REDUCING MORTALITY IN ADULTS WITH ADVANCED HIV DISEASE (REVIVE)

A randomised, double blinded, placebo-controlled, multicentre trial of azithromycin prophylaxis for advanced HIV disease

CLINICAL TRIAL PROTOCOL

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Version Number: 3.0

7 December 2023

INVESTIGATOR'S AGREEMENT

I, _____, the investigator, have examined this REVIVE trial protocol and have fully discussed the objectives of this trial and the contents of this amended protocol with the REVIVE Coordinating Centre representative(s) from the Population Health Research Institute at Hamilton Health Sciences Corporation (Sponsor).

I agree to conduct the study according to this protocol and to comply with its requirements, subject to ethical and safety considerations.

I agree to comply with the International Council for Harmonization Tripartite Guideline on Good Clinical Practice (GCP), Tri-Council Policy Statement (TCPS2) and other applicable guidelines, and all applicable laws and regulations.

I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

I agree the Sponsor owns all data and intellectual property generated from this trial. I further agree that the first publication will be led by the trial's Steering Committee, after which I will receive access to the full dataset for internal education and non-commercial research purposes only.

I acknowledge the Sponsor will indemnify my hospital and I for any third-party claims resulting directly from PHRI's negligence or wilful misconduct in this trial, provided that such claim is not caused by my hospital's or my negligence or wilful misconduct.

Investigator Name: _____

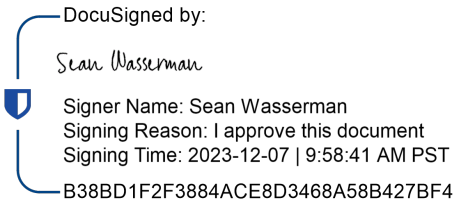
Investigator Signature: _____ Date: _____

Facility/Site: _____

Country: _____

PROTOCOL APPROVAL

Signature:



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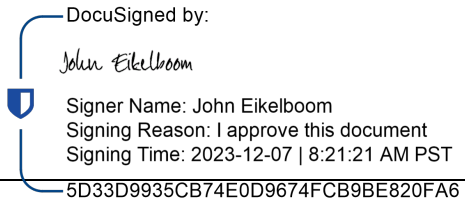

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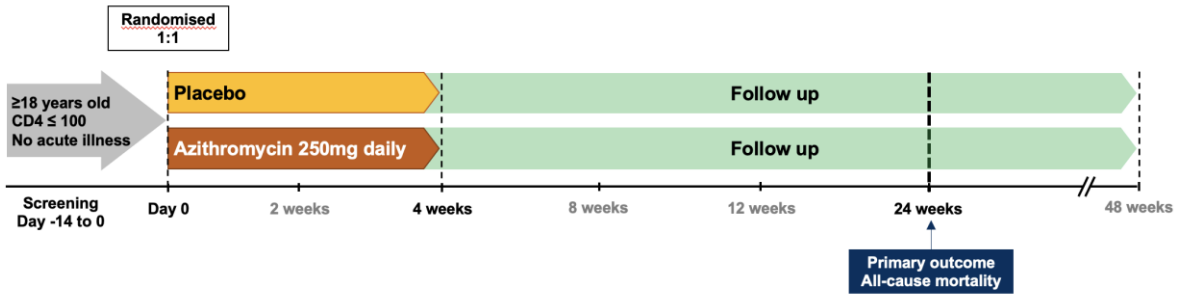
1.0 SYNOPSIS

Acronym	REVIVE
Trial title	Reducing mortality in adults with advanced HIV disease
Study design	A double blinded, placebo-controlled, event-driven multicentre trial to evaluate effectiveness of azithromycin prophylaxis for prevention of mortality in advanced HIV. All participants will be randomised (1:1) at the time of study entry to receive azithromycin prophylaxis or placebo for 28 days and will be followed for 24 weeks to determine the primary outcome measure. An overview of the study is provided in Figure 1.
Hypothesis	Azithromycin prophylaxis, provided at study entry as a 250 mg daily tablet for 4 weeks, will reduce all-cause mortality at 24 weeks compared with placebo, in patients receiving local standards of care.
Population	HIV-positive adults with CD4 counts ≤ 100 who are initiating, reinitiating, or switching to suppressive antiretroviral therapy.
Intervention and control	Enhanced antimicrobial therapy with azithromycin 250 mg once daily for 4 weeks, started at the time of study entry. The intervention will be compared with matching placebo and given on a background of standard of care for advanced HIV disease as determined by relevant local guidelines.
Primary outcome measure	All-cause mortality at 24 weeks after randomisation
Secondary outcome measures	▪ All-cause mortality at 12 weeks ▪ Hospitalisation at 24 weeks ▪ Composite of hospitalisation and all-cause mortality at 24 weeks
Exploratory outcomes	▪ Cause of death (verbal autopsy) ▪ Incident severe infection episodes through 24 weeks. ▪ Mortality and retention in care at 48 weeks after study entry.
Randomisation	Participants will be allocated 1:1 to azithromycin or placebo
Number of participants	8,000 adults aged 18 years or older
Duration	Each participant will be followed for 48 weeks in the trial
Ancillary studies	▪ Economics and cost-effectiveness ▪ Social science research (acceptability of intervention, determinants of adherence)

1.1 Figure 1. Trial schema



2.0 BACKGROUND/RATIONALE

2.1 Advanced HIV disease remains a public health problem in the ART era

The impact of HIV has been most profound in low- and middle-income countries and particularly in the African region, where 25.3 million people are living with HIV [1]. The introduction of ART has drastically improved the prognosis of HIV infection, with clinical trials demonstrating durable suppression of plasma HIV-RNA (viral load) in 95% of patients living with HIV [2-4]. While individuals who attain virological suppression may still encounter health risks related to HIV, their life expectancy approaches that of their HIV-negative counterparts [5]. The worldwide roll-out of ART constitutes a remarkable public health achievement, with approximately 77% coverage in the African region where 19.5 million people living with HIV were receiving ART by the end of 2020 [1]. In countries with high HIV-related mortality, ART has prevented millions of HIV-related deaths and has contributed to rising population-level trends in life expectancy [6, 7]. Despite the widespread availability of ART, however AIDS-related illnesses still cause a large number of deaths [1]. The greatest burden of premature death is in Africa where HIV is still consistently reported in the top five causes of mortality [1, 8].

ART modifies the natural history of HIV through suppression of HIV RNA, restitution of CD4 counts which improves cellular immunity, and reduced HIV-associated inflammation,[9, 10] thereby preventing opportunistic infections and HIV-related mortality.[11, 12] The Joint United Nations Programme on HIV/AIDS (UNAIDS) estimates that globally 1.2 million deaths have been averted by ART; in sub-Saharan Africa, where access to ART was delayed, HIV-related deaths have been reduced by 64% since the peak in 2004 and by 47% since 2010. Nevertheless, the risk of disease progression and death remains much higher in sub-Saharan Africa than in high-income countries, driven primarily by high early mortality due to opportunistic infections. CD4 cell count at baseline is the strongest prognostic factor for progression to AIDS and death, even after starting ART [13-15]. The risk of opportunistic diseases is greatest at CD4 < 200 cells/mm^3 and persists for longer if ART is started at a lower CD4 baseline. With ART and effective viral suppression, the expected CD4 cell response is an increase of approximately 50 to 150 cells/mm^3 at one year. Accordingly, individuals starting ART at low counts remain at high risk for longer [16], and patients with advanced HIV initiating ART at CD4 count ≤ 100 cells/mm^3 have up to 17% mortality during the first 6 months, and particularly in the first 4 weeks [15-18]. These high mortality rates are despite routine concomitant use of the WHO-recommended package of supportive care (cotrimoxazole prophylaxis, TB preventive therapy, and screening for cryptococcal disease) [15]. The majority of adults living with HIV in sub-Saharan Africa have advanced disease when they start ART [19], and approximately one-quarter start with a CD4 counts ≤ 100 cells/mm^3 [20].

Late presentation is not the only driver of advanced HIV; even when ART is initially successful, virological suppression rates in clinical practice in low-and middle-income countries remain far below those observed in clinical trials, and failure of ART occurs in 10-20% of individuals within the first years of treatment [21-23]. Loss of virological suppression may result in progression to advanced HIV disease during treatment and substantially increases the risk of death [24-26]. Over one-third of people living with HIV in the African region are lost to follow-

up within 3 years of ART initiation [27]. As a result of treatment failure and loss to follow-up, a substantial proportion of people with advanced HIV are ART-experienced, re-engaging in care after declining CD4 counts [28, 29]. The large number of incident cases further contributes to the persistently high mortality burden due to HIV in sub-Saharan Africa [17, 30, 31]. **Additional strategies to reduce early mortality are needed for people with advanced HIV disease (AHD), and WHO has prioritised research in this area.**

2.2 Bacterial infections among adults with AHD

Bacterial infections (mainly pneumonia and bacteraemia) are leading causes of hospitalization and mortality among adults with HIV, particularly in SSA [32]. The highest hospitalization rates are seen in patients with CD4 counts ≤ 100 cells/mm^3 [33]. In two large trials of intensified adverse event monitoring on ART in SSA, the most frequent causes of death were 'septicaemia/meningitis' and unknown causes. Identified pathogens included *Streptococcus pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* [15]. Febrile patients with HIV in Africa are over 3-fold more likely to have bloodstream infection compared with adults without HIV [34, 35]. At a single hospital in Malawi, the incidence rate of bloodstream infection was much higher in the first 3 months after ART initiation (68/1000 person years) compared with ART-naïve (incidence rate ratio 80; 95% CI, 46 - 139) and HIV-negative (incidence rate ratio 568; 95% CI, 302 - 1069) patients and associated with a case fatality of 14%. That study estimated a minimum annual case fatality from confirmed or suspected BSI of 18 deaths per 1000 adults in the first 3 months of ART in Blantyre [36].

In the most recent systematic review, bloodstream infections among febrile adults hospitalised in Africa were dominated by typhoidal (11%) and non-typhoidal *Salmonella* (19%), *E. coli* (11%), and pneumococcus (14%); *S. aureus* was also an important cause [34]. HIV, particularly at low CD4 counts, is a major risk factor for invasive non-typhoidal *Salmonella* infection [35, 37], which may cause up to 40% of community-acquired bloodstream infections in SSA with a case fatality close to 20% [37]. *Streptococcus pneumoniae* is the leading cause of lower respiratory tract (LRTI) morbidity and mortality globally - resulting in 55.4% (1,189,937) of all LRTI deaths - with the highest number of cases in Africa [38]. Invasive pneumococcal disease (IPD) is associated with high morbidity and in-hospital mortality, ranging between 8 and 25% [39-43]. PLWH are at much higher risk of IPD and community-acquired pneumonia (CAP) - up to 100-fold pre-ART - than among age-matched adults without HIV [44-46]. Illustrating this, almost 90% of IPD cases in South Africa are HIV infected [44]. Risk is greatest at lower CD4 counts [47], and although ART reduces incidence of IPD [48], the risk for both IPD and CAP remains high for PLHIV, even in those who have high CD4 cell counts and are virally suppressed (up 60-fold increased risk on ART compared with HIV-negative people) [43, 46]. Introduction of conjugate pneumococcal vaccination into childhood immunisation programmes has not resulted in significant reduction of IPD among adults in Malawi and the Gambia [49], with only modest indirect benefits in other SSA countries [50, 51]. Pneumococcal carriage, a prerequisite for invasive disease, remains high among PLWH after starting ART suggesting a continued defect in respiratory mucosal immunity [52], persistent reservoir for transmission, and ongoing risk for IPD [53, 54].

Most bacterial infections in people with AHD in SSA are not ascertained because of inherent limitations of diagnostic testing, poor access to resources such as radiology, microbiology, and routine laboratory tests, and lack of systematic investigation of causes of death in this population. Consequently, identified bacterial infections are likely to be far outnumbered by undetected cases in AHD. *Post hoc* analyses in the REALITY trial (described below) indirectly suggested a role of bacterial infection as an undiagnosed contributor to mortality given that azithromycin was potential explanation for reduction in undiagnosed causes of death [55]. Early mortality from invasive bacterial infection in AHD occurs despite ART initiation and other interventions such as cotrimoxazole prophylaxis and childhood pneumococcal vaccination. There are high levels of cotrimoxazole resistance among invasive pneumococcal, *Salmonella*, and *S. aureus* isolates in Africa (22 - 99%), threatening the benefit of this longstanding intervention [34, 35, 41, 56-60] and a likely reason for lack of impact on BSI incidence and outcome on ART [36]. **Given that severe bacterial disease is under-recognised as a cause of mortality in this population and is not easily identifiable within HIV programmes, we hypothesise that a broad prophylactic strategy to reduce the incidence of invasive infection targeting common bacterial pathogens, particularly pneumococcus and Salmonella, with broad-spectrum antibiotic prophylaxis will improve early ART outcomes in this high-risk group.**

2.3 Azithromycin as a strategy to reduce early mortality in AHD

Azithromycin, an azalide antibiotic that inhibits bacterial protein synthesis, is highly active against a broad spectrum of bacterial pathogens that cause morbidity and mortality in AHD. These include sensitive strains of *S. pneumoniae, S. pyogenes, S. aureus*; 'atypical' bacteria causing pneumonia; gram-negative respiratory pathogens (*H. influenzae, M. catarrhalis*); and enteric pathogens (*Salmonella sp, E. coli*, Shigella *sp, V. cholera, C. jejuni*). Azithromycin also has activity against other infections that contribute to morbidity in AHD in SSA, including syphilis, leptospirosis, malaria, pneumocystis pneumonia, non-tuberculous mycobacteria, and other sexually transmitted infections.

Like other macrolide antibiotics, azithromycin has potent and wide-ranging immunomodulatory properties which may favourably modulate immune dysregulation in AHD. Documented effects include reduced release of pathogen-associated molecular patterns (PAMPs) by bacteria and inhibition of toll-like receptor surface expression and signalling by monocytic immune cells; enhanced phagocytosis of bacteria; inhibition of pro-inflammatory cytokines and chemokines; and stimulation of neutrophil apoptosis [61, 62]. Possibly and in part attributable to these effects, there is evidence of survival benefit with macrolide use in severely ill patients with pneumonia, as well as in pneumonia-derived sepsis, with most pronounced benefit in pneumococcal pneumonia [61]. Although 'off-target' immune effects may be beneficial in AHD, the dominant mechanism of protection from azithromycin is likely to be prevention of invasive bacterial infection during the early high-risk period of profound immunosuppression following initiation (or re-initiation) of ART and prior to significant recovery of immune function. **Azithromycin potentially provides additional protection over cotrimoxazole prophylaxis because of broader spectrum of antimicrobial activity, high rates of cotrimoxazole resistance, and anti-inflammatory properties.**

Azithromycin has favourable pharmacokinetic (PK) characteristics and an excellent safety profile [62]. It undergoes rapid clearance from plasma with extensive tissue distribution and intracellular accumulation - up to 800-fold higher concentrations than plasma - within polymorphonuclear leucocytes, monocytes, lymphocytes, and alveolar macrophages [63, 64]. Slow-release accounts for the long elimination half-life in plasma (~70 hours) and maintenance of intracellular pharmacodynamic target concentrations for over 7 days after dosing [62, 65-67]. Accumulation occurs at sites of inflammation, possibly through transfer from fibroblast reservoirs to nearby phagocytes for transport to the site of infection [64]. Azithromycin is a weak inhibitor of CYP3A4 with low potential for PK drug-drug interactions with ART and other agents used in recommended AHD packages of care [68].

Azithromycin is widely used in sub-Saharan Africa for treatment of other bacterial infections, including as mass administration for trachoma control, with no serious safety signals [69, 70]. Discontinuation rates were only 0.8% in RCTs for short-course azithromycin (n = 4,870) [71]. Azithromycin is well-tolerated and safe among HIV-positive patients in clinical trials of prolonged therapy for non-tuberculous mycobacterial infection [72-74]. Long-term azithromycin prophylaxis for chronic lung disease is remarkably safe with rates of adverse events similar to placebo in multiple trials [75-78]. Azithromycin does not require dose adjustment in patients with renal impairment and is the most used macrolide in pregnancy [79]. The most common side effects are gastrointestinal (nausea, vomiting, diarrhoea, abdominal pain) but these are generally mild and do not usually require discontinuation. Azithromycin has been associated with transient sensorineural hearing loss among patients on long term prophylaxis and high dose therapy [76, 80], although the evidence is weak and inconclusive [81]. A Cochrane review of four randomised controlled trials concluded that macrolide antibiotics were associated more often with hearing loss compared to controls, but the odds ratio was borderline statistically significant, 1.30 (95% CI 1.00 to 1.70) [80]. Hearing loss has been occasionally reported in HIV-positive patients receiving long-term azithromycin prophylaxis for *M. avium* complex, but this is almost always transient [82, 83].

Azithromycin is associated with minor (~10 ms) QT prolongation, a much smaller effect than other macrolides [79]. A population-based study among older patients in the US reported a prolonged QTc interval in association with azithromycin use, with an estimated 0.047 additional deaths per 10,000 azithromycin courses, almost entirely among patients with a high baseline risk of cardiovascular disease [84]; only one death was observed in 144,165 persons in the lowest four deciles of risk scores for cardiovascular disease [85]. Other observational studies in high income countries have produced inconsistent results [84, 86] , and the increased risk is more likely attributable to underlying patient factors associated with azithromycin prescription [87]. Supporting this, the risk of cardiac events was not increased with azithromycin use in pooled data from 7 randomised controlled trials (n = 1715) [80], and there was no evidence of excess cardiac deaths in large population-based analyses involving younger patients at low baseline risk for cardiovascular disease [85, 86]. A recent systematic review and meta-analysis including data from 33 studies and 22,601,032 individuals found no association between the risk of arrhythmia or cardiovascular mortality and macrolide use. Overall, there was a small increased risk of myocardial infarction, but on network meta-analysis, this was significantly higher for erythromycin and clarithromycin compared with azithromycin [88]. Taken together, extensive data from clinical trials and observational studies strongly support the cardiac safety of azithromycin, particularly in young populations with low underlying propensity for cardiac disease.

There are much stronger signals from clinical trials that azithromycin prophylaxis may reduce mortality among high-risk populations in SSA. The REALITY trial (n = 1805) evaluated azithromycin 500 mg daily for 5 days as part of package of enhanced prophylaxis (including fluconazole, IPT, and albendazole) provided at the time of ART initiation among adults and older children with AHD in three SSA countries [17]. The group allocated enhanced prophylaxis experienced a 27% relative reduction in the primary outcome of 24-week mortality compared with standard of care (HR 0.73; 95% CI 0.55 - 0.98); the absolute mortality reduction was over 3% (8.9% vs. 12.2% for standard prophylaxis). The greatest effect on mortality was observed in the subgroup with CD4 counts ≤ 50 cells/mm^3. Enhanced prophylaxis was associated with fewer early deaths from unknown causes (3.8% vs 6.1% for standard prophylaxis) and fewer hospitalisations, but not a significant reduction in deaths due to bacterial infection (although this was poorly ascertained). 30% of deaths were from unknown causes, and most deaths from potentially azithromycin-responsive infections occurred within 4 weeks after starting ART, suggesting that azithromycin contributed to overall mortality reduction [55].

Findings from the MORDOR trial support an independent effect of azithromycin on survival in populations at high risk of death from bacterial infection. This large cluster randomised trial (n = 190,000 individuals) allocated communities across three SSA countries to provide young children with either single dose azithromycin or placebo every 6 months for 2 years. All-cause mortality was 14% lower in the azithromycin communities, with the largest effect in Niger which had the highest mortality rate [89].

Azithromycin use can promote antibiotic resistance in both commensal and potentially pathogenic bacteria. Mass azithromycin provision selects for macrolide resistance in some potentially pathogenic organisms, with a possible population-level dose–response resulting in increased resistance selection as the number of distribution cycles increases [90-92]. Additionally, azithromycin may cause important changes in the human gut microbiome (reductions in observed richness and Shannon diversity) during or shortly after exposure [93, 94]. However, increased carriage of azithromycin-resistant pathogens appears to be transient, with replacement by susceptible strains soon after discontinuation of azithromycin prophylaxis and removal of selective pressure [95, 96]. The clinical and population-level impact of pathogen-specific and genetically-expressed resistance is unknown, and the health effects from shifts in gut microbiome structure also remain unclear [94]. Any negative public health consequence needs to be weighed against the much larger relative reduction in pathogen carriage and potential benefit on important clinical outcomes [75].

The enhanced prophylaxis package that improved survival in the REALITY trial has not changed policy because of uncertainty around the independent effect of azithromycin. Other components of that package - presumptive cryptococcosis therapy and TB preventive therapy - are recommended as standard of care for AHD based on conclusive evidence of benefit for each individual intervention. **Azithromycin prophylaxis in AHD is not recommended by international guidelines because evidence only exists as part of a package of interventions. Stand-alone evidence for use is needed to justify cost, resistance issues, and investment into establishing supply chains for large scale deployment. Given the proven safety and accessibility of azithromycin, plus a strong possibility of efficacy, WHO has prioritised research for "approaches to antibiotic therapy within a public health approach, and specifically, the independent effect of short-course azithromycin on mortality [92]."**

2.4 Justification of trial design

People with advanced HIV disease are at high risk of hospitalisation and early mortality from undetected bacterial infections. In the absence of accurate and accessible diagnostics, interventions with proven ability to reduce severe bacterial disease could improve early outcomes in this population. There is indirect evidence for the intervention being tested in the REVIVE trial, but several factors (cost, concern about resistance, supply chain requirements) preclude large scale deployment prior to definitive demonstration of benefit. REVIVE will provide a pragmatic assessment of a cost-efficient intervention to save lives among people with advanced HIV disease, a high-risk group that represents a substantial proportion of ART-naïve and experienced persons living with HIV in sub-Saharan Africa. The trial population is representative of typical patients accessing ART care in programmatic settings. The intervention is already widely used in sub-Saharan Africa for other indications and could readily be implemented in ART programmes in this setting. REVIVE has a pragmatic design with a simple parallel group comparison and an important clinical outcome measure that can be ascertained across diverse clinical trial sites, thereby also serving as a possible platform to evaluate other promising interventions.

3.0 OBJECTIVE AND HYPOTHESIS

3.1 Objectives

The primary objective is to determine whether azithromycin is an effective and safe intervention to reduce excess mortality in adults with advanced HIV (CD4 ≤ 100 cells/mm^3).

Secondary objectives include exploring effects on mortality and hospitalisation at early and late timepoints, impact on incident infection, and cause of death.

3.2 Hypothesis

The primary hypothesis is that azithromycin prophylaxis, provided at study entry as a 250 mg daily tablet for 4 weeks, will reduce all-cause mortality at 24 weeks compared with placebo, in patients receiving local standards of care.

4.0 STUDY DESIGN

A multi-centre, placebo-controlled, double-blinded, parallel group, event-driven, randomised controlled trial.

4.1 RANDOMISATION

Participants will be allocated 1:1 to azithromycin or identical placebo. Randomisation (azithromycin vs. placebo) will occur at the time of study entry, stratified by site. Randomisation will occur via a secure web-based system pre-programmed with a computer generated, sequentially numbered randomisation list.

5.0 INTERVENTION

5.1 Study treatment

Azithromycin 250 mg once daily orally, starting at the time of randomisation, for 4 weeks (28 days); OR identical placebo, at the same dosing schedule.

The azithromycin dosing strategy is selected to balance likelihood of efficacy with cost, any side effects, and adherence. The labelled dose for treatment of respiratory tract infections is a loading dose of 500 mg followed by 250 mg daily for up to 5 days. The REVIVE trial will not provide a loading dose because rapid attainment of therapeutic targets is not required for prophylactic use. Dosing at 250 mg daily will be extended for 4 weeks to cover the early risk period after ART (re)initiation when most bacterial deaths are hypothesised to occur prior to immune reconstitution. Pharmacokinetic targets for antibacterial effect have not been established. However, with a 250 mg daily dose, intracellular azithromycin concentrations (the site of infection by *S. pneumonia* and *Salmonella* spp., the most common bacterial pathogens in AHD) are expected to greatly exceed the minimum inhibitory concentrations for susceptible organisms over the full dosing interval, and for over 21 days post-dosing [67]. Further supporting this dose, daily azithromycin dosing at 250 mg for up to one year was safe and effective as prophylaxis against exacerbations of chronic obstructive pulmonary disease and reduced nasopharyngeal colonisation by respiratory pathogens in a large RCT and in meta-analyses [76, 93].

5.2 Standard of care and concomitant medications

The trial will be conducted on a background of standard of care (SOC) according to relevant guidelines (national and/or local) which is expected to include:

- Antiretroviral therapy
- TB preventive therapy
- Cotrimoxazole prophylaxis
- Cryptococcal antigen screening plus fluconazole prophylaxis if positive

All SOC interventions will be provided by routine care; the trial will be restricted to sites able to provide SOC as recommended by local or national guidelines at that site. National guidelines vary with respect to TB preventive therapy and cryptococcal preventive strategies across countries in Africa.

There are no absolute contraindications for concomitant medications. Antacids should not be administered simultaneously because they reduce absorption.

6.0 STUDY POPULATION

Patients with suspected or known AHD will be referred by local clinicians at the recruitment sites. Both outpatients and hospitalised patients may be screened for enrolment.

6.1 Inclusion criteria

- Age ≥ 18 years
- Documented HIV infection
- CD4 count criteria:
    - CD4 count ≤ 100 cells/mm^3 within past 4 weeks; or
    - Documented CD4 nadir ≤ 100 cells/mm^3 and complete interruption of ART for ≥ 6 months; or
    - Documented CD4 count ≤ 100 cells/mm^3 if ART-naive
- Ability to initiate or re-initiate ART, or switch to an effective ART regimen if failing current therapy, within 4 weeks of enrolment

6.2 Exclusion criteria

- Contraindications to azithromycin:
    - Hypersensitivity to azithromycin, erythromycin, or any macrolide antibiotic; or
    - Personal or family history of QT-prolongation
- Severe illness requiring immediate or continued hospitalisation*
- Off-label azithromycin prophylaxis or requirement for prolonged (> 7 days) azithromycin (or macrolide) therapy

Most patients with HIV-associated TB will be eligible as WHO guidelines recommend ART initiation within 2 weeks of TB diagnosis for this group and there are no significant drug-drug interactions between azithromycin and anti-tuberculosis agents. Individuals with newly diagnosed cryptococcal and TB meningitis will be eligible if ART initiation is planned within around 4 weeks of study entry. Potentially eligible patients currently on systemic antibiotic therapy and/or who require hospitalisation for an acute severe illness may be rescreened once stable and ready for discharge or referred to a step-down facility for longer-term care. Pregnant and breastfeeding women will not be excluded as azithromycin has demonstrable safety profiles in pregnancy, and this population group may be at even higher risk of severe outcomes with AHD, potentially benefiting from the intervention and trial participation.

*This will be in the judgement of site investigators.

7.0 OUTCOME MEASURES

7.1 Primary outcome

All-cause mortality over the first 24 weeks after randomisation.

7.2 Secondary outcomes

- All-cause mortality at 12 weeks.
- Hospitalisation at 24 weeks.
- Composite of hospitalisation or all-cause mortality at 24 weeks.

*Hospitalisation is defined as an overnight stay in a medical facility, including inpatient wards and emergency units, because of an acute illness (i.e., new, or worsened pre-existing illness).

7.3 Safety outcome

The main safety outcome is adverse drug reactions that lead to permanent discontinuation of study drug or that lead to death.

7.4 Exploratory (at selected sites)

- Cause of death (using a validated shortened verbal autopsy instrument [94]).
- Incident severe infection episodes through 24 weeks, defined as a clinical diagnosis of infection leading to hospitalisation and/or isolation of pathogenic bacteria from a sterile site.
- Mortality and retention in care at 48 weeks after study entry.

7.5 Ancillary studies

1. Quality of life and cost-effectiveness
2. Social science research (acceptability of intervention, determinants of adherence)

8.0 STUDY VISITS AND PROCEDURES

8.1 Screening, consent, and randomisation

Referred patients will be pre-screened for inclusion after providing verbal consent. Eligibility information (HIV status, CD4 count) will be collected by site investigators or a designee from medical records or direct interaction with patients and recorded in an electronic case report form (eCRF). If a current CD4 count is required for eligibility assessment, verbal consent will be obtained from otherwise eligible patients to perform CD4 count testing; this will be done either using a point of care test or at the local laboratory and results shared with treating clinicians to inform routine care. Screening evaluations to determine eligibility must be completed within 14 days prior to study entry. In addition to data being collected for participants who enrol into the study, anonymised demographic, clinical, and laboratory data on screening failures will be captured in a Screening Failure Log form and stored in a separate database for non-randomized patients. Written informed consent (electronic and/or hardcopy, dependent on site) will be obtained from all eligible participants before enrolment into the study. Consenting participants will be randomised in real-time using a central web-based randomisation tool; study medication will be dispensed within 24 hours of randomisation.

8.2 Baseline information

Baseline information to be recorded on eCRFs by the site investigator, attending clinician, or delegate will include:

- Demographics
- ART history
- Concomitant medications
- Vital signs and biometrics
- Major comorbidities, including current and previous opportunistic diseases

8.3 Follow up information

<u>All participants</u>
Follow up visits will be conducted in accordance with the schedule of events (Table 1). Information to be ascertained at all follow up visits or at the time of awareness of death (whichever is sooner) includes:

- Adherence
- Concomitant medications, including ART
- Incident opportunistic diseases
- Vital status (alive/dead, with date and presumed cause of death, if appropriate)
- Hospitalisation status (inpatient/discharged, diagnosis, and date of discharge, if appropriate)

This information will be obtained and captured onto eCRFs by a member of the clinical or research team. Follow-up information is to be collected on all study participants, irrespective of whether they complete the scheduled course of allocated study treatment. Study staff will

seek follow-up information through various means including medical staff, telephonic contact, home visits, reviewing information from medical records, routine healthcare systems, and registries.

Exploratory outcomes

Severe infection episodes and cause of death will be ascertained using various methods, including participant interviews, medical staff, telephonic contact with friends or relatives, home visits, reviewing information from medical records, routine healthcare systems, and registries. Verbal autopsies will be conducted by trained study staff using a validated shortened verbal autopsy instrument.

Mortality and retention in care will be assessed remotely at 48 weeks (telephone/hospital records/death register) to investigate long-term benefits of the intervention.

Cost-effectiveness analysis

The trial will measure healthcare-related costs, starting at randomisation and continuing for the duration of follow-up. Costs incurred by the participants and their families (transport, indirect and companion person's costs) will be obtained by participant reports. Reported transport costs will be confirmed using local information on distance and cost of transport. Information on hospitalisations (number, reason, and duration of stay) will be collected from hospital records, and data on other healthcare resource utilisation (outpatient visits, medications, and procedures) will be collected by abstraction of medical notes and by participant interview.

We will evaluate quality of life for cost-utility analysis using the European Quality of Life-5 Dimensions (EQ-5D) instrument which is comprised of two parts. The first consists of five items that are measured along a 3-point Likert scale assessing domains of patients' mobility, self-care, usual activities, pain/discomfort, and anxiety/depression, with scoring combinations resulting in a health utilities index. The second consists of a visual analogue scale where the participant is asked to rate his/her health state with endpoints labeled as 'The best health you can imagine' and 'The worst health you can imagine', resulting in a score from 0-100. The EQ-5D is validated for use in clinical trials has been translated into more than 130 languages; if required, translations of the questionnaire will be made and verified using standard forward and back translation procedures.

8.4 Study withdrawal

Any participant who discontinues study treatment will continue follow-up assessments through to the final study visit at 48 weeks except for those participants who withdraw consent for follow-up. If this occurs, the reason for withdrawal from further follow up must be documented. Data already collected from participants requesting withdrawal will not be deleted. Withdrawn participants will not be replaced.

8.5 Table 1. Schedule of events.

	Screening and Entry	Week 2	**Week 4**	Week 8	Week 12	**Week 24**	Week 48
Window (days)	*-14 to 0*	*±5*	*±7*	*±7*	*±14*	*±28*	
Screening and informed consent	X						
Randomisation	X						
Dispense azithromycin/placebo	X						
Study entry and baseline procedures	X						
Vital signs and biometrics			X				
Adherence check		X[a]	X[b]				
EQ-5D questionnaire	X		X			X	
Vital status and hospitalisation episodes		X[a]	X[b]	X[a]	X[a]	X[b]	
Outcomes[b]		X	X	X	X	X	X[c]

[a] Remote/telephonic visit

[b] Ascertain remotely if in-person visit not possible (using telephonic/hospital records/lab results/death register)

[c] Ascertain remotely (using telephonic/hospital records/death register)

9.0 ADMINISTRATION AND MANAGEMENT OF STUDY TREATMENT

9.1 Product Supply, Distribution, and Accountability

Study medication will be pre-packaged, labelled, and distributed to sites from a central repository. It will dispensed by a central or on-site pharmacy in each country and provided to participants by either site investigators or local clinicians at participating sites. Study drugs will be dispensed for 4 weeks. The tablets should be swallowed whole and may be taken with or without food. If a daily dose is missed, the participant will be instructed to administer the missed dose as soon as possible, unless the next dose is due within 6 hours. If the next dose is due within 6 hours, the missed dose will be skipped and the next dose will be administered as originally scheduled. Participants should not double the next dose of study drug to "make up" what had been missed. No dose adjustment is necessary in patients with renal impairment.

9.2 Clinical management

The routine clinical service at study sites will be responsible for ART management (choice of regimen, timing, adverse events) and provision of other HIV care (OI prophylaxis, screening for cryptococcal meningitis). Drug toxicity will be managed by clinical staff according to standard clinical practice.

10.0 SAFETY MONITORING AND REPORTING

10.1 Recording of adverse events

Azithromycin has been extensively studied and used for many years in millions of patients, and its adverse effects profile is well described. Similarly, the manifestations and clinical outcomes of advanced HIV disease are well known.

All serious adverse events[1] (SAEs) and adverse drug reactions that lead to permanent discontinuation of study drug will be recorded. Adverse events that do not result in permanent drug discontinuation will not be collected.

Serious events that are efficacy endpoints (including death and hospitalisation), safety endpoints (including adverse drug reactions that lead to permanent discontinuation of study drug or death), other known adverse effects of study medication (e.g., nausea and vomiting), consequences of the underlying disease (e.g., weight loss) or that are common in the study population (e.g., malnutrition), will be exempted from expedited reporting.

All adverse events will be reviewed by the DSMB on an ongoing basis and included in the final study report.

10.2 Expedited reporting

Serious adverse events with a reasonable causal relationship to study interventions, unexpected for the patient population under study or inconsistent with the product information (i.e., SUSARs) will be reported to regulatory agencies in an expedited manner.

10.3 Clinical Site Monitoring and Record Availability

Study personnel will monitor clinical research sites to ensure that the trial is being conducted in accordance with the protocol and local regulatory requirements. The Sponsor or its designee may also conduct for cause site-monitoring visits as detailed in the clinical monitoring plan.

[1] Serious Adverse Events are defined as those adverse events that result in death; are life-threatening; require in-patient hospitalization or prolongation of existing hospitalization; result in persistent or significant disability or incapacity; result in congenital anomaly or birth defect; or are important medical events in the opinion of the responsible investigator (that is, not life-threatening or resulting in hospitalization, but may jeopardise the participant or require intervention to prevent one or other of the outcomes listed above).

11.0 STATISTICAL CONSIDERATIONS

11.1 Sample size

An 8,000-patient trial targeting 713 primary events will provide 84% power to detect a 20% relative reduction in 24-week all-cause mortality with azithromycin compared with placebo (Table 2). These calculations assume a somewhat lower mortality rate and smaller relative risk reduction than those seen in the REALITY trial (12% control mortality rate, 27% relative risk reduction), because of improved standard of care (more potent and better tolerated ART, better access to prophylaxis for opportunistic infections) and because REVIVE is testing a single intervention (versus a package of care). The sample size calculation accounts for up to a 2% loss to follow up by 24 weeks; this is expected to be a worst-case scenario because of relatively short follow up, ability to ascertain primary outcomes remotely, recruitment from many experienced trial sites, and the pragmatic nature of the trial.

11.2 Table 2. Estimated sample sizes

Relative risk reduction = 20%			
Control Event Rate	Sample Size	Power	Expected number of events
10%	6,000	0.73	535
	7,000	0.79	624
	8,000	0.84	713
	9,000	0.88	802

11.3 Analysis plan

Azithromycin prophylaxis is hypothesised to be superior to placebo and will be analysed according to intention to treat including all randomised participants. The primary analysis will use time-to-event methods Cox proportional hazards regression, censoring at 24 weeks. We will explore treatment effects in pre-specified subgroup defined by sex, weight (≥40 vs <40 kg), active TB at randomization, cotrimoxazole prophylaxis (yes/no), isoniazid preventive therapy (yes/no), country, and CD4 count ≤50 or >50-100 cells/mm^3.

We will also explore whether the effects of azithromycin may differ by sex on cost-effectiveness and quality of life. We hypothesise that azithromycin will be clinically superior and cost-neutral, or cost saving compared to placebo (i.e., dominant).

For safety analysis, counts and percentages of and events will be presented by randomised group.

Detailed analysis plans, including for secondary aims, will be provided in a separate Statistical Analysis Plan, to be finalised prior to database lock.

11.4 Data management

Data will be collected on electronic case report forms (eCRFs) using an electronic data capture system; paper CRFs/worksheets may be used to capture information for periods where electronic data capture is unavailable. It is the Investigator's responsibility to ensure the accuracy, completeness, legibility, and timeliness of the data reported on the participant's eCRF. All data will be kept secure, and confidentiality of all study participants will be carefully protected. Data will be validated, managed, and stored in a de-identified database on a secure server at the Population Health Research Institute (PHRI).

12.0 ORGANISATIONAL STRUCTURE AND MANAGEMENT

12.1 Principal Investigators

The Principal Investigators will have overall responsibility for design and conduct of the study, and preparation of the Protocol and subsequent revisions, in collaboration with the Steering Committee.

12.2 Steering Committee

This group, consisting of the Principal Investigators, study chair, study statistician, involved subject matter experts, and National Leaders (representatives of site countries) will meet on a regular basis to assess study progress and discuss necessary interventions or protocol amendments as required. Specific responsibilities include:

- Agreement of the final Protocol and the Statistical Analysis Plan
- Reviewing progress of the study and, if necessary, deciding on protocol changes
- Review and approval of study publications and sub-study proposals
- Reviewing new studies that may be of relevance

12.3 Central coordination and study management

The study will be coordinated through PHRI, a joint institute of Hamilton Health Sciences and McMaster University, Hamilton, Canada. Regular meetings will be coordinated between a primary team consisting of the Principal Investigators, central research coordinators, and relevant team members from the PHRI and the University of Cape Town to assess trial progress on an ongoing basis, review recruitment rates by site, and address any potential need for site visits or direct intervention. Specific responsibilities include:

- Study planning and organisation of Steering Committee meetings
- Ensuring necessary regulatory and ethics committee approvals
- Development of Standard Operating Procedures and computer systems
- Monitoring overall progress of the study
- Provision of study materials to study sites
- Monitoring and reporting safety information in line with the protocol and regulatory requirements
- Dealing with technical, medical, and administrative queries from study sites

12.4 Study sites

Participating countries will be represented by National Leaders. Each site will be led by a local Principal Investigator. The Investigators undertake to perform the study in accordance with Good Clinical Practice and will provide documentation of their qualifications to the project office prior to study start. The Investigator is required to ensure compliance with respect to the visit schedule and procedures required by the protocol. The Investigator agrees to provide all information requested in the case report forms in an accurate and timely manner according to instructions provided. Random or for cause monitoring visits may be done by PHRI representatives. National Leaders and Principal Investigators are responsible for:

- Obtaining all relevant local permissions (assisted by the coordinating centres)
- All trial activities at the local site, including appropriate training and supervision for clinical staff
- Conducting trial procedures at the local site in line with all relevant local policies and procedures
- Dealing with enquiries from participants and others

12.5 Data Safety Monitoring Committee (DSMC)

An independent DSMC will review the accumulating study data on a quarterly basis (the frequency of review can be adjusted at their discretion based on emerging data) and make recommendations to the study leadership about the conduct of the trial, integrity of the data and trial discontinuation to ensure the overall safety of participants. The DSMC will be provided with summaries of safety (all reportable AEs), efficacy (deaths and hospitalisation), and administrative (including accrual, retention, compliance with study requirements) data. The guiding policies and operating procedures governing the DSMC will be described in a separate DSMC charter. Formal interim analyses will occur after enrolment of 50% and 75% of the target sample size. The DSMC will follow a flexible pragmatic monitoring approach for efficacy, based on a modified Haybittle-Peto boundary of 3 SD which must be crossed on two occasions 3 months apart in order to make a recommendation for stopping. Because these boundaries are extreme, no modification of the level of significance of the final results is necessary. The DSMC will also be monitoring the safety of the participants; there will be no pre-specified safety stopping rule.

13.0 REGULATORY AND ETHICS CONSIDERATIONS

13.1 Institutional Review Board (IRB) Review and Informed Consent

The study will be conducted in accordance with Good Clinical Practice, all applicable privacy requirements, and the guiding principles of the Declaration of Helsinki. This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the IRB responsible for oversight of the study. A signed consent form will be obtained from all participants. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation.

13.2 Participant Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain participant confidentiality. All records will be kept locked. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the Sponsor, IRB, or other local and international regulatory entities as part of their duties.

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