TITLE PAGE

Benefits of enhanced infection prophylaxis at antiretroviral therapy initiation by cryptococcal antigen status (111 chars)

Running title: Enhanced OI-prophylaxis and CrAg status (40 chars)

Sarah L PETT[1,2,3], Moira SPYER[2], Lewis J HADDOW[1], Ruth NHEMA[4], Laura A BENJAMIN[5,6], Grace NAJJUKA[7], Sithembile BILIMA[8], Ibrahim DAUD[9], Godfrey MUSORO[4], Juliet KITABALWA[7], George SELEMANI[8], Salome KANDIE9, K Magut CORNELIUS[9], Chrispus KATEMBA[7], Jay A BERKLEY[10], Amin S HASSAN[10], Cissy KITYO[7], James HAKIM[4], Robert S HEYDERMAN[8,11], Diana M GIBB[2] *, A Sarah WALKER[2] * and the REALITY trial team

* Contribution considered equal

1 Institute for Global Health, UCL, London, UK

2 MRC CTU at UCL, Institute of Clinical Trials and Methodology, UCL, London, UK

3 Kirby Institute for Infection and Immunity in Society, University of New South Wales, Australia

4 University of Zimbabwe Clinical Research Centre, Harare, Zimbabwe

5 Institute of Neurology, UCL, UK

6 Institute of Infection and Global Health, University of Liverpool, Liverpool, UK

7 Joint Clinical Research Centre, Kampala, Uganda

8 Department/College of Medicine and Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Blantyre, Malawi

9 Moi University School of Medicine, Eldoret, Kenya

10 KEMRI Wellcome Trust Research Programme, Kilifi, Kenya

11 Division of Infection and Immunity, UCL, London, UK

Length: abstract 250 words

text 3241 words

2 tables plus 1 supplementary table

3 figures plus 2 supplementary figures

Corresponding author and for reprints:

Professor Sarah L. Pett, FRACP, FRCPE, PhD

ORCID: 0000-0002-4851-4727

MRC Clinical Trials Unit at UCL, 90 High Holborn, 2nd Floor, London, WC1V 6LJ, UK.

Tel +44 (0) 20 7670 4726. Fax +44 (0) 20 7670 4949.

Email: s.pett@ucl.ac.uk

Alternative corresponding author:

Professor (Ann) Sarah Walker, PhD

MRC Clinical Trials Unit at UCL, 90 High Holborn, 2nd Floor, London, WC1V 6LJ, UK.

Tel +44 (0) 20 7670 4726. Fax +44 (0) 20 7670 4949.

Email: rmjlasw@ucl.ac.uk

FUNDING STATEMENT

This work was supported by the Joint Global Health Trials Scheme (JGHTS) of the UK Department for International Development (DFID), the Wellcome Trust and Medical Research Council (MRC) [G1100693]. Additional funding support was provided by the PENTA foundation and core support to the MRC Clinical Trials Unit at UCL [MC_UU_12023/23] [MC_UU_12023/26]. Cipla Ltd, Gilead Sciences, ViiV Healthcare/GlaxoSmithKline, Merck Sharp & Dohme donated drugs for REALITY and Ready-to-Use-Supplementary-Food (RUSF) was purchased from Valid International. The Malawi–Liverpool–Wellcome Trust Clinical Research Programme, University of Malawi

College of Medicine [101113/Z/13/Z], and the KEMRI/Wellcome Trust Research Programme, Kilifi [203077/Z/16/Z] are supported by strategic awards from the Wellcome Trust, United Kingdom. Permission to publish was granted by the Director of KEMRI. ASW is a National Institutes of Health Research Senior Investigator.

The full trial protocol can be accessed at https://www.ctu.mrc.ac.uk/media/1293/reality-protocol.pdf [accessed 21 December 2019].

DISCLAIMERS

In addition to the support from organisations for the submitted work as described above the institution of ASW has received other funding from Janssen and Gilead Sciences for DSMB membership and lectures; JH has received funding for advisory board membership from Mylan Pharmaceuticals; SLP has received grants to support academic research from ViiV Healthcare and Gilead Sciences; all other authors have no other relationships or activities that could appear to have influenced the submitted work.

Abstract (250 words)

Objectives: To assess baseline prevalence of cryptococcal antigen (CrAg) positivity; and its contribution to reductions in all-cause mortality, deaths from cryptococcus and unknown causes, and new cryptococcal disease in the REALITY trial.

Design: Retrospective CrAg testing of baseline and week-4 plasma samples in all 1805 African adults/children with CD4<100 cells/μL starting antiretroviral therapy who were randomised to receive 12-week enhanced-prophylaxis (fluconazole 100mg/day, azithromycin, isoniazid, cotrimoxazole) vs. standard-prophylaxis (cotrimoxazole).

Methods: proportional hazards models were used to estimate the relative impact of enhanced-prophylaxis vs. standard-cotrimoxazole on all, cryptococcal and unknown deaths, and new cryptococcal disease, through 24-weeks, by baseline CrAg positivity..

Results: Excluding 24(1.4%) participants with active/prior cryptococcal disease at enrolment (all treated for cryptococcal disease), 133/1781(7.5%) participants were CrAg-positive. By 24-weeks, 105 standard-cotrimoxazole vs. 78 enhanced-prophylaxis participants died. Of 9 standard-cotrimoxazole and 3 enhanced-prophylaxis cryptococcal deaths, 7 and 2 respectively were CrAg-positive at baseline. Among deaths of unknown cause, only 1/46 standard-cotrimoxazole and 1/28 enhanced-prophylaxis were CrAg-positive at baseline. There was no evidence that relative reductions in new cryptococcal disease associated with enhanced-prophylaxis varied between baseline CrAg-positives (hazard-ratio, HR=0.36 (95% CI 0.13-0.98), incidence 19.5 vs. 56.5/100 person-years) and CrAg-negatives (HR=0.33 (0.03-3.14), incidence 0.3 vs. 0.9/100 person-years; $p_{heterogeneity}$=0.95); nor for all deaths, cryptococcal deaths or unknown deaths ($p_{heterogeneity}$>0.3).

Conclusions: Relative reductions in cryptococcal disease/death did not depend on CrAg status. Deaths of unknown cause were unlikely to be cryptococcus-related; plausibly azithromycin contributed to their reduction. Findings support including 100mg fluconazole in an enhanced-prophylaxis package at ART initiation where CrAg screening is unavailable/impractical.

Keywords: HIV; Africa; cryptococcus; prophylaxis; late presentation.

INTRODUCTION

Cryptococcal disease continues to have high morbidity and mortality in advanced HIV disease in sub-Saharan Africa, despite improved antifungal regimens for treatment[1], and combination antiretroviral therapy (ART)[2]. The screening test of choice is for cryptococcal antigen (CrAg), for asymptomatic individuals in blood, and for symptomatic individuals in cerebrospinal fluid (CSF) to identify meningitis[3]. The global prevalence of cryptococcal antigenaemia in HIV-infected adults with CD4 <100 cells/μL is ~6%, although higher prevalences have been reported[4]. Using the CrAg latex agglutination assay, the average time between CrAg detection in blood and the onset of symptomatic cryptococcal disease is ~3 weeks[5], and is likely even longer with more sensitive lateral flow assays (LFA)[6], allowing the opportunity to intervene with antifungal prophylaxis or treatment[5]. A recent cross-sectional study in South Africa[7] confirmed that 90% of CrAg-positive patients with headache as their only reported symptom had CrAg-positive CSF, as did 34% of asymptomatic CrAg-positive patients.

WHO guidelines[3] recommend a “[CrAg] screen and treat” approach to preventing cryptococcal disease, with CrAg-positive individuals receiving pre-emptive fluconazole treatment (800 mg/day for 2 weeks) then maintenance (400mg/day for 8 weeks). This recommendation was based on the REMSTART trial[8] which showed significant mortality reductions in HIV-infected adults in Tanzania and Zambia initiating ART with CD4 <200 cells/μL with this approach. One challenge with “screen and treat” in high-risk populations is that CrAg testing kits are frequently unavailable in low and middle-income countries (LMIC), especially at primary healthcare centres where ART is increasingly initiated. Furthermore, even when kits are available, waiting for CrAg results can considerably delay starting ART in patients at high risk of immediate morbidity/mortality, particularly if the CrAg test is not performed on the same residual specimen from CD4 testing, if the latter is requested[9].

An alternative approach is universal prophylaxis in high-risk populations. The REALITY trial (ISRCTN43622374) demonstrated that a package of enhanced-prophylaxis, comprising cotrimoxazole (as fixed dose combination with isoniazid/pyridoxine), fluconazole (100mg/day for 12 weeks) azithromycin (500mg/day for 5 days) and albendazole (single dose), significantly reduced all-cause mortality, deaths from cryptococcus and unknown causes, and incidence of new cryptococcal disease and tuberculosis, compared to standard-cotrimoxazole prophylaxis alone. Patients were African adults and children >5 years initiating ART with CD4 <100 cells/μL[10]. The total dose per week (700mg) and duration of fluconazole used in REALITY was based on a previous trial in Uganda, showing benefit of fluconazole 200mg three times weekly (total 600mg per week) until CD4 reached ≥200 cells/μL[11]. However, dosing was daily in REALITY to match ART dosing schedules.

Given these findings, current WHO cryptococcal guidelines[3] also recommend that, where there is no access to CrAg testing or delays in returning results, fluconazole can be offered as primary prophylaxis in advanced HIV at the time of ART initiation or switch, using the REALITY dose of 100mg/day or alternatively 200mg three times/week[3].

Baseline CrAg testing was not routinely performed in real-time in REALITY. Therefore, it was unknown whether reductions in cryptococcal disease and deaths were restricted to baseline CrAg-positives, and whether the significant reductions in deaths from unknown causes associated with enhanced-prophylaxis could have been due to missed cryptococcal disease (and hence plausibly reduced by fluconazole prophylaxis), or whether reductions might be driven by other components of the package. The aims of this substudy were therefore to estimate baseline CrAg prevalence, and to assess its contribution to the significant reductions in all-cause and cryptococcal-related/unknown mortality, and cryptococcal-related morbidity observed in the REALITY trial.

METHODS

CrAg LFA qualitative and quantitative testing was performed retrospectively between May 2017 and February 2018, blinded to randomised group and patient characteristics, using 40uL of frozen plasma samples stored at baseline (day of enrolment) and 4 weeks after ART initiation, from all REALITY participants. If CrAg-positive, CrAg titre was estimated using the semi-quantitative dilution technique as per package insert. Any sample that was positive on qualitative testing, but not using any of the dilution steps, was assigned a titre of 1:2.5 (half the lowest titre of 1:5). Results were verified, blinded to randomisation, by central review of photographs of the testing strips. Testing was performed at one central laboratory within each country by staff trained in qualitative and semi-quantitative CrAg testing using the IMMY LFA kits supplied by Alpha Laboratories Limited, UK. CrAg testing was included in the main trial protocol and approved by Ethics Committees in Zimbabwe, Uganda, Malawi, Kenya and the UK.

All clinical events in the trial up to 48 weeks were ascertained at pre-specified trial visits or additional visits for acute illness. Nurse visits at weeks 2, 4, 8, 12, 18, 24, 36, and 48 included a symptom checklist which included severe headache amongst solicited symptoms; history and examination by a clinician was performed at weeks 4, 12, 24, 36 and 48. All defaulting participants were traced through home visits and telephone calls. An Endpoint Review Committee (ERC) (majority independent members) adjudicated causes of death and non-fatal events (WHO 3/4 events/grade 3/4 AEs/SAEs) using clinical narratives written by treating clinicians, incorporating imaging scans/reports and laboratory results, including CrAg-positive status (usually in blood) if this was measured locally in real-time. Definitive cryptococcal meningitis was defined as clinical meningitis (severe headache, meningism, photophobia) with a positive CSF CrAg test and/or CSF microscopy (positive India ink stain and/or CSF culture positive). A probable diagnosis was defined as a consistent clinical history and a positive plasma CrAg test (or fungaemia) in the absence of any CSF results. Events were adjudicated retrospectively by ≥2 ERC members, blinded to randomised

groups, against protocol-defined criteria and grading tables[10, 12]. Compatibility of fatal and non-fatal event(s) with immune reconstitution inflammatory syndrome (IRIS) was documented based on clinical and diagnostic information (often limited) and the time course after ART initiation. No distinction was made between paradoxical and unmasking IRIS given the limited information available. The ERC did not have access to viral load data as these were done retrospectively; the earliest post-randomisation CD4 results were at week 4. Therefore for early events, a clinical judgement was made using baseline data (including CD4) and previously published definitions[13, 14] in modified form, to determine whether event(s) represented an atypical/exaggerated presentation of an opportunistic infection or tumour soon after ART initiation (i.e. were IRIS-compatible).

Statistical analysis

As our first aim was to estimate the prevalence of latent cryptococcal infection prior to ART initiation with CD4 <100 cells/μL, participants with a diagnosis of cryptococcal meningitis at or prior to baseline were excluded from all analyses; all were treated for cryptococcal disease. Logistic regression with backwards elimination ($p>0.1$ to fit an exploratory model, including non-linearity by fractional polynomials where $p<0.05$) was used to identify independent predictors of baseline CrAg status in the remaining asymptomatic individuals, from age, gender, WHO stage, body mass index (BMI), CD4, VL and haemoglobin, adjusting for site of enrolment.

We then considered the time-to-event outcomes of mortality (all-cause, cryptococcal and from unknown causes as determined by the ERC (where enhanced-prophylaxis had significant benefits in the trial overall)), new cryptococcal disease (fatal and non-fatal), cryptococcal IRIS, and determined or undetermined central nervous system (CNS) events (fatal or non-fatal) (**Supplementary Table 1**). Analyses used competing risks methods for patients dying of other causes without having recorded the event of interest[15]. Absolute rates of each outcome by baseline CrAg status were calculated through 24 weeks on ART

(time of the main trial primary endpoint) when most clinical events had occurred[16]. Proportional (sub)hazards models were used to estimate heterogeneity in the relative impact of enhanced-prophylaxis vs. standard-cotrimoxazole by CrAg status over the first 24 weeks using interaction tests.

RESULTS

All 1805 REALITY participants (98% aged ≥13 years) had a baseline CrAg test using stored plasma. We excluded 23 (1.3%) participants being treated for local physician-identified active cryptococcal disease at baseline (on stored samples 22 were CrAg-positive with titres 1:1280-1:2560; one CrAg-negative) and one participant with previous cryptococcal disease (CrAg-positive on stored sample at enrolment, titre 1:2560, on 400mg fluconazole), leaving 1781 participants without identified cryptococcal disease at baseline in the analyses (**Table 1**).

Prevalence of CrAg positivity at baseline (ART initiation)

133/1781 (7.5%, 95% CI 6.3-8.8%) participants were CrAg-positive at ART initiation, 69/888 (7.8%) in the standard-cotrimoxazole vs. 64/893 (7.2%) in enhanced-prophylaxis group (p=0.65, **Table 1**). In CrAg-positives, the median CrAg titre was 1:80 (IQR 1:10-1:640) (range <1:5-1:2560) (1:80 standard-cotrimoxazole vs. 1:20 enhanced-prophylaxis, p=0.06) (**Figure 1A).**

As expected, the median CD4 was slightly lower in CrAg-positives (30 vs. 38 cells/μL in CrAg-negatives, p=0.003), but there was no evidence of differences in VL (median $\log_{10}$ VL 5.5 vs. 5.4 respectively, p=0.76). 173 (9.6%) participants enrolled in the trial had received fluconazole in the 14 days before randomisation, (mostly (79%) 200mg daily for oral candida infection). However, baseline CrAg-positivity did not differ by receipt of prior fluconazole (14/173 (8.1%)) or not (119/1608 (7.4%), p=0.76, **Table 1**). 59 (3.3%) participants reported severe headache at enrolment (as a nurse-solicited symptom), but baseline CrAg-positivity

did not differ in those reporting (2/59 (3.4%)) and not reporting (131/1710 (7.7%)) severe headache (p=0.32, **Table 1**). Considering factors in **Table 1**, the only independent predictors of CrAg-positivity were a lower CD4 count (OR=0.89 per 10 cells/μL higher (95% CI 0.83-0.95) p=0.001) and being older (OR per 10 years older=1.19 (1.00-1.42) p=0.046) at ART initiation. Accounting for CD4 and age, CrAg-positivity was significantly lower amongst individuals recruited from Kilifi, Kenya (p=0.03). Even considering many other factors reflecting clinical status[17], only night sweats (OR=1.67 (0.98-2.85) p=0.06) added weak predictive power to the model.

Mortality

Enhanced-prophylaxis significantly reduced all-cause mortality and deaths from cryptococcus and of unknown cause, with no evidence of effect on deaths from tuberculosis or other causes[10, 16]. Of the 12 deaths before 24 weeks adjudicated by the ERC as due to cryptococcal disease, nine were CrAg positive at baseline on retrospective testing (7/9 deaths on standard-cotrimoxazole, 2/3 deaths on enhanced-prophylaxis; **Table 2**). In contrast, of the 74 deaths before 24 weeks adjudicated as due to unknown causes (many dying away from a healthcare facility), only two were CrAg-positive at baseline (1/46 deaths on standard-cotrimoxazole, 1/28 deaths on enhanced-prophylaxis) (**Table 2**). Proportions who were baseline CrAg-positive were similarly low for deaths adjudicated to be from tuberculosis, severe bacterial infections or other causes (**Table 2**).

As expected, absolute rates of cryptococcal deaths were very high in those who were CrAg-positive at baseline (solid symbols in **Figure 2**), and low in those who were CrAg-negative at baseline (hollow symbols in **Figure 2**). However, there was no evidence that relative benefits from enhanced-prophylaxis differed by baseline CrAg status for cryptococcal deaths ($p_{heterogeneity}$=0.73) (**Figure 3**); nor was there any evidence of variation for all-cause mortality ($p_{heterogeneity}$=0.39), or deaths from unknown causes ($p_{heterogeneity}$=0.67).

Cryptococcal disease and cryptococcal IRIS-compatible events

Over the first 24 weeks on ART, new cryptococcal meningitis occurred in 17 standard-cotrimoxazole vs. 6 enhanced-prophylaxis participants (p=0.03), diagnosed a median 20 days post-ART initiation (IQR 15-45) (16 vs. 5 adjudicated as cryptococcal-IRIS respectively). Two additional cases were diagnosed after 24 weeks, both in the standard-cotrimoxazole group (not included in time-to-event analyses through 24 weeks). 14/17 standard-cotrimoxazole vs. 5/6 enhanced-prophylaxis cryptococcal meningitis cases were CrAg-positive at baseline (13/16 vs. 4/5 cryptococcal IRIS-compatible cases respectively) (**Table 2**). Most CrAg-positives who developed cryptococcal disease had baseline titres of 1:2560 (**Table 2**), with no clear gradient below 1:2560 (**Figure 1B/C**). Of these 23 patients with new cryptococcal disease during the trial, 53% (9/17) and 50% (3/6) died in the standard-cotrimoxazole and enhanced-prophylaxis groups respectively (exact p=1.00).

As expected, similarly to cryptococcal deaths, the absolute incidence of cryptococcal disease was significantly greater in those who were CrAg-positive vs. CrAg-negative at baseline (p<0.0001), regardless of randomisation (solid vs. hollow symbols respectively, **Figure 2**). However, similarly to cryptococcal mortality, there was no evidence that the relative benefits of enhanced prophylaxis differed by baseline CrAg status ($p_{heterogeneity}$=0.95 for cryptococcal disease, 0.97 for cryptococcal IRIS-compatible disease), with an overall risk reduction of 0.36 (95%CI 0.13,0.98) in cryptococcal disease associated with enhanced-prophylaxis (including 100mg fluconazole daily) in those CrAg-positive at baseline. Results were not influenced by the small proportion of participants prescribed fluconazole at enrolment outside of the randomisation (138/1781 (7.7%), predominantly (83%) at a dose of 200mg daily for oral/oesophageal candida (**Supplementary Results**).

Determined CNS events (**Supplementary Table 1**) were predominantly cryptococcal meningitis, so results were similar to those for new cryptococcal disease. In contrast undetermined CNS events occurred similarly between the randomised groups (**Table 2,**

Figure 3).

CrAg titres

In baseline CrAg-positives with titres between 1:2.5 and 1:1280, cryptococcal disease occurred during the first 24 weeks on ART in 5/56 (9%) standard-cotrimoxazole vs. 1/55 (2%) enhanced-prophylaxis participants (**Figure 1B/C**). At week 4, overall CrAg positivity was 7.9% (95% CI 6.7-9.3%) (130/1642 participants with data, excluding those developing cryptococcal disease between enrolment and week 4). 95 (5.8%) were positive at both baseline and week 4, with median no change in doubling dilution (IQR 0 to +1; p=0.78 comparing standard-cotrimoxazole vs. enhanced-prophylaxis) (**Supplementary Figure 1**). 35 (2.1%) became positive at week 4 having been negative at baseline (18 enhanced-prophylaxis, 17 standard-cotrimoxazole), whereas 15 (0.9%) became negative having been positive at baseline (9 enhanced-prophylaxis (one presumptively treated with 200mg fluconazole for oral candida; others receiving 100mg), 6 standard-cotrimoxazole (one presumptively treated with 1200mg fluconazole daily for headache, others not receiving fluconazole)) (McNemar p=0.005; **Supplementary Results**).

DISCUSSION

In the four Sub-Saharan African countries that enrolled participants with advanced HIV starting ART into the REALITY trial, we found a 7.5% prevalence of CrAg positivity with no clinically apparent cryptococcal disease. Whilst CrAg positivity increased as baseline CD4 decreased from 100 to 0 cells/μL, the impact of baseline CD4 on CrAg positivity was relatively small. CrAg positivity rates were higher in older individuals, consistent with the known epidemiology[18]. We found no other predictors of CrAg positivity that could be used to target fluconazole prophylaxis or pre-emptive treatment where CrAg screening is not available.

As previously reported[10, 16], the REALITY enhanced-prophylaxis package was associated

with significantly lower mortality from ERC-adjudicated cryptococcus and unknown causes of death. Here we demonstrate that undiagnosed cryptococcus at baseline was not a driver of reductions in early deaths from unknown causes, since very few of these participants were CrAg-positive. As the CrAg test we used is both highly sensitive and specific, precedes clinical disease by several weeks and, in turn, remains positive for several weeks, this finding strongly suggests that deaths from unknown causes were not predominantly due to cryptococcus. Instead, the reduction in early deaths from unknown causes in the enhanced-prophylaxis group is plausibly due to another component of the enhanced-prophylaxis package. Possible candidates are isoniazid or azithromycin. Tuberculosis was a relatively common diagnosis in the trial[16], with most sites using GeneXpert. Enhanced-prophylaxis was associated with reductions in tuberculosis disease, but not tuberculosis-related deaths, making this a less likely explanation, although tuberculosis can be difficult to diagnose in this population with advanced HIV. Azithromycin is a broad-spectrum macrolide with a long intracellular half-life in macrophages[19] and potential efficacy against severe respiratory and gastrointestinal bacterial infections common in Africa, especially in advanced HIV. In this setting, azithromycin could also have had activity against toxoplasmosis[20], atypical mycobacteria[21], malaria[22] and/or as an anti-inflammatory agent[19, 23]. However, it is also theoretically possible that fluconazole could have contributed to reduction in unknown deaths through non-cryptococcal pathways, e.g. by affecting other fungi (e.g. candida oesophagitis leading to bacterial translocation) or through gut microbiome changes[24].

While there was no evidence that the relative clinical benefits of enhanced-prophylaxis differed among CrAg-positive and CrAg-negative participants, as expected the absolute benefits were much greater amongst CrAg-positives, who are at much higher absolute risk of developing overt cryptococcal disease. Reasons for observing clinical benefits from fluconazole prophylaxis in baseline CrAg-negative participants include false-negatives at baseline, unmasking of cryptococcal disease post-ART initiation (particularly given the low CD4 counts at ART initiation), or new acquisition of cryptococcus after ART initiation. False-

negative CrAg are relatively rare, but even a 0.5% false-negative rate would have led to 8 false-negatives in our population. The latter two scenarios (i.e. unmasking of cryptococcal disease post-ART initiation or new acquisition of cryptococcus after randomisation) are supported by the fact that 2% of participants converted from being CrAg-negative at enrolment to CrAg-positive at week 4. A disease incidence of 10-20% over 24 weeks (**Figure 2**) in the 35 participants who CrAg-converted could account for the new cases we observed post-enrolment in those CrAg-negative at baseline.

There were fewer cryptococcal deaths in baseline CrAg-positives in the enhanced-prophylaxis group (2 deaths) vs. the standard-cotrimoxazole group (7 deaths). We cannot directly assess the contribution of the emergence of fluconazole resistance to these deaths because no samples were stored; nor are resistance data available from those with non-fatal cryptococcal disease. However, although numbers are small, 50% (3/6) enhanced-prophylaxis participants with incident cryptococcus survived vs 47% (8/17) standard-cotrimoxazole participants, suggesting that receipt of low-dose fluconazole does not increase the risk of treatment failure with current standard-of-care for treatment in Africa, i.e. high dose fluconazole monotherapy or high dose fluconazole plus amphotericin. While a recent study suggested that 100mg/day fluconazole could lead to subtherapeutic levels for treating cryptococcal disease in 40% of patients[25], in REALITY this fluconazole dose was given synchronously with ART, which was associated with substantial early immune reconstitution[10]. In those patients who were CrAg-positive at baseline, 24 week all-cause mortality was 7.8% with enhanced-prophylaxis (plus immediate ART) and 15.9% with standard-prophylaxis (plus immediate ART) (**Figure 2**), only slightly lower than the ~20% in a pooled analysis of 4 CrAg-positive cohorts.with titre ≤1:80[26]. This is consistent with generally better outcomes observed in trials, either due to more consistent management (e.g. no stockouts, little delay in ART initiation) or less sick patients being enrolled (although death rates were very high shortly after enrolment in REALITY[16] suggesting the trial was not doing this to a large degree). Moreover, time from screening to trial enrolment was very

short (median only 5 days (IQR 2-8)), meaning there was little opportunity for sites to recruit only ‘non-progressors’, and CrAg testing was done on the sample taken at enrolment (day of ART initiation), not screening. However, it is possible that cryptococcal disease was more likely to be identified at trial screening than in a general programmatic setting.

An important study limitation includes the limited diagnostic information available in some cases, reflecting real-world settings, but making it difficult to distinguish between newly acquired, latent or undiagnosed cryptococcal infection in those without clinically apparent disease at baseline, or between paradoxical or unmasking IRIS. However, practically the distinction between these is probably small. Although delaying ART initiation for 5 weeks after starting treatment with amphotericin B and 800mg daily fluconazole for cryptococcal meningitis was associated with improved survival[27], all participants in our study initiated 100mg daily fluconazole at the same time as ART, so we cannot assess whether reductions in cryptococcal disease/death in CrAG-positives would have been even greater had ART been delayed.

The REALITY trial was designed to be pragmatic and relevant to real-life settings. As such, the trial did not mandate CrAg screening in the inclusion criteria. Ideally a cheap point-of-care CrAg test may become available. However, even then, our findings show that an enhanced-prophylaxis package containing fluconazole at 100mg/day for 12 weeks is effective in this population of HIV-infected adults, adolescents and older children without overt cryptococcal disease, when started concurrently with first-line combination ART. Moreover, the finding of significant benefit in reducing early deaths from unknown causes in those CrAg-negative at baseline suggests that another component of the enhanced-prophylaxis package, possibly azithromycin, is providing this benefit, and supports the use of the enhanced-prophylaxis package in its entirety, in these populations with advanced HIV.

ACKNOWLEDGEMENTS

We thank all the participants and staff from all the centres participating in the REALITY trial.

The REALITY trial group consists of:

Participating Centres: Joint Clinical Research Centre (JCRC), Kampala, Uganda (coordinating centre for Uganda): P Mugyenyi, C Kityo, V Musiime, P Wavamunno, E Nambi, P Ocitti, M Ndigendawani. **JCRC, Fort Portal, Uganda:** S Kabahenda, M Kemigisa, J Acen, D Olebo, G Mpamize, A Amone, D Okweny, A Mbonye, F Nambaziira, A Rweyora, M Kangah and V Kabaswahili. **JCRC, Gulu, Uganda:** J Abach, G Abongomera, J Omongin, I Aciro, A Philliam, B Arach, E Ocung, G Amone, P Miles, C Adong, C Tumsuiime, P Kidega, B Otto, F Apio. **JCRC, Mbale, Uganda:** K Baleeta, A Mukuye, M Abwola, F Ssennono, D Baliruno, S Tuhirwe, R Namisi, F Kigongo, D Kikyonkyo, F Mushahara, D Okweny, J Tusiime, A Musiime, A Nankya, D Atwongyeire, S Sirikye, S Mula, N Noowe. **JCRC, Mbarara, Uganda**: A Lugemwa, M Kasozi, S Mwebe, L Atwine, T Senkindu, T Natuhurira, C Katemba, E Ninsiima, M Acaku J Kyomuhangi, R Ankunda, D Tukwasibwe, L Ayesiga. **University of Zimbabwe Clinical Research Centre, Harare, Zimbabwe:** J Hakim, K Nathoo, M Bwakura-Dangarembizi, A Reid, E Chidziva, T Mhute, GC Tinago, J Bhiri, S Mudzingwa, M Phiri, J Steamer, R Nhema, C Warambwa, G Musoro, S Mutsai, B Nemasango, C Moyo, S Chitongo, K Rashirai, S Vhembo, B Mlambo, S Nkomani, B Ndemera, M Willard, C Berejena, Y Musodza, P Matiza, B Mudenge, V Guti. **KEMRI Wellcome Trust Research Programme, Kilifi, Kenya:** A Etyang, C Agutu, J Berkley, K Maitland, P Njuguna, S Mwaringa, T Etyang, K Awuondo, S Wale, J Shangala, J Kithunga, S Mwarumba, S Said Maitha, R Mutai, M Lozi Lewa, G Mwambingu, A Mwanzu, C Kalama, H Latham, J Shikuku, A Fondo, A Njogu, C Khadenge, B Mwakisha. **Moi University Clinical Research Centre, Eldoret, Kenya:** A Siika, K Wools-Kaloustian, W Nyandiko, P Cheruiyot, A Sudoi, S Wachira, B Meli, M Karoney, A Nzioka, M Tanui, M Mokaya, W Ekiru, C Mboya, D Mwimali, C Mengich, J Choge, W Injera, K Njenga, S Cherutich, M Anyango Orido,G Omondi Lwande, P Rutto, A Mudogo, I Kutto, A Shali, L Jaika, H Jerotich, M Pierre. **Department of Medicine and Malawi-Liverpool Wellcome Trust Clinical Research**

Programme, College of Medicine, Blantyre, Malawi: J Mallewa, S Kaunda, J Van Oosterhout, B O'Hare, R Heydermann, C Gonzalez, N Dzabala, C Kelly, B Denis, G Selemani, L Nyondo Mipando, E Chirwa, P Banda, L Mvula, H Msuku, M Ziwoya, Y Manda, S Nicholas, C Masesa , T Mwalukomo, L Makhaza, I Sheha, J Bwanali, M Limbuni.

Trial Coordination and Oversight: MRC Clinical Trials Unit at UCL, London, UK: D Gibb, M Thomason, AS Walker, S Pett, A Szubert, A Griffiths, H Wilkes, C Rajapakse, M Spyer, A Prendergast, N Klein. Data Management Systems: M Rauchenberger, N Van Looy, E Little, K Fairbrother.

Social Science Group: F Cowan, J Seeley, S Bernays, R Kawuma, Z Mupambireyi.

Independent REALITY Trial Monitors: F Kyomuhendo, S Nakalanzi, J Peshu, S Ndaa, J Chabuka, N Mkandawire, L Matandika, C Kapuya**.**

Trial Steering Committee: I Weller (Chair), E Malianga, C Mwansambo, F Miiro, P Elyanu, E Bukusi, E Katabira, O Mugurungi, D Gibb, J Hakim, A Etyang, P Mugyenyi, J Mallewa.

Data Monitoring Committee: T Peto (Chair), P Musoke, J Matenga, S Phiri.

Endpoint Review Committee (independent members): H Lyall (Co-Chair), V Johnston (Co-Chair), F Fitzgerald, F Post, F Ssali, A Prendergast, A Arenas-Pinto, A Turkova, A Bamford.

AUTHORS CONTRIBUTIONS

SLP, LJH and LAB designed the cryptococcal substudy. RN, GN, SB, ID, JK, GS, SK, KMC, CK carried out the assays and also conducted laboratory testing for the main trial. MJS organised sample retrieval and quality control of assays. DMG, JH, ASW, JAB, RSH contributed to design of the overall trial. JH, GM, JAB, AH collected data for the trial. ASW analysed the data; ASW vouches for data and analysis and is the guarantor; SLP and ASW wrote the first draft; all authors approved the final version and decided to publish.

PRESENTATION

These data were first presented at a themed oral discussion and as a poster (Poster 784) at the annual Conference on Retroviruses and Opportunistic Infections (CROI), held 4-7th

March 2018 in Boston, Mass, USA.

DATA SHARING

The REALITY trial data are held at MRC CTU at UCL, which encourages optimal use of data by employing a controlled access approach to data sharing, incorporating a transparent and robust system to review requests and provide secure data access consistent with the relevant ethics committee approvals. All requests for data are considered and can be initiated by contacting mrcctu.ctuenquiries@ucl.ac.uk

REFERENCES

1. Molloy SF, Kanyama C, Heyderman RS, Loyse A, Kouanfack C, Chanda D, et al. **Antifungal Combinations for Treatment of Cryptococcal Meningitis in Africa**. *The New England journal of medicine* 2018; 378(11):1004-1017.

2. Oladele RO, Bongomin F, Gago S, Denning DW. **HIV-Associated Cryptococcal Disease in Resource-Limited Settings: A Case for "Prevention Is Better Than Cure"?** *Journal of fungi* 2017; 3(4).

3. World Health Organisation. **Guidelines for the diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children: Supplement to the 2016 consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection**. In. Geneva: World Health Organisation; 2018.

4. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, et al. **Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis**. *The Lancet Infectious diseases* 2017; 17(8):873-881.

5. French N, Gray K, Watera C, Nakiyingi J, Lugada E, Moore M, et al. **Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults**. *Aids* 2002; 16(7):1031-1038.

6. Binnicker MJ, Jespersen DJ, Bestrom JE, Rollins LO. **Comparison of four assays for the detection of cryptococcal antigen**. *Clin Vaccine Immunol* 2012; 19(12):1988-1990.

7. Wake RM, Britz E, Sriruttan C, Rukasha I, Omar T, Spencer DC, et al. **High Cryptococcal Antigen Titers in Blood Are Predictive of Subclinical Cryptococcal Meningitis Among Human Immunodeficiency Virus-Infected Patients**. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2018; 66(5):686-692.

8. Mfinanga S, Chanda D, Kivuyo SL, Guinness L, Bottomley C, Simms V, et al. **Cryptococcal meningitis screening and community-based early adherence support in people with advanced HIV infection starting antiretroviral therapy in Tanzania and Zambia: an open-label, randomised controlled trial**. *Lancet* 2015; 385(9983):2173-2182.

9. Ministry of Health. **2016 Clinical Management of HIV in Children and Adults**. In. Lilongwe, Malawi: Ministry of Health; 2016.

10. Hakim J, Musiime V, Szubert AJ, Mallewa J, Siika A, Agutu C, et al. **Enhanced Prophylaxis plus Antiretroviral Therapy for Advanced HIV Infection in Africa**. *The New England journal of medicine* 2017; 377(3):233-245.

11. Parkes-Ratanshi R, Wakeham K, Levin J, Namusoke D, Whitworth J, Coutinho A, et al. **Primary prophylaxis of cryptococcal disease with fluconazole in HIV-positive Ugandan adults: a double-blind, randomised, placebo-controlled trial**. *The Lancet Infectious diseases* 2011; 11(12):933-941.

12. Division of AIDS. **Table for Grading the Severity of Adult and Pediatric Adverse Events v1.0 (https://rsc.tech-res.com/docs/default-source/safety/table_for_grading_severity_of_adult_pediatric_adverse_events.pdf?sfvrsn=6, accessed 21 December 2019)**. In; 2004.

13. French MA, Price P, Stone SF. **Immune restoration disease after antiretroviral therapy**. *Aids* 2004; 18(12):1615-1627.

14. Haddow LJ, Colebunders R, Meintjes G, Lawn SD, Elliott JH, Manabe YC, et al. **Cryptococcal immune reconstitution inflammatory syndrome in HIV-1-infected individuals: proposed clinical case definitions**. *The Lancet Infectious diseases* 2010; 10(11):791-802.

15. Fine JP, Gray RJ. **A proportional hazards model for the subdistribution of a competing risk**. *Journal of the American Statistical Association* 1999; 94(496-509).

16. Post FA, Szubert AJ, Prendergast AJ, Johnston V, Lyall H, Fitzgerald F, et al. **Causes and Timing of Mortality and Morbidity Among Late Presenters Starting Antiretroviral Therapy in the REALITY Trial**. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2018; 66(suppl_2):S132-S139.

17. Siika A, McCabe L, Bwakura-Dangarembizi M, Kityo C, Mallewa J, Berkley J, et al. **Late Presentation With HIV in Africa: Phenotypes, Risk, and Risk Stratification in the**

526 **REALITY Trial**. *Clinical infectious diseases : an official publication of the Infectious Diseases*
527 *Society of America* 2018; 66(suppl_2):S140-S146.
528 18. Meya D, Rajasingham R, Nalintya E, Tenforde M, Jarvis JN. **Preventing**
529 **Cryptococcosis-Shifting the Paradigm in the Era of Highly Active Antiretroviral**
530 **Therapy**. *Current tropical medicine reports* 2015; 2(2):81-89.
531 19. Jain R, Danziger LH. **The macrolide antibiotics: a pharmacokinetic and**
532 **pharmacodynamic overview**. *Current pharmaceutical design* 2004; 10(25):3045-3053.
533 20. Dunay IR, Gajurel K, Dhakal R, Liesenfeld O, Montoya JG. **Treatment of**
534 **Toxoplasmosis: Historical Perspective, Animal Models, and Current Clinical Practice**.
535 *Clinical microbiology reviews* 2018; 31(4).
536 21. Uthman MM, Uthman OA, Yahaya I. **Interventions for the prevention of**
537 **mycobacterium avium complex in adults and children with HIV**. *The Cochrane database*
538 *of systematic reviews* 2013; (4):CD007191.
539 22. van Eijk AM, Terlouw DJ. **Azithromycin for treating uncomplicated malaria**. *The*
540 *Cochrane database of systematic reviews* 2011; (2):CD006688.
541 23. Huckle AW, Fairclough LC, Todd I. **Prophylactic Antibiotic Use in COPD and the**
542 **Potential Anti-Inflammatory Activities of Antibiotics**. *Respiratory care* 2018; 63(5):609-
543 619.
544 24. Wheeler ML, Limon JJ, Bar AS, Leal CA, Gargus M, Tang J, et al. **Immunological**
545 **Consequences of Intestinal Fungal Dysbiosis**. *Cell host & microbe* 2016; 19(6):865-873.
546 25. Chesdachai S, Rajasingham R, Nicol MR, Meya DB, Bongomin F, Abassi M, et al.
547 **Minimum Inhibitory Concentration Distribution of Fluconazole against Cryptococcus**
548 **Species and the Fluconazole Exposure Prediction Model**. *Open Forum Infect Dis* 2019;
549 6(10).
550 26. Rajasingham R, Boulware DR. **Cryptococcal antigen screening and preemptive**
551 **treatment - how can we improve survival?** *Clinical infectious diseases : an official*
552 *publication of the Infectious Diseases Society of America* 2019.

553 27. Boulware DR, Meya DB, Muzoora C, Rolfes MA, Huppler Hullsiek K, Musubire A, et al.
554 **Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis**. *The New*
555 *England journal of medicine* 2014; 370(26):2487-2498.
556