·35–0·64]; p<0·0001). Among survivors, the median length of hospital admission in the flucytosine group was 11 days (IQR 8–15) versus 17 days (13–21) in the comparison group (p=0·0010).

Interpretation In-hospital mortality among patients treated with a flucytosine-containing regimen was comparable to reduced mortality reported in patients receiving a flucytosine-containing regimen in a recent multicentre African clinical trial. Flucytosine-based treatment can be delivered in routine care in a middle-income country with a substantial survival benefit.

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Introduction

Cryptococcal meningitis is a major cause of death among people living with HIV in low-income and middle-income countries, with up to 75% mortality in routine hospital care.[1] Globally, cryptococcal meningitis was estimated to affect 223 100 people annually in 2014, resulting in 181 100 deaths.[1] Sub-Saharan Africa has the highest burden of HIV-associated cryptococcal meningitis, estimated at 162 500 annual cases (73% of the total) with 135 900 deaths.[1] Although the incidence of cryptococcal meningitis has declined in high-income countries with close-to-universal antiretroviral treatment

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For the Zulu translation of the abstract see Online for appendix 1

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Panel: Research in context

Evidence before the study

HIV-associated cryptococcal meningitis affects 223 100 people and causes approximately 181 100 deaths globally on an annual basis, with the highest mortality in sub-Saharan Africa. Data from Botswana indicates that 2 weeks of amphotericin B plus fluconazole, the current South African standard induction treatment for cryptococcal meningitis, is associated with a 2-week mortality of 26% and 10-week mortality of 50%. However, WHO recommends flucytosine as a key component of any cryptococcal meningitis induction regimen. This recommendation is based on the results of the Advancing Cryptococcal Treatment in Africa (ACTA) trial which found that flucytosine was superior to fluconazole in combination with amphotericin B, and that 1 week of amphotericin B plus flucytosine followed by 1 week of fluconazole was associated with the lowest 10-week mortality (24%) of five tested regimens. We searched Google Scholar and PubMed with no language restrictions to identify relevant publications from Jan 1, 2008, to July 31, 2021, using the search terms "flucytosine" AND "cryptococcal meningitis" AND "treatment" AND "HIV" AND "Africa". Although randomised controlled trials are the gold standard to establish the efficacy of treatment regimens and several clinical trials done in low-resource settings in sub-Saharan Africa have evaluated the efficacy of flucytosine-containing regimens, no effectiveness data have been published to support these findings. We used a cross-sectional study design nested within established surveillance for cryptococcal meningitis in an upper-middle-income country, to determine the effectiveness of flucytosine-containing regimen used in routine care.

Added value of this study

This multicentre observational study with a large sample size allowed us to determine the effectiveness of flucytosine-containing induction regimens in routine health-care settings in South Africa among inpatients with cryptococcal meningitis and advanced HIV disease. The in-hospital mortality among patients treated with flucytosine was significantly lower than that observed among those treated with other regimens and similar to the mortality reported by recent clinical trials evaluating flucytosine-containing regimens. The WHO-recommended regimens can be delivered in routine care in a resource-limited country with a substantial survival benefit. The addition of flucytosine also has the potential to reduce the length of hospital stay, with cost benefits. We expect that these results would be broadly generalisable to urban health-care facilities in sub-Saharan Africa.

Implications of all the available evidence

Currently, only a minority of patients with cryptococcal meningitis in Africa are treated with flucytosine, thus urgent action is needed to facilitate registration by regulatory authorities, reduce costs, increase the availability of generic products, and train clinicians to use flucytosine as part of an induction regimen.

(ART) access, it remains a persistent health problem in settings where HIV diagnosis is late, ART coverage is suboptimal, and particularly in sub-Saharan African countries where HIV prevalence is high. Many people remain unaware of their HIV infection status and are thus ART-naïve, and among those who start ART, interruption of therapy is a common occurrence.[2]

Antifungal treatment of HIV-associated cryptococcal meningitis in low-income and middle-income countries is complex. In 2018, WHO recommended a 1-week combination of amphotericin B deoxycholate and flucytosine for the induction phase.[3] Flucytosine, developed in 1957, is the preferred partner antifungal agent in any treatment combination, even in resource-limited settings. This preference is based on the results of the Advancing Cryptococcal Treatment in Africa (ACTA) trial, which found that a 1-week regimen of amphotericin B 1 mg/kg per day and flucytosine 100 mg/kg per day (followed by 1-week of fluconazole 1200 mg/day) had the lowest 10-week mortality of 24% (95% CI 16–32) and less toxicity than did the previous standard of 2 weeks of amphotericin B deoxycholate and flucytosine.[4] Despite its inclusion in the WHO Essential Medicines List (EML), flucytosine is manufactured at low volumes globally and is not widely available outside of high-income countries. Flucytosine was registered by the South African Health Products Regulatory Authority (SAHPRA) in December, 2021, 2 years after a dossier was submitted by Viatris (formerly Mylan).[5] However, the South African standard treatment guidelines currently recommend a 2-week induction course of amphotericin B deoxycholate and fluconazole for cryptococcal meningitis.[6] According to the results of a cost-effectiveness analysis, South Africa's National EML Committee recommended in July, 2019, that flucytosine be considered for inclusion in the national EML for cryptococcal meningitis, pending SAHPRA registration and a substantial price reduction.[7] The standard treatment guidelines are likely to be updated to include flucytosine on the basis of the recent registration, although the price of flucytosine still needs to be negotiated. Cost savings were anticipated with a short-course regimen with a length of hospital stay of 10 days or fewer. However, implementation science research is essential to confirm if flucytosine-containing regimens reduce the length of hospital stay and improve outcomes compared with other regimens in routine-care settings.

Within the final 6 months of 2018, a South African flucytosine access programme was established at selected large academic and regional hospitals.[8] We aimed to use enhanced laboratory-based surveillance data collected at

these sites to compare the in-hospital outcomes of adults with incident laboratory-confirmed cryptococcal meningitis treated with induction regimens with or without flucytosine.

Methods

Study design and participants

We did a cross-sectional study, nested within an active national laboratory-based surveillance programme (GERMS-SA) for cryptococcal meningitis. The study was done in 19 GERMS-SA enhanced surveillance sites, all of which were public-sector urban large academic and regional hospitals in South Africa. A case of cryptococcosis was defined as illness in an adult aged 18 years and older with: (1) positive cerebrospinal fluid (CSF) India ink microscopy; (2) a positive CSF cryptococcal antigen (CrAg) test; or (3) culture of *Cryptococcus neoformans* or *Cryptococcus gattii* from CSF or any other specimen. The definition included cases of disseminated disease without meningitis. We included patients with a first episode of laboratory-confirmed cryptococcosis and excluded patients without a case report form, those with an unknown or negative HIV serology result, those with a recurrent episode (based on a standardised surveillance definition of a positive cryptococcal test >30 days after the first positive test), and those recorded to not have received antifungal treatment in hospital. Participants were required to provide informed consent to receive an unregistered medicine. The access programme was initiated by Médecins Sans Frontières with formal approval from the National Department of Health and later coordinated by the Clinton Health Access Initiative. We obtained annual approvals for GERMS-SA surveillance from the human research ethics committees of the University of the Witwatersrand (1809107), University of Pretoria (19/2014), Sefako Makgatho Health Sciences University (MREC/P/44/2010), University of the Free State (UFS-HSD2018/0858), University of KwaZulu-Natal (BF130/11), University of Cape Town (115/2009), and University of Stellenbosch (N04/01/001). For cases in which surveillance data were collected prospectively through interview, participants provided written informed consent.

Procedures and outcomes

Trained nurse surveillance officers used a standardised case report form to collect demographic and clinical data such as age, sex, HIV infection status, $CD4^+$ T-cell (CD4) count at diagnosis of cryptococcal meningitis, ART use, comorbidities, severity of cryptococcal meningitis (by assessment of mental status at diagnosis), previous and in-hospital antifungal therapy, hospital admission duration, and in-hospital outcome. During the study period, we collected additional information from individuals treated with a flucytosine-containing regimen. This additional information included laboratory test results (serum haemoglobin concentrations; peripheral blood neutrophil and platelet counts; and serum alanine aminotransferase, creatinine, potassium, and magnesium concentrations) during induction treatment and clinical adverse events (gastrointestinal symptoms, phlebitis, and serious allergic reactions) during or after initiation of flucytosine-based therapy. We also recorded therapeutic lumbar punctures, potassium or magnesium supplementation, and blood transfusions during the hospital stay. Adverse events were defined according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events.[9,10]

Bulk stock of flucytosine was procured by the coordinating agency and in-country use was authorised after Section 21 application to SAHPRA.[11] Stock was delivered to a total of 36 participating facilities (including the 19 aforementioned GERMS-SA enhanced surveillance sites) in sufficient quantities for projected case numbers and stored in registered hospital pharmacies for dispensing. Senior clinicians were identified at these hospitals to supervise the prescription of flucytosine. Clinicians at participating sites were also invited to complete an online training module offered by the Southern African HIV Clinicians Society. The access programme was initiated at different times at these hospitals. Not all patients with cryptococcal meningitis at these sites received a flucytosine-containing regimen, even after the programme was launched.

The primary outcome of the study was cumulative in-hospital mortality at 14 days and 30 days. A 10-week outcome post-diagnosis was determined for individuals treated with a flucytosine-containing regimen at the relevant sites by telephone interview with the patient or their next-of-kin, as a secondary outcome. We also did an exploratory analysis to assess factors associated with being prescribed a flucytosine-containing regimen among patients who received flucytosine.

Statistical analysis

We restricted this analysis to patients admitted to the GERMS-SA enhanced surveillance sites with flucytosine access. Descriptive statistics were used to summarise demographic, clinical, and laboratory variables of all participants with laboratory-confirmed cryptococcal meningitis and the additional information for the subset of participants who received a flucytosine-containing regimen. We compared categorical variables between the two treatment groups using the χ^2 test or the Kruskal-Wallis tests, and reported unadjusted odds ratios (ORs) and 95% CIs using univariable logistic regression. To account for variations in outcomes between clusters (ie, enhanced surveillance sites), we used random-effects logistic regression analysis and simultaneously adjusted for age, sex, ART status, CD4 count at diagnosis of cryptococcal meningitis, mental status at diagnosis and tuberculosis treatment on admission when examining the association between treatment group and in-hospital

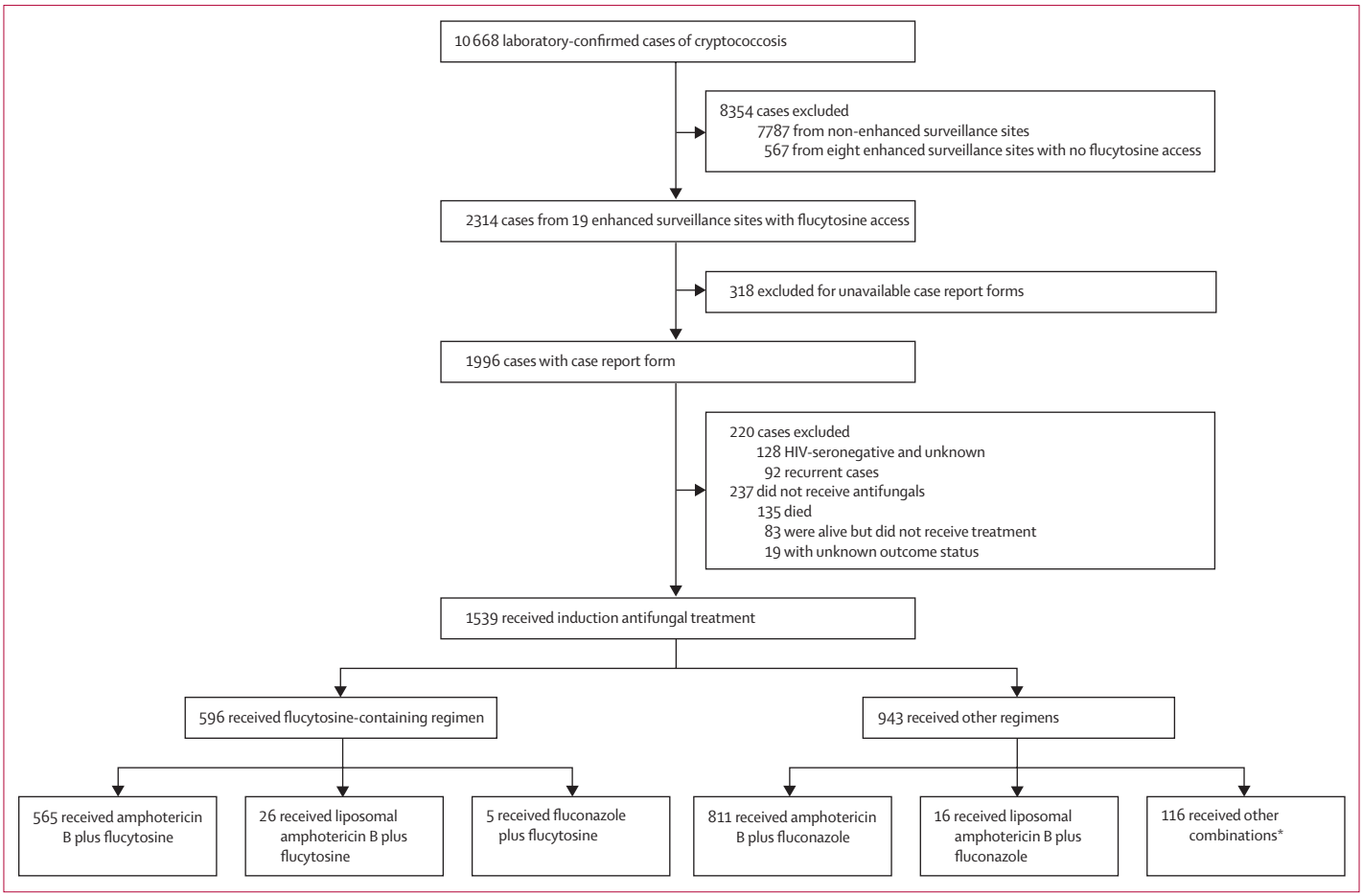

Figure 1: **Study profile**
*91 received fluconazole monotherapy and 25 received amphotericin B monotherapy.

mortality. We could not adjust for HIV viral load due to missing data. A multivariable logistic regression analysis was done to determine factors that were independently associated with receiving a flucytosine-containing regimen. We plotted the in-hospital survival experience of all participants, and differences in 30-day mortality between treatment groups were compared using a log rank test. Data analysis was done with Stata (version 15.1).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

During the study period from July 1, 2018, to March 31, 2020, 10 668 individuals aged 18 years or older were diagnosed with laboratory-confirmed cryptococcal meningitis (or disseminated culture-confirmed cryptococcosis) nationally (figure 1). We excluded 7787 cases diagnosed at non-enhanced surveillance sites and 567 cases from eight enhanced surveillance sites with no access to flucytosine. Of 2314 adults with a first episode of cryptococcosis diagnosed at the 19 facilities with access to flucytosine, 1996 (86·3%) had a case report form and of these, 1539 (77·1%) received induction antifungal treatment and were confirmed HIV-seropositive first-episode cases. Of these, 596 (38·7%) individuals received a flucytosine-containing regimen and 943 (61·3%) received another regimen. 565 patients (94·7%) who were prescribed flucytosine received 1-week of amphotericin B plus flucytosine as their induction regimen. Of the 943 treated with regimens without flucytosine, 811 (86·0%) received amphotericin B plus fluconazole, for close to the recommended 2-weeks in most cases. We excluded 237 people who did not receive antifungal treatment: 135 died before treatment was prescribed (median time from admission to death was 1 day [IQR 1–3 days]), 83 did not receive any treatment yet had a vital status recorded as alive, and 19 had an unknown in-hospital outcome.

Of 1539 cases at the 19 facilities who received antifungal treatment, the median age was 36 years (IQR 32–43) and 906 (58·9%) were male and 633 (41·1%) were female. Most of the patients were admitted to hospital at sites in

Gauteng (525 [34·1%]), KwaZulu-Natal (316 [20·5%]), and Eastern Cape provinces (308 [20·0%]). Among those with available data, 1154 (80·4%) of 1435 had a CD4 count of less than 100 cells/µl at cryptococcal meningitis diagnosis and 941 (62·0%) of 1517 were ART-experienced (table 1). Of 941 who were ART-experienced, 106 (11·3%) of 941 had started ART within a 30-day period before their cryptococcal meningitis diagnosis and 302 (32·1%) of 941 had previously taken ART but were no longer on treatment. At the time of diagnosis, 383 (27·8%) of 1377 had an altered mental status (Glasgow Coma Scale score of <15), 283 (73·7%) of 384 had a CSF opening pressure of more than 20 cm H_2O, and 415 (27·4%) of 1513 were on anti-tuberculosis treatment. During the study period, we collected additional information from individuals treated with a flucytosine-containing regimen at 19 of the 27 sites (figure 2)

Among the 596 patients who received flucytosine, we collected additional data from 386 (64·8%) and for these patients, we recorded reasons for discontinuing treatment, baseline and follow-up laboratory tests, adverse events, and additional therapeutic interventions (appendix 2 pp 3, 6, 7).

In a multivariable logistic regression analysis, ART-experienced patients had an increased adjusted odds of receiving flucytosine compared with those who were ART-naïve (adjusted [a]OR 1·30 [95% CI 1·02–1·67], p=0·033). Patients were more likely to be prescribed a flucytosine-containing regimens at a non-academic hospital (aOR 1·95 [95% CI 1·53–2·48], p<0·0001; appendix 2 p 5).

The crude in-hospital case-fatality ratio was 32·1% (95% CI 30·0–34·0; 494 of 1539). Overall, 24·0% (95% CI 20·0–27·0; 143 of 596) died among those treated with flucytosine-containing regimens and 37·2% (95% CI 34·0–40·0; 351 of 943) among those on other regimens. The crude odds of death among patients receiving a flucytosine-containing regimen was 47% lower than those treated with any other regimen (OR 0·53 [95% CI 0·42–0·67], p<0·0001; table 2). After adjusting for age, sex, ART status, CD4 count, mental status, and concurrent tuberculosis treatment at the time of diagnosis, patients receiving a flucytosine-containing regimen were 53% less likely to die (aOR 0·47 [95% CI 0·35–0·64]; p<0·0001).

14 days post-diagnosis, the in-hospital case-fatality ratio was 21·6% (91 died while on flucytosine-containing regimens and 240 on other regimens) among 1530 patients; an in-hospital outcome date was not recorded for two patients treated with flucytosine and seven patients treated with non-flucytosine regimens. Within 30 days of diagnosis, 458 of the 1530 (29·9%) had died, 133 on flucytosine-containing regimens and 325 on other regimens. A difference in mortality between people on flucytosine-containing regimens versus other regimens was observed after 2 days and the survival curves remained roughly parallel until day 30 (log rank test p-value of 0·015; figure 3). 28 patients died in hospital after 30 days, eight on the flucytosine-containing regimen and 20 on other regimens. Of 453 patients who received a flucytosine-containing regimen and were discharged from hospital, we collected information on vital status at 70 days

	Participants (n/N)	Flucytosine-containing regimens (n=596)	Other regimens (n=943)	p value
Age, years				
Median (IQR)	..	37 (32–43)	36 (31–43)	0·08
≥18–29	280/1537 (18·2%)	97 (16·3%)	183/941 (19·4%)	0·11
30–39	679/1537 (44·2%)	269 (45·1%)	410/941 (43·6%)	..
40–49	396/1537 (25·7%)	167 (28·0%)	227/941 (24·1%)	..
>49	184/1537 (12·0%)	63 (10·6%)	121/941 (12·9%)	..
Sex				
Male	906/1539 (58·9%)	341 (57·2%)	565 (59·9%)	0·29
Female	633/1539 (41·1%)	255 (42·8%)	378 (40·1%)	..
Province of sentinel site hospital				
Gauteng	525/1539 (34·1%)	255 (42·8%)	270 (28·6%)	<0·0001
KwaZulu-Natal	316/1539 (20·5%)	25 (4·2%)	291 (30·9%)	..
Eastern Cape	308/1539 (20·0%)	168 (28·2%)	140 (14·8%)	..
Limpopo	43/1539 (2·8%)	3 (0·5%)	40 (4·2%)	..
North West	131/1539 (8·5%)	56 (9·4%)	75 (8·0%)	..
Western Cape	66/1539 (4·3%)	23 (3·9%)	43 (4·6%)	..
Mpumalanga	86/1539 (5·6%)	48 (8·1%)	38 (4·0%)	..
Free State	52/1539 (3·4%)	16 (2·7%)	36 (3·8%)	..
Northern Cape	12/1539 (0·8%)	2 (0·3%)	10 (1·1%)	..
CD4$^+$ T-cell count, cells per mm^3				
<100	1154/1435 (80·4%)	438/544 (80·5%)	633/793 (79·8%)	0·76
≥100	281/1435 (19·6%)	106/544 (19·5%)	160/793 (20·2%)	..
Viral load, log_{10} RNA copies per mL				
<400–10 000	331/1015 (32·6%)	155/451 (34·4%)	176/564 (31·2%)	0·29
>10 000	684/1015 (67·4%)	296/451 (65·6%)	388/564 (68·8%)	..
Antiretroviral therapy				
Naive	576/1517 (38·0%)	215/592 (36·3%)	361/925 (39·0%)	0·013
Experienced	941/1517 (62·0%)	377/592 (63·7%)	564/925 (70·0%)	..
Glasgow Coma Scale score <15				
No	994/1377 (72·2%)	377/517 (72·9%)	617/860 (71·7%)	0·64
Yes	383/1377 (27·8%)	140/517 (27·1%)	243/860 (28·3%)	..
CSF opening pressure, cm H_20				
Median (IQR)	..	37 (29–38)	39 (27–50)	0·70
CSF opening pressure of >20 cm H_20				
No	101/384 (26·3%)	60/229 (26·2%)	41/155 (26·5%)	0·96
Yes	283/384 (73·7%)	169/229 (73·8%)	114/155 (73·5%)	..
Concurrent tuberculosis treatment				
No	1098/1513 (72·6%)	412/586 (70·3%)	686/927 (74·0%)	0·12
Yes	415/1513 (27·4%)	174/586 (29·7%)	241/927 (26·0%)	..
Headache at diagnosis				
No	179/1482 (12·1%)	52/577 (9·0%)	127/905 (14·0%)	0·0040
Yes	1303/1482 (87·9%)	525/577 (91·0%)	778/905 (86·0%)	..

Data are n (%) or n/N (%), unless otherwise specified. We compared categorical variables between the two treatment groups using the χ^2 test or the Kruskal-Wallis tests. Differing denominators were due to missing data from participants. CSF=cerebrospinal fluid.

Table 1: **Characteristics of people with cryptococcal meningitis on flucytosine-based combination treatment versus other induction regimens**

See **Online** for appendix 2

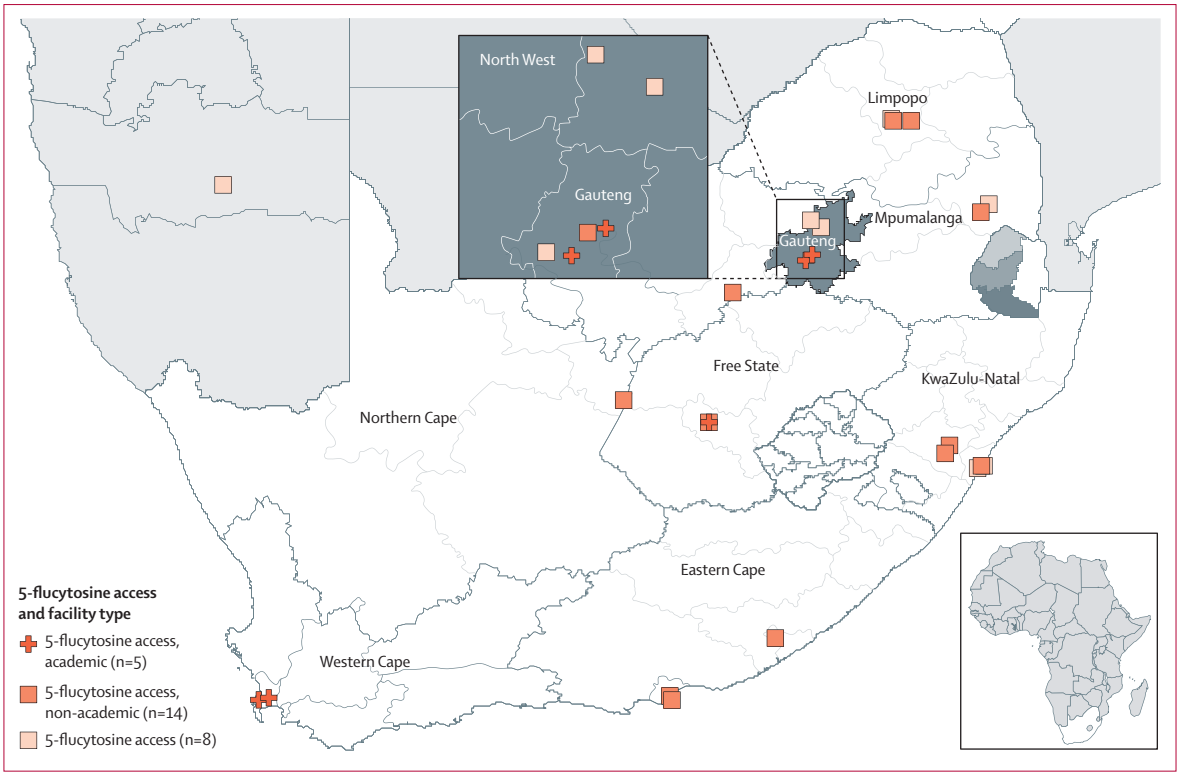

Figure 2: **GERMS-SA enhanced surveillance sites with or without access to flucytosine, by type of hospital facility, between July 1, 2018–March 31, 2020, South Africa**

(ie, 10 weeks) post-diagnosis for 267 (58·9%). 57 (21·3%) of these 267 patients treated with flucytosine died within 10 weeks of discharge from hospital.

The median length of antifungal treatment for 594 patients on the flucytosine-containing regimen (including those who died) was 8 days (IQR 7–10) versus 13 days (6–14) for 936 patients in the other treatment group (p=0·0010), reflective of the recommended treatment duration (ie, 1 week for amphotericin B plus flucytosine and 2 weeks for amphotericin B plus fluconazole). The median length of hospital admission for patients receiving a flucytosine-containing regimen was shorter than for patients on any other regimen (all patients including those who died [n=1530): 10 days [IQR 7–15] *vs* 14 days [8–18]; p=0·0010); survivors [n=1044]: 11 days [8–15] *vs* 17 days [13–21]; p=0·0010). Among those who died, the median time to death for 141 patients receiving flucytosine-containing treatment was 7 days (IQR 2–14) versus 8 days (2–16) for 345 patients on other treatment (p=0·84).

Discussion

In the context of a national access programme, we found that treatment with a flucytosine-containing regimen for HIV-associated cryptococcal meningitis was associated with a 53% reduction in the adjusted odds of in-hospital mortality compared with induction treatment without flucytosine. Patients who received a flucytosine-containing regimen also had a significantly shorter hospital stay.

The overall in-hospital case-fatality ratio of 32·1% was probably underestimated in this study because we excluded patients with cryptococcal meningitis who did not receive any antifungal treatment, including those who died before they could be started on induction treatment. Nevertheless, the overall in-hospital mortality of 23·9% among patients treated with a flucytosine-containing regimen is consistent with the 2-week mortality of 22% reported from the ACTA trial.[4] Because this was an observational study, the effectiveness estimate that we report might be subject to bias. Only 38·7% of eligible patients in our analysis received a flucytosine-containing regimen during the study period. Clinicians might have selected patients to receive a flucytosine regimen on the basis of their baseline characteristics. For example, we found that ART-experienced patients were more likely to be prescribed a flucytosine-containing regimen. In several cohorts, ART-experienced patients with cryptococcal meningitis had similar mortality at 2 weeks compared with those who were ART-naïve.[12–14] Thus, this difference between treatment groups is unlikely to have affected our findings. Importantly, all major prognostic factors were similar between those treated with and without flucytosine-containing regimens so that issues of patient selection are unlikely to account for the differences in outcome observed. For example, we found that a similar proportion of patients in both treatment groups had altered mental status, which is

	Alive (n=1045)	Died (n=494)	Unadjusted OR for mortality (95% CI)	p value	Adjusted OR for mortality (95% CI)	Wald p value
Treatment regimen						
Other regimen	592/943 (62·8%)	351/943 (37·2%)	1 (ref)	..	1 (ref)	..
Flucytosine-containing regimen	453/596 (76·0%)	143/596 (24·0%)	0·53 (0·42–0·67)	<0·0001	0·47 (0·35–0·64)	<0·0001
Age category, years						
≥18–29	195/280 (69·6%)	85/280 (30·3%)	1 (ref)	..	1 (ref)	..
30–39	501/679 (73·8%)	178/679 (26·2%)	0·82 (0·60–1·11)	0·20	1·04 (0·71–1·51)	0·85
40–49	245 /396 (61·7%)	151/396 (38·6%)	1·41 (1·02–1·96)	0·039	1·81 (1·221–2·70)	0·0040
≥50	104/184 (56·5%)	80/184 (43·5%)	1·76 (1·20–2·60)	0·0040	2·05 (1·26–3·34)	0·0040
Sex						
Male	611/906 (67·4%)	295/906 (32·6%)	1 (ref)	..	1 (ref)	..
Female	434/633 (68·6%)	199/633 (31·4%)	0·96 (0·77–1·19)	0·70	1·22 (0·93–1·59)	0·15
CD4⁺ T-cell count, cells per mm³						
<100	770/1154 (66·7%)	384/1154 (33·3%)	1 (ref)	..		..
≥100	221/281 (78·6%)	60/281 (21·4%)	0·54 (0·40–0·74)	<0·0001	0·42 (0·75–0·61)	<0·0001
Antiretroviral therapy						
Naive	383/576 (66·5%)	193/576 (33·5%)	1 (ref)	..	1 (ref)	..
Experienced	656/941 (69·7%)	285/941 (30·3%)	0·86 (0·69–1·07)	0·18	1·00 (0·75–1·32)	0·98
Glasgow Coma Scale score <15						
No	755/994 (76·0%)	239/994 (24·0%)	1 (ref)	..	1 (ref)	..
Yes	194/383 (50·7%)	189/383 (49·3%)	3·13 (2·44–4·02)	<0·0001	3·00 (2·25–4·00)	<0·0001
Concurrent tuberculosis treatment						
No	768/1098 (69·9%)	330/1098 (30·1%)	1 (ref)		1 (ref)	
Yes	266/415 (64·1%)	149/415 (35·9%)	1·33 (1·05–1·69)	0·029	1·24 (0·91–1·67)	0·17

Data are n/N (%), unless otherwise specified. OR=odds ratio. CSF=cerebrospinal fluid.

Table 2: **Random-effects logistic regression analysis of the effect of a flucytosine-containing treatment regimen versus any other regimen on in-hospital mortality among HIV-seropositive patients with cryptococcal meningitis**

among the strongest prognostic indicators for mortality. Our adjusted multivariable analysis was done in a smaller group because of missing confounder data. Nevertheless, the halving of the in-hospital mortality among those treated with flucytosine-containing regimens was similar in both crude and adjusted analyses. We were also unlikely to have misclassified either the main exposure (treatment group) or outcome (mortality).

Based on our data, only a small fraction of patients diagnosed with cryptococcal meningitis in South Africa during the study period were recorded to have received a flucytosine-containing regimen. Although the access programme was recently expanded to include almost 60 health-care facilities in South Africa, including all hospitals in the Western Cape Province, the coverage is still inadequate for this crucially important life-saving antifungal medicine. Our data from a large access programme were instrumental in fast-tracking SAHPRA's registration of flucytosine. This registration will facilitate inclusion of flucytosine on the South African EML and ensure its availability at all levels of hospital care. The standard treatment guidelines need to be updated as soon as possible and health-care workers need to be trained to prescribe, dispense, and monitor flucytosine for HIV-associated cryptococcal meningitis. Detailed guidance on prescribing and monitoring

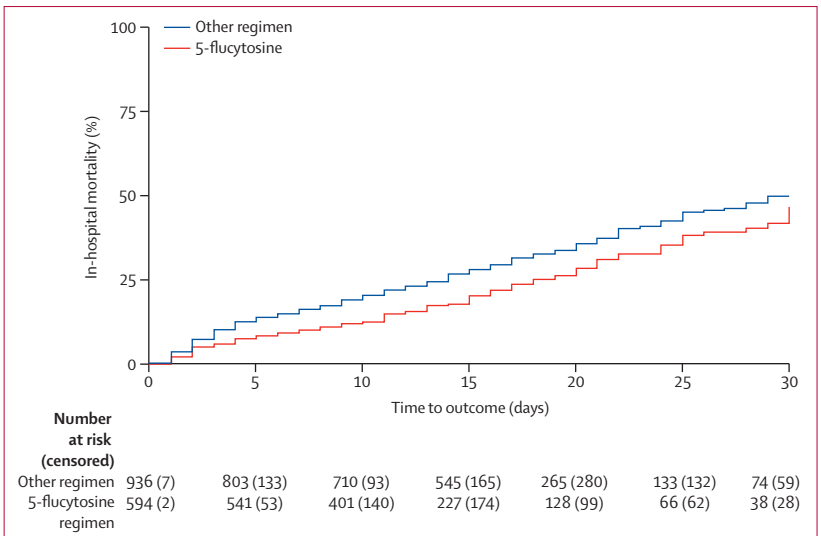

Figure 3: **Mortality by regimen for 1530 patients during a 30-day period from diagnosis of culture-confirmed cryptococcal meningitis to in-hospital outcome**

flucytosine was published by the Southern African HIV Clinicians Society in 2019.[10] Similar efforts in terms of registration, guideline updates, and health-care worker training are required to expand access to flucytosine

across low-income and middle-income countries on the African continent and globally.

A full economic costing and cost-effectiveness comparison of different treatment combinations used in the ACTA trial demonstrated that the 1-week amphotericin B and flucytosine regimen was less costly and more efficacious at reducing mortality than were regimens based on 2 weeks of amphotericin B.[15] A recent cost-effectiveness decision-analysis modelling exercise, conducted independently by the South African National EML Committee and using input parameters for mortality from the ACTA trial and local costs, confirmed these findings.[7] However, in both models, the authors could not incorporate the cost benefit associated with the length of hospital admission owing to a lack of evidence for a reduction in hospital stay among patients. Our multicentre study now provides robust evidence that inclusion of flucytosine in a treatment regimen reduces the median length of stay from 17 (in the ACTA trial) to 11 days among those who survive their hospital admission. Thus, in routine practice, the costs of flucytosine-containing regimens might be significantly less than in the ACTA trial and the aforementioned health economic analyses, and the cost savings resulting from flucytosine access would further increase from the perspective of a national government.

When considering inclusion of flucytosine in a national EML and in standard treatment guidelines, understanding clinicians' knowledge and preferences in terms of prescribing a flucytosine-containing regimen versus another induction regimen and barriers to prescribing flucytosine is important. In our study, clinicians at non-academic hospitals were more likely to prescribe flucytosine than those at academic hospitals. The variability in prescribing flucytosine by hospital tier might reflect the ease of interdepartmental communication and thus more uniform clinician training and prescribing practices in smaller non-academic versus larger academic facilities. Alternatively, decreased prescribing of flucytosine at large academic hospitals might be a consequence of stock-outs. During the surveillance period, the flucytosine access programme had a sufficient supply of flucytosine; however, stock-outs in some facilities might have occurred due to the additional administrative work required to order flucytosine.

A strength of our multicentre study was a large sample size which allowed us to gauge the effectiveness of adding flucytosine to the cryptococcal meningitis induction regimen as a programmatic intervention. We believe our results are broadly generalisable to urban health-care facilities in African low-income and middle-income countries. However, this cross-sectional study had several limitations, in addition to those mentioned previously described. We nested the study in routine surveillance for cryptococcal meningitis. This was a secondary analysis of enhanced surveillance data and was not specifically designed or powered to detect differential mortality outcomes between patients receiving flucytosine-containing regimens and other regimens. This was a non-randomised study and thus is subject to biases inherent to this design.[16] We had missing data for some important confounder variables—for example, HIV viral load was excluded from the models. Residual confounding is possible because we did not measure or adjust for some factors known to affect cryptococcal-related mortality such as high baseline CSF fungal burden. We also did not collect information on severe anaemia and renal dysfunction at baseline or during treatment in both groups. Finally, 10-week outcome data were only collected for a selected subgroup of patients treated with flucytosine and thus this limits any meaningful comparisons with clinical trial data.

In conclusion, the low in-hospital mortality among inpatients with cryptococcal meningitis treated with flucytosine in this observational study was similar to the mortality reported from the ACTA trial. We found that flucytosine combination treatment could be delivered in routine care in a resource-limited country with a substantial survival benefit. Only a minority of South Africans with cryptococcal meningitis are currently treated with flucytosine, thus urgent action to address access has the potential to avert thousands of avoidable deaths from cryptococcal meningitis in South Africa and other countries in the African region each year.

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Contributors

RCM was involved in conceptualisation of the study, study design, acquisition of data, data analysis, writing of the original manuscript, and revision according to feedback from co-authors. STM was involved in conceptualisation, data collection plan, analysis plan, accessed and verified the data, reviewed the manuscript, and provided final approval for the version to be submitted. JN, VCQ, GSG, AG, CM, DLR, MV, SS, MM, LB, TSH, GM, AS, LT-D, and JB were involved in conceptualisation, methodology, reviewing the manuscript and providing final approval for the version to be submitted. NPG was involved in conceptualisation, study design and data collection plan, analysis plan, accessed and verified the data, wrote and critically revised the manuscript, and provided final approval for the version to be submitted. The authors had full access to all study data and accepted responsibility to submit for publication.

Declaration of interests

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Data sharing

The data used for this analysis can be made available upon reasonable request once all relevant sub-studies from the National Institute for Communicable Diseases are reported and completed. However, should other researchers request access to this data or require additional information, they should communicate with the corresponding author.

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References

1 Rajasingham R, Smith RM, Park BJ, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis* 2017; **17:** 873–81.

2 Abassi M, Boulware DR, Rhein J. Cryptococcal meningitis: diagnosis and management update. *Curr Trop Med Rep* 2015; **2:** 90–99.

3 WHO. Guidelines on the diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children. 2018. https://apps.who.int/iris/bitstream/handle/10665/260399/9789241550277-eng.pdf (assessed Jan 13, 2021).

4 Molloy SF, Kanyama C, Heyderman RS, et al. Antifungal combinations for treatment of Cryptococcal meningitis in Africa. *NEJM* 2018; **378:** 1004–17.

5 South African Health Products Regulatory Authority. Registered Health Products. 2022. https://www.sahpra.org.za/registered-health-products/ (accessed June 9, 2022).

6 National Department of Health. Standard Treatment Guidelines And Essential Medicines List for South Africa: Hospital Level 2019 Edition. 2019. https://www.sapc.za.org/Media/Default/Documents/STG hospital level adult 2019_v2.0.pdf (assessed May 21, 2021).

7 Miot J, Leong T, Takuva S, et al. Cost-effectiveness analysis of flucytosine as induction therapy in the treatment of cryptococcal meningitis in HIV-infected adults in South Africa. *BMC Health Serv Res* 2021; **21:** 1–11.

8 Shroufi A, Govender NP, Meintjes G, et al. Time to embrace access programmes for medicines: lessons from the South African flucytosine access programme. *Int J Infect Dis* 2020; **95:** 459–61.

9 US Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases D of A. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events. 2017. https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf (assessed May 12, 2021).

10 Govender NP, Meintjes G, Mangena P, et al. Southern African HIV Clinicians Society guideline for the prevention, diagnosis and management of cryptococcal disease among HIV-infected persons: 2019 update. *Sout Afr J HIV Med* 2019; **20:** 1–16.

11 Leong TD, Ndlovu T, Jugathpal J, et al. Accessing essential medicines in the South African public healthcare sector via section 21. *SA Pharm J* 2019; **86:** 37–40.

12 Rhein J, Hullsiek KH, Evans EE, et al. Detrimental outcomes of unmasking cryptococcal meningitis with recent ART initiation. *Open Forum Infect Dis* 2018; **5:** 1–7.

13 Jarvis JN, Bicanic T, Loyse A, et al. Determinants of mortality in a combined cohort of 501 patients with HIV-associated cryptococcal meningitis: implications for improving outcomes. *Clinical Infect Dis* 2014; **58:** 736–45.

14 Kalata N, Ellis J, Kanyama C, et al. Short-term mortality outcomes of HIV-associated Cryptococcal meningitis in antiretroviral therapy-naïve and -experienced patients in sub-Saharan Africa. *Open Forum Infect Dis* 2021; **8:** 397.

15 Chen T, Mwenge L, Lakhi S, et al. Healthcare costs and life-years gained from treatments within the Advancing Cryptococcal Meningitis Treatment for Africa (ACTA) trial on Cryptococcal meningitis: a comparison of antifungal induction strategies in sub-Saharan Africa. *Clinical Infect Dis* 2019; **69:** 588–95.

16 Sterne JA, Hernán MA, Reeves BC, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ* 2016; **355:** 4–10.