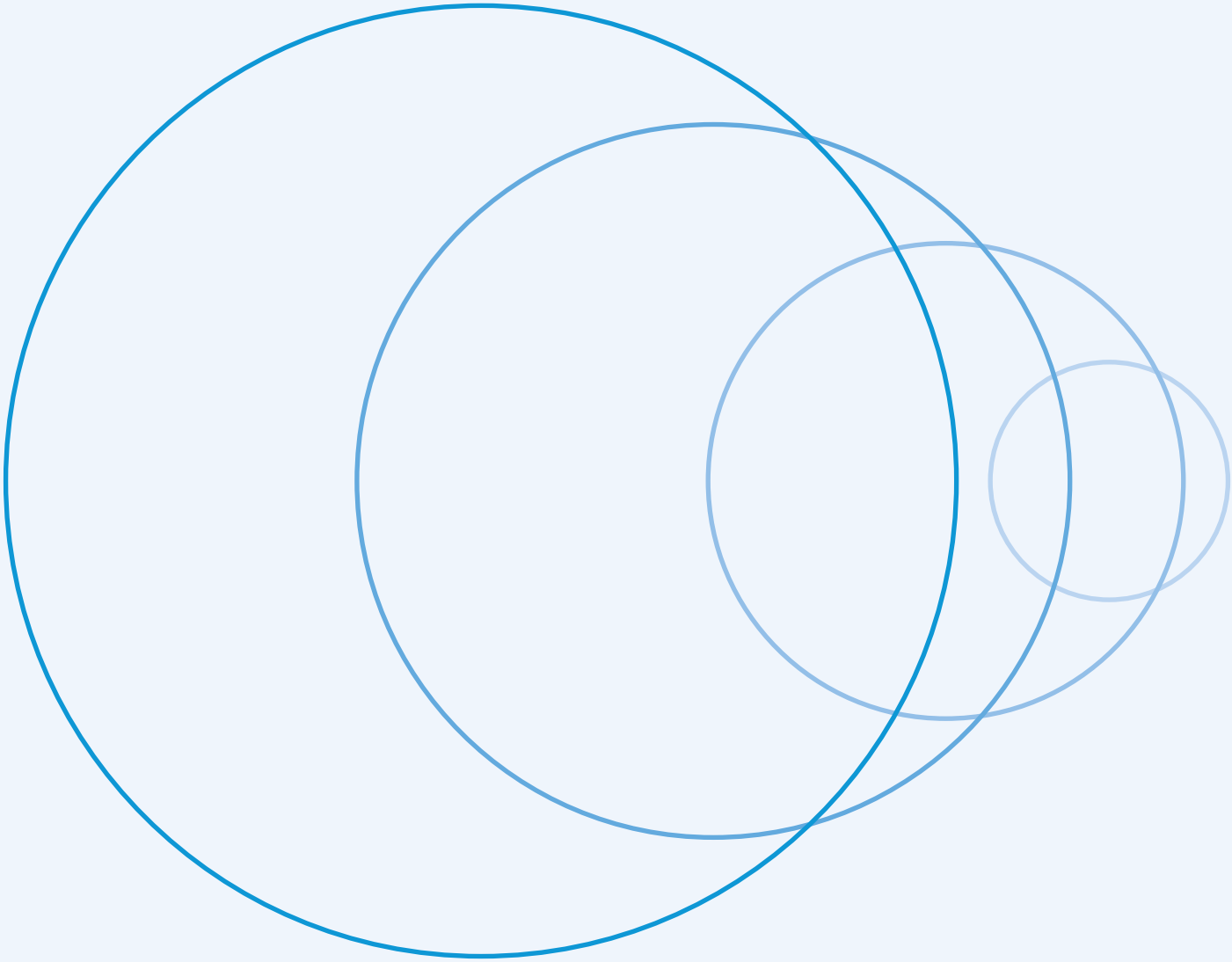
The advanced HIV disease research landscape



World Health Organization

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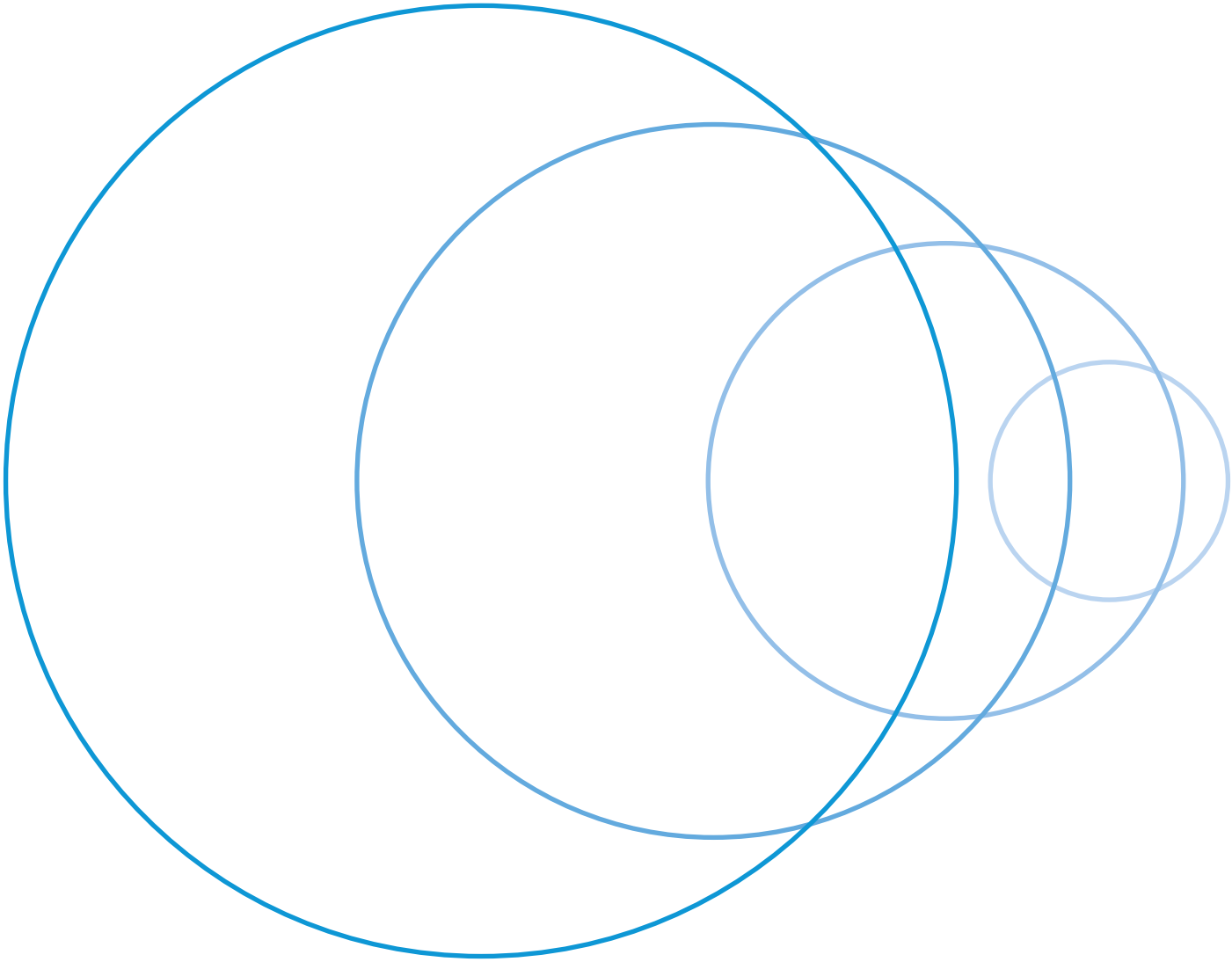

Introduction

WHO defines advanced HIV disease as CD4 cell count <200 cells/µL or WHO stage 3 or 4 disease for adults and adolescents *(1)*. All children younger than five years are considered to have advanced HIV disease regardless of immune or clinical stage. Despite widespread roll-out of antiretroviral therapy (ART), many people are still unable to access treatment until late in their disease course and present with advanced HIV disease. An increasing population is also at risk of developing advanced HIV disease after treatment failure because of difficulty in engaging with treatment services and adhering to lifelong medications or developing drug resistance. As a result, the proportion of individuals initiating or reinitiating ART with advanced HIV disease remains between 15% and 30% or higher in Africa *(2–7)*, with similar data reported from Latin America and the Caribbean *(8)* and Asia and the Pacific *(4)*. Individuals presenting for care with advanced HIV disease are at very high risk of AIDS-related morbidity and mortality *(2,5,9)*; UNAIDS estimates suggest that 650 000 people died from AIDS-related causes in 2021 *(10)*, although detailed data on the numbers and causes of HIV-related deaths are limited. Three opportunistic infections are primarily responsible for most AIDS-related deaths: invasive bacterial infections, tuberculosis (TB) and cryptococcal meningitis *(11)*. Other opportunistic infections occurring frequently in the context of advanced HIV disease with a global distribution include *Pneumocystis* pneumonia, cytomegalovirus and toxoplasmosis. In specific geographical areas, other infections including talaromycosis (South-East Asia and China), histoplasmosis (the Americas) and HHV8 and Kaposi sarcoma (Africa) cause a considerable burden of disease. Emerging infections can also disproportionately affect people living with advanced HIV disease; COVID-19 has been shown to cause increased morbidity and mortality among individuals with low CD4 counts *(12)*, and over the past year it has become apparent that mpox causes severe disseminated multiorgan disease among people with advanced HIV disease *(13)*.

Alongside efforts to expand HIV testing and early initiation of ART and improve monitoring and engagement of individuals in ART programmes, effective identification and management of individuals with advanced HIV disease are essential in comprehensive HIV care and treatment and a critical component of efforts to meet the ambitious target of ending AIDS as a public health threat by 2030 *(10)*. WHO has developed several guidelines and resources related to advanced HIV disease *(1,14–20)*.

This research landscaping work has been used to inform a roadmap for WHO scoping and future guidelines on Advanced HIV disease.

Roadmap for WHO scoping and future guidelines on Advanced HIV disease

	Expected results/policy changes		
Topic area	2024	2025–2026	2027–2028
CD4 testing	📋		
Cryptococcal meningitis			✔
Severe Bacterial infections			✔
Tuberculosis	📋	✔	
PCP		✔	
Toxoplasmosis		⌛	
CMV		⌛	
Talaromycosis	📋	✔	
Histoplasmosis		✔	
Kaposi Sarcoma	📋		

📋 Included for WHO guidelines scoping ✔ Study results expected ⌛ Pending new evidence, no changes expected

Methods

This research landscape report aims to provide an overview of nine key focus areas in advanced HIV disease, starting with CD4 testing as the entry point into differentiated advanced HIV disease care, followed by sections on the three leading causes of mortality in advanced HIV disease – TB, cryptococcal meningitis and severe bacterial infections. The research pipelines for three other opportunistic infections with a global distribution – *Pneumocystis* pneumonia, toxoplasmosis and cytomegalovirus – are described, followed by sections on talaromycosis and histoplasmosis. Finally, the report discusses other potentially relevant conditions associated with advanced HIV disease, including emerging and re-emerging pathogens. When data or guidance is specific to a certain age group, the text mentions this; if not specifically mentioned, it is assumed to be applicable to both children and adults. However, most trials for conditions related to advanced HIV disease discussed here did not include children or infants.

This report includes information up to 20 September 2023. It is based on publicly available information identified from databases (PubMed and ClinicalTrials.gov), conference abstracts (International AIDS Society and Conferences on Retroviruses and Opportunistic Infections); published and unpublished reports; and interviews with developers, researchers and manufacturers. Given the primary focus on the ongoing research pipeline and research in progress, a formal systematic review approach was not feasible and would not have yielded the required information. The findings were verified through a WHO virtual expert consultation convened on 11–12 September 2023.

Findings

1. CD4 testing

CD4 testing is critical for timely recognition of advanced HIV disease and as an entry point into advanced HIV disease care pathways; clinical staging has been shown to have very low sensitivity for identifying individuals with CD4 counts below 200 cells/µL *(5)*. Centralized laboratory-based CD4 testing using flow cytometry necessitates sending blood samples to laboratories for testing and delays obtaining results and the timely implementation of prophylactic, screening, diagnostic and treatment interventions.

Current WHO advanced HIV disease guidelines state that "CD4 cell count testing at baseline for all people living with HIV remains important" and that, to detect advanced HIV disease among ART-experienced individuals, "CD4 cell count testing should be specifically prompted for people with a viral load exceeding 1000 copies/mL and for everyone whose clinical presentation suggests advanced HIV disease regardless of whether they have been exposed to ART or not" *(1)*.

It is noted that "a simplified tool to perform CD4 cell count testing to ensure people with advanced immune disease are identified" is a research priority, and that "ongoing research is being undertaken, including using a semiquantitative CD4 cell count lateral flow assay (LFA) for which operational research will be required to evaluate feasibility under field conditions".

Since the WHO advanced HIV disease guidelines were published in 2017, there have been several marked changes in the CD4 testing landscape.

- As "treat all" has been rolled out globally, there has been a decline in donor and programme support for centralized laboratory CD4 testing infrastructure and commodities, and evidence from several African countries has shown a marked decline in the proportion of individuals initiating ART who receive baseline CD4 testing *(21–23)*, making diagnosing advanced HIV disease challenging.
- Two near-patient or point-of-care CD4 testing devices using flow-cytometry have undergone further validation and feasibility studies, demonstrating their utility for rapid near-patient CD4 testing *(24–26)*. However, for commercial reasons, both manufacturers are planning to discontinue production of the CD4 testing devices in 2024.
- A point-of-care semiquantitative CD4 LFA has come to market that aims to identify blood samples with CD4 counts of less than 200 cells/µL. The CD4 LFA received WHO prequalification in August 2020 and is being implemented in several ART programmes in Africa (personal communication: James Conroy, Clinton Health Access Initiative, Boston, MA, USA, April 2023).
  - Published studies of the performance characteristics of the point-of-care LFA test are limited, with just two published studies *(27,28)* and one conference poster *(29)* describing diagnostic performance (Table 1). Using flow cytometry as a reference standard, sensitivity of the test for categorizing the samples as CD4 ≤200 cells/µL in venous blood ranged from 94% to 95% and specificity ranged from 82% to 86% in published studies.
  - This compares to a reported sensitivity of 86–92% and specificity of 90–93% in the LFA test product literature. The use of finger-prick capillary blood increased the number of individuals classified as having advanced HIV disease using the assay, increasing sensitivity to 98% but reducing specificity to 77%.
  - Although the point-of-care test is an instrument-free LFA, performing the test requires a number of timed steps; blood is added to the test strip, followed by the addition of one drop of buffer after three minutes, the addition of three drops of buffer to a different well after a further 17 minutes, and a five-minute visual reading window comparing the intensity of two lines after a further 20 minutes.
  - Batched testing of six samples is possible.

Table 1. Currently published literature reporting point-of-care CD4 LFA advanced disease test performance

Reference	Key findings
Lechiile et al. *(27)*	A laboratory evaluation comparing point-of-care LFA to flow cytometry in Botswana. In a sample of 1053 venous blood samples, sensitivity of the LFA was 94.1% (95% CI: 88.3–97.6%) and specificity 85.9% (95% CI: 83.5–88.0%).
Ndlovu et al. *(28)*	An evaluation in the Democratic Republic of the Congo, Malawi and Zimbabwe. Compared point-of-care LFA to flow cytometry in (1) 708 venous blood samples in a laboratory-based evaluation, demonstrating a sensitivity of 95.0% (95% CI: 91.3–97.5%) and specificity of 81.9% (95% CI: 78.2–85.2%); and (2) 433 finger-prick samples collected at the point of care, demonstrating a sensitivity of 98.3% (95% CI: 95.0–99.6%) and specificity of 77.2% (95% CI: 71.6–82.2%).
Otobu et al. *(29)*	An evaluation across four Nigerian states comparing point-of-care LFA and CD4 flow cytometry. LFA testing was performed by health-care workers "in facilities" using venous or capillary blood (the relative proportions not specified). 603 participants were included, with point-of-care LFA sensitivity of 93.4% and specificity of 87.7%.
Gils et al. *(30)*	Implementation study of point-of-care LFA CD4 testing among 676 people living with HIV in Lesotho and South Africa. Point-of-care testing was administered by clinic staff to 672 of 676 (99.4%) of eligible clinic attendees. This enabled almost universal coverage of TB lipoarabinomannan (LAM) and cryptococcal antigen (CrAg) screening.

Research pipeline

The key data that will inform further guideline updates relate to the diagnostic performance of the point-of-care CD4 LFA advanced HIV disease test which, following the planned discontinuation of the two near-patient or point-of-care CD4 testing devices using flow-cytometry, will be the only near-patient or point-of-care CD4 test available for programmes to procure. Key questions about the diagnostic performance of the test in real-world conditions remain to be answered. Further data are required regarding performance on finger-prick capillary blood, information regarding feasibility, acceptability and task shifting and how testing could be operationalized, particularly in higher throughput clinics.

Costing and cost–effectiveness data would help to assess:

- the consequences of classifying a larger number of individuals as having advanced HIV disease who actually have CD4 counts exceeding 200 cells/μL and would then undergo screening and receive prophylaxis for opportunistic infections: 14–23% of all individuals with CD4 counts exceeding 200 cells/μL on flow cytometric testing would be misidentified as having advanced HIV disease based on the reported test specificity *(27–30)*; and
- the implications of semiquantitative testing that provides a single cut point at about 200 cells/μL, requiring all screening and prophylactic guidelines to use a nominal cut-off of about 200 cells/μL.

Data from several service evaluations of point-of-care LFA CD4 testing, including Nigeria (personal communication: Rita Oladele, Lagos University Teaching Hospital, Nigeria, April 2023) and Lesotho and South Africa (personal communication: Tinne Gils, Médecins Sans Frontières, May 2023) are anticipated by 2024. The Lesotho and South Africa evaluation will provide additional data regarding test performance and feasibility (a mixed-methods evaluation of acceptability, intervention delivery, process compliance and early effectiveness) of implementation of the point-of-care LFA advanced HIV disease test as part of an enhanced care package. Multiple other smaller country validation studies are underway for the purposes of registration in individual countries, including but not limited to: African Region: Democratic Republic of the Congo, Equatorial Guinea, Eswatini, Gambia, Kenya, Liberia, Malawi, Mali, Mozambique, Senegal, Sierra Leone, South Sudan, Togo, Uganda, United Republic of Tanzania and Zambia; Region of the Americas: Argentina, Brazil, Panama, Peru and Venezuela (Bolivarian Republic of) (personal communication: Eman Aleksic, United Kingdom, April 2023). It is unclear whether these data will be published or publicly available.

Two larger diagnostic evaluation studies of the point-of-care LFA advanced HIV disease test have been performed: one in collaboration with the Kenyan Medical Research Institute and the United States Centers for Disease Control and Prevention used in part for WHO prequalification and a second multicountry evaluation performed by FIND. The FIND study used finger-prick blood samples from 1600 individuals and compared with flow cytometry testing; the results are expected in late 2023. One ongoing randomized controlled trial (ENCORE) with retention in care and survival outcomes is ongoing, including an evaluation of the utility of point-of-care LFA advance HIV disease testing (of venous blood) versus conventional laboratory based CD4 testing plus a qualitative-methods study to understand health-care workers' perception of the test and an economic evaluation.

Key trial

ENCORE: an enhanced package of care to reduce mortality in advanced HIV disease

International registry identifier: NCT05085171

Type: Phase 3 trial.

Description: A community-based Phase 3, cluster randomized trial that seeks to determine 24-week survival with retention in care of point of care LFA CD4 testing and an enhanced package of screening and prophylaxis for opportunistic infections among people with advanced HIV disease.

Sample size and population: 2400 adult outpatients >18 years old with CD4 counts <200 cells/μL.

Study sites: Uganda

Interventions: An enhanced intervention package including point-of-care LFA CD4 testing, screening for TB and cryptococcal meningitis using TB LAM and semiquantitative CrAg LFA respectively and pre-emptive treatment with isoniazid and rifapentine for one month. Those with high CrAg titres will receive treatment for central nervous system (CNS) cryptococcal disease. Randomization will be factorial, with (1) comparison between point-of-care CD4 testing and standard flow cytometry and (2) enhanced package of opportunistic infection screening and prophylaxis, versus current WHO standard.

Primary outcome: 24-week survival with retention in care.

Status: Recruiting. 2021–2024. To date (September 2023), 1040 of the projected 2400 participants have been enrolled.

Summary points

- CD4 testing is essential to identify and stratify individuals appropriately for targeted advanced HIV disease interventions.
- Rapid near-patient or point-of-care CD4 testing enables same-day implementation of the advanced HIV disease package.
- Both currently available near-patient CD4 testing devices are being withdrawn from the market.
- Point-of-care LFA advanced HIV disease testing provides a possible alternative means to rapidly identify individuals with advanced HIV disease but has suboptimal specificity. Significant evidence gaps remain with regards to test performance in real-world settings and the feasibility of implementation in outpatient clinic and hospital settings.
- Additional diagnostic performance data and programmatic implementation data will be available in 2024–2025.
- Given the likely reduction in access to CD4 testing in the medium term, alternative strategies to identify individuals at risk of advanced HIV disease for stratification into differentiated care pathways may be required.

Implications

Updated guidelines will need to consider the scaling back of conventional laboratory based CD4 testing, restricted availability of near-patient rapid CD4 testing devices and the introduction of the point-of-care LFA advanced HIV disease test. Evidence to guide these updates will be available by late 2024 or early 2025 and included in scoping for the advanced HIV disease guidelines update in 2024.

2. TB

TB is the leading cause of morbidity and mortality among people living with HIV *(31)*. The prevention, diagnosis and treatment of TB among people living with HIV is summarized in the WHO consolidated HIV guidelines, with prevention and diagnosis screening of TB in the context of advanced HIV disease specifically also covered in the advanced HIV disease guidelines. Many aspects of TB diagnosis, screening, prevention and treatment are applicable to both people with and without HIV and to all people living with HIV regardless of CD4 counts. For a comprehensive review of the research pipelines, see the Treatment Action Group (TAG) 2022 pipeline reports for TB treatment *(32)* and diagnostics *(33)*.

WHO held Guideline Development Group meetings focusing on TB diagnostics and on TB preventive therapy in 2023. These topics are therefore not included in this pipeline document, with the focus restricted to other aspects of TB management specific to advanced HIV disease: TB screening and treatment of disseminated TB disease in the context of advanced HIV disease.

Further studies on systematic TB screening have led to revised WHO guidance on systematic screening for TB, chest X-ray , including artificial intelligence-based computer-aided. design tools for digital X-rays and C-reactive protein in addition to the WHO-recommended for symptoms screen for TB screening among people living with HIV. The uptake of these screening tools can help to optimize TB screening, depending on the national screening objectives. However, few of these screening interventions have been specifically designed for individuals with advanced HIV disease, and specificity in this group is generally low, particularly in inpatient settings. For example, the WHO-recommended four-symptom screen has a specificity of 0.30 (0.18–0.45) among adults with CD4 counts <200 cells/µL and 0.11 (0.08–0.14) among inpatients. Chest X-ray has a specificity of 0.14 (0.07–0.25) in adults with CD4 counts <200 cells/µL, and 0.07 (0.03–0.19) among inpatients. Using C-reactive protein as a screen for TB disease at a cut off of >5 mg/L has a specificity of 0.40 (0.22–0.62) for adults with CD4 counts <200 cells/µL and 0.12 (0.09–0.17) among inpatients *(16,35)*.

Current WHO advanced HIV disease guidelines recommend molecular rapid diagnostic testing as the first test for TB diagnosis among symptomatic people at any CD4 count. Molecular WHO-recommended rapid diagnostics (mWRD) can be used as a diagnostic test on sputum as well as the following specimens: urine, blood (for suspected disseminated TB in PLHIV), CSF, lymph node samples, pleural, peritoneal, pericardial or synovial fluid. The WHO advanced HIV disease guidelines additionall recommend the use of LF-LAM to assist in the diagnosis of active TB among adults, adolescents and children with HIV and signs and symptoms of TB (pulmonary and/or extrapulmonary) or with advanced HIV disease or who are seriously ill regardless of CD4 count, based on evidence from randomized controlled trials *(34)*. The guidelines also recommend using LF-LAM regardless of signs and symptoms of TB among hospitalized people with a CD4 cell count below 200 cells/µL or below 100 cells/µL in outpatient settings. Currently only one LF-LAM test is commercially available for TB, although several others are under development or currently undergoing validation.

Research pipeline

Few studies were identified focusing on TB screening among individuals with advanced HIV disease or inpatient populations. Data on the utility of urine Xpert Ultra screening combined with urine LAM testing among inpatients with advanced HIV disease were reported; among 238 inpatients with advanced HIV disease and TB symptoms, urine Ultra had a yield of 68% versus 45% for urine LAM for definite TB. Combined Xpert Ultra and LF-LAM testing had a yield of 73% *(36)*. A single study in Malawi – the CASTLE Trial – investigated an enhanced TB screening intervention (digital chest X-ray with computer-aided diagnosis plus urine LAM testing) among adults living with HIV who were admitted to hospital for acute care. The trial has now completed recruitment (see key trial details below), and the results are anticipated in late 2023 or 2024.

CASTLE: computer-aided screening for tuberculosis in low-resource environments

International registry identifier: NCT04545164

Type: Phase 2 trial

Description: The CASTLE trial aims to determine whether systematic screening for TB using digital chest X-ray with computer-aided diagnosis plus urine lipoarabinomannan testing plus usual care can improve admission outcomes for hospitalized people living with HIV versus usual care alone. A single-centre, unblinded, cluster-randomized (by day of admission) trial.

Sample size and population: 498 adult inpatients aged 18 years and older; people living with HIV regardless of CD4 count

Study sites: Malawi

Interventions: digital chest X-ray with computer-aided diagnosis plus TB LAM plus usual care versus usual care alone for screening for TB among unselected adults living with HIV admitted to a district general hospital in Malawi.

Primary outcome: TB treatment initiation

Status: Completed. 2020–2022.

Two trials are underway examining intensified TB treatment and the utility of corticosteroids for reducing the high mortality seen among inpatients with advanced HIV disease and disseminated TB. The DATURA trial is examining a combination intervention of increased dose of rifampicin and isoniazid plus prednisone. The combination intervention will mean that it is not possible to determine which components of the modified regimen are effective if the trial yields a positive result or whether one of the intervention components would have been effective alone in the event of a negative result. The NEW-STRAT TB trial is also examining an intensified drug regimen, in this case high-dose rifampicin and levofloxacin, and steroids using a factorial design that should enable the individual effects of the two interventions to be assessed. A third trial, the ATLAS trial (early empiric anti-*Mycobacterium tuberculosis* therapy for sepsis in sub-Saharan Africa, NCT04618198) is focusing on the role of TB treatment among people living with HIV presenting with sepsis syndromes based on data suggesting that TB causes 25–50% of bloodstream infections and is the leading cause of sepsis among people living with HIV *(37–39)*. The hypothesis of this study is that immediate optimally dosed anti-TB therapy will improve 28-day mortality among people with sepsis in Uganda and the United Republic of Tanzania. The trial is a Phase 3, open-label, 2-by-2 factorial clinical trial of (1) immediate empiric anti-TB therapy plus standard care versus diagnosis-dependent anti-TB therapy plus standard care and (2) sepsis-specific dose anti-TB therapy plus standard care versus conventional WHO weight-based dose anti-TB therapy plus standard care for treating hospitalized people living with HIV for sepsis.

DATURA: determination of adequate tuberculosis regimen in patients hospitalized with HIV-associated severe immune suppression

International registry identifier: NCT04738812

Type: Phase 3 trial.

Description: the DATURA trial is a Phase 3, multicentre, two-arm, open-label, randomized superiority trial to compare the efficacy and safety of an intensified TB regimen versus standard TB treatment for adults and adolescents living with HIV who are hospitalized for TB with CD4 ≤100 cells/µL over 48 weeks. The intensified TB treatment regimen includes increased doses of rifampicin and isoniazid together with standard dose of pyrazinamide and ethambutol for eight weeks in addition to prednisone for six weeks and albendazole for three days.

Sample size and population: 1330 people living with HIV 15 years and older with CD4 counts ≤100 cells/µL.

Study sites: Cambodia, Cameroon, Guinea, Mozambique, Uganda and Zambia.

Interventions: Rifampicin (R) 35 ± 5 mg/kg daily and isoniazid (H) 10 ± 2 mg/kg daily together with standard dose of pyrazinamide (Z) 20–30 mg/kg daily + ethambutol (E) 15–20 mg/kg daily for eight weeks plus prednisone 40–80 mg once a day according to weight bands for two weeks, followed by 20–40 mg once a day according to weight bands for two weeks and then 10–20 mg once a day according to weight bands for the last two weeks (total duration: six weeks) followed by standard continuation phase versus WHO standard TB treatment.

Primary outcomes: Mortality at 48 weeks.

Status: Recruiting. 2022–2025. As of September 2023, 380 participants were enrolled.

New-Strat: TB: testing new strategies for patients hospitalized with HIV-associated disseminated tuberculosis

International registry identifier: NCT04951986

Type: Phase 3 trial.

Description: The New Strat-TB trial is a superiority Phase 3 randomized controlled clinical trial with a 2-by-2 factorial design. The main aim of the study is to assess the efficacy and safety of high-dose rifampicin and levofloxacin for 14 days in addition to standard TB therapy with or without steroids among adults hospitalized with HIV-associated disseminated TB.

Sample size and population: 732 people living with HIV older than 18 years admitted to hospital with disseminated TB.

Study sites: South Africa.

Interventions: randomization 1: standard first-line anti-TB therapy plus additional rifampicin to reach 35 mg/kg per day for 14 days plus levofloxacin 750 mg/day for weight <50 kg and 1 g/day for weight >50 kg for 14 days versus standard TB therapy. After 14 days, both study arms will continue standard TB therapy. Randomization 2: prednisone 1.5 mg/kg per day for 14 days versus identical placebo for 14 days.

Primary outcomes: All-cause mortality at 12 weeks.

Status: Recruiting. Due to complete end 2024.

Several trials are exploring novel treatment approaches for tuberculous meningitis for people living with HIV, one of the most severe forms of TB. These are outside the direct scope of this review but are included in Annex 1.

Summary points

- WHO Guideline Development Group meetings focusing on TB diagnostics and on TB preventive therapy at the end of 2023 will review and update guidance applicable to advanced HIV disease in these areas.
- Updated 2021 WHO guidance on TB screening applies to individuals with advanced HIV disease; however, the applicability of the screening interventions to advanced HIV disease populations, especially inpatients, has yet to be well defined. Further research is required.
- Studies investigating the utility of urine Xpert Ultra screening in inpatient populations with advanced HIV disease and computer-aided chest X-ray screening in inpatient populations with advanced HIV disease have been completed and will report results in 2023.
- Updated evidence on the reworked TB LAM is projected to be available in mid-2024.
- Two trials investigating enhanced treatment for TB among hospitalized people with advanced HIV disease will report in 2025–2026.

Implications

Advanced HIV disease guidelines will require updates to ensure that TB-focused recommendations incorporate updates from TB diagnostic and TB preventive therapy guideline development groups and data from TB screening studies due to report in 2023; the findings are to be included in scoping review for advanced HIV disease guidelines update in 2024.

3. Cryptococcal meningitis

Cryptococcal meningitis accounts for about 20% of all AIDS-related deaths, with most cases occurring in Africa *(40)*. WHO published guidelines on managing HIV-associated cryptococcal disease in July 2022 *(18)*.

Research pipeline

Ongoing research likely to affect future WHO guidelines is ongoing in diagnostics, screening and treatment; each are considered below.

Diagnostics

The mainstay of diagnosis of symptomatic cryptococcal disease is through CrAg detection in cerebrospinal fluid (CSF) and/or blood using a widely available and affordable point-of-care LFA. Alternative point-of-care CrAg LFAs are commercially available, but their performance characteristics vary *(41,42)*. Two semiquantitative LFAs have been produced and are either commercially available or undergoing final validation and licensing studies; a third is under development. These semiquantitative tests offer the potential to risk-stratify individuals with either cryptococcal antigenaemia identified through CrAg screening programmes or individuals presenting with cryptococcal meningitis based on CrAg levels. The semiquantitative CrAg levels have been shown to correlate with CrAg titre, and CrAg LFA titre is strongly correlated with mortality risk for both people with cryptococcal antigenaemia and people with cryptococcal meningitis *(43–46)*. Several published articles and conference abstracts have reported semiquantitative CrAg test performance, with variable results in terms of diagnostic accuracy, but all demonstrating the potential utility of semiquantitative CrAg testing in risk stratification *(47-54)*; however, to date, no prospective studies have shown any impact on clinical outcomes.

The two semiquantitative CrAg assays have important differences in terms of performance and diagnostic accuracy *(47-54)*. Notably, there is variable sensitivity for cryptococcal serotypes B/C *(Cryptococcus gattii)* between the tests; among people with meningitis, sensitivity of one of the semiquantitative CrAg assays was 100% for *Cryptococcus neoformans* and 84% for C. gattii in Botswana *(47)*. The poor sensitivity for *C. gattii* may account in part for the suboptimal sensitivity of this test seen in a Botswana CrAg screening cohort *(50)*, in which *C. gattii* caused up to 30% of cryptococcal disease.

One of the two semi-quantitative tests provides five antigen quantification levels and is more complex to read, but studies have shown that the semiquantitative scores are strongly associated with the presence of CNS infection and mortality *(51)*.

Further evaluations of semiquantitative CrAg testing for diagnosis and prognostication are underway. Secondary analysis of data and samples from the AMBITION-cm Trial *(55)* are being performed to definitively determine the prognostic value of testing of blood and CSF using CrAG LFA tests among patients with cryptococcal meningitis.

The prospective ENCORE randomized controlled trial (NCT05085171) is assessing the utility of prospective stratified management of individuals with advanced HIV disease and cryptococcal antigenaemia. The EFFECT study (ISRCTN30579828) includes an evaluation of a semi-quantitative test among individuals undergoing CrAg screening in South Africa, United Republic of Tanzania and Viet Nam, including for the diagnosis of subclinical cryptococcal meningitis in a substudy.

Screening

CrAg screening is recommended as a key component of advanced HIV disease care, with current WHO guidelines strongly recommending screening all adults and adolescents before initiating or reinitiating ART with CD4 cell counts below 100 cells/μL and conditionally recommending for those with CD4 cell counts below 200 cells/μL (as a result of less robust evidence for clinical and cost–effectiveness for those with CD4 counts of 100–200 cells/μL). The guidelines recommend that everyone with a positive CrAg on screening should be carefully evaluated for signs and symptoms of meningitis and undergo lumbar puncture if feasible with CSF examination to exclude meningitis; ART initiation should be delayed by 4–6 weeks if cryptococcal meningitis is identified while initiating treatment for cryptococcal meningitis.

- CrAg-positive individuals without evidence of meningitis should be given pre-emptive antifungal therapy (fluconazole 800–1200 mg/day for adults and 12 mg/kg per day for adolescents for two weeks), followed by consolidation and maintenance fluconazole therapy. The evidence underpinning these recommendations comes primarily from observational and cohort studies demonstrating that cryptococcal antigenaemia is highly predictive of the subsequent development of cryptococcal meningitis and death among people with advanced HIV disease *(43)*.
- Fluconazole monotherapy prevents most cases of overt cryptococcal meningitis in this CrAg-positive population *(9,56)*.
- Screening and pre-emptive treatment is likely to be a highly cost-effective intervention *(56–58)*.

A single randomized controlled trial, the REMSTART Trial *(59)*, demonstrated that a combined intervention of a short period of community support to supplement clinic-based services plus serum cryptococcal antigen screening led to an 28% mortality reduction among outpatients initiating ART with CD4 counts less than 200 cells/μL versus standard of care. The individual effects of the two components of this combined intervention could not be disaggregated.

Subsequent observational data and programmatic evaluations have demonstrated that, despite pre-emptive fluconazole therapy, mortality risk remains two- to three-fold higher among CrAg-positive individuals than CrAg-negative individuals with comparable CD4 counts *(9,44,60,61)* Limited postmortem studies have provided preliminary evidence that undertreated cryptococcal infection may be contributing to this excess mortality risk *(60)*. These findings have led to two areas of research: the first is examining whether individuals with advanced HIV disease and cryptococcal antigenaemia can be risk-stratified into differentiated treatment groups depending on rapid assessment of disease severity using semiquantitative CrAg assays (see the diagnostics section above); the second is evaluating enhanced cryptococcal treatment strategies for CrAg-positive individuals identified through CrAg screening programmes. Two such enhanced approaches are currently being trialled – an enhanced oral treatment combining high-dose fluconazole and flucytosine (5-FC) based on the oral arm of the ACTA Trial *(62)* and treatment with a single high dose of liposomal amphotericin plus fluconazole, adapted from the AMBITION-cm Trial *(55,63)*.

Key trial

EFFECT: efficacy of fluconazole and 5-FC for early cryptococcal treatment. Treatment of cryptococcal antigen-positive people identified through screening using fluconazole plus 5-FC versus fluconazole alone.

International registry identifier: ISRCTN30579828

Type: Phase 3 trial.

Description: This study aims to compare how well an oral combination of fluconazole plus 5-FC works compared with fluconazole alone (the current recommended treatment) in reducing six-month mortality among asymptomatic people with advanced HIV disease (CD4 count <100 cells/µL) and a CrAg-positive blood test.

Sample size and population: 600 people living with HIV older than 18 years with CD4 counts ≤100 cells/µL.

Study sites: South Africa, United Republic of Tanzania and Viet Nam

Interventions: (1) Fluconazole 1200 mg/day plus 5-FC 25 mg/kg four times daily orally (intervention arm) for 2 weeks; (2) fluconazole alone 1200 mg/day orally (standard dose control arm) for two weeks. All participants will then receive fluconazole 800 mg/day to 10 weeks and fluconazole 200 mg/day thereafter for a minimum of 12 months in accordance with national guidelines. ART will commence on day 14 in accordance with current international (WHO) and national guidelines.

Primary outcome: All-cause mortality at six months.

Status: Recruiting. 2022–2025. Likely end late 2025 or early 2026.

Key trial

ACACIA: single-dose liposomal amphotericin for asymptomatic cryptococcal antigenaemia

International registry identifier: NCT03945448

Type: Phase 3 trial.

Description: A randomized controlled trial of asymptomatic CrAg-positive people in Uganda. They will be randomized to receive pre-emptive treatment with one dose of liposomal amphotericin (10 mg/kg) in addition to standard-of-care fluconazole therapy. How the enhanced antifungal therapy prevents progression to meningitis in the first 24 weeks and overall survival among those who receive the intervention compared with participants receiving fluconazole in accordance with WHO and national standard-of-care therapy will be evaluated.

Sample size and population: 600 (100 stage 1, 500 stage 2) outpatients with HIV 15 years or older

Study sites: Uganda

Interventions: Experimental: single-dose liposomal amphotericin 10 mg/kg and fluconazole 800 mg for two weeks, 400 mg for eight weeks and 200 mg up to six months; comparator: standard-of-care pre-emptive treatment fluconazole 800 mg for two weeks, 400 mg for eight weeks and 200 mg for up to six months.

Primary outcome: Number of people who develop meningitis in the liposomal amphotericin arm versus the fluconazole arm within 24 weeks.

Status: Recruiting. 285 enrolled. 2022–2025. Projected 2026 finish.

These two clinical trials could greatly affect public health policy. In addition to the EFFECT Trial, a nested substudy called Sub-EFFECT is investigating outcomes among individuals diagnosed with early asymptomatic or minimally symptomatic cryptococcal meningitis identified through CrAg screening and the potential for ambulatory therapy using the AMBITION-cm single dose liposomal amphotericin treatment regimen in this population (personal communication: Síle Molloy, St George's, University of London, United Kingdom, May 2023). Both studies are incorporating alongside-trial cost–effectiveness analyses.

Several caveats may affect the outcomes of these two trials. The first is that the CrAg-positive populations most likely to benefit from enhanced cryptococcal treatment are those with high disease burdens and subclinical cryptococcal meningitis. In routine care, in which detailed symptomatic assessment and lumbar punctures may not be widely performed, many of these high-risk individuals would receive pre-emptive therapy; whereas in a trial setting with rigorous symptom screening and access to lumbar puncture, many of these high-risk people will be screened out of the trial. The trials may thus underestimate the potential benefits of enhanced treatments. The second is that the optimal treatment strategy for CrAg-positive individuals may depend on risk stratification, potentially based on semiquantitative CrAg results. The EFFECT trial is including all CrAg-positive individuals regardless of CrAg titre and will be able to perform stratified analysis examining the effects by CrAg-SQ result, although it will not be powered to draw definitive conclusions from this subgroup analysis. The ACACIA Study has modified its inclusion criteria between the initial stage 1 pilot and the follow-on stage 2 of the trial to only include individuals with high CrAg titres (personal communication: Radha Rajasingham, University of Minnesota, Minneapolis, USA, March 2023). This has the benefit of providing a definitive result in this high-titre population, but as a consequence may not be generalizable to those with low CrAg titres or populations for whom CrAg titres have not been obtained. Also notable is that the primary outcome in ACACIA is not mortality but meningitis-free survival.

Treatment

Current WHO treatment recommendations for HIV-associated cryptococcal meningitis are primarily based on two large randomized controlled trials, the ACTA Trial *(62)* and the AMBITION-cm Trial *(55)*. Two ongoing studies may impact treatment guidelines in the medium term (see key trial details below): the ENACT Trial investigating a novel oral encochleated amphotericin B formulation and the 5-FC HIV-Crypto Trial developing and testing long-acting formulations of 5-FC. An embedded pharmacokinetic substudy within the EFFECT trial (see above) will provide further information on long-acting 5-FC formulations. A second novel amphotericin B formulation is at an early stage, with Phase 1 and 2 human studies in cryptococcal meningitis unlikely to happen before 2025. Other potential drug treatment evaluations are at a very early stage, with promising rabbit model data for APX2039 *(64)*, belonging to a novel class of broad-spectrum antifungal agents that inhibit Gwt1, an enzyme required for cell wall localization of glycosylphosphatidylinositol-anchored mannoproteins in fungi. It is currently unclear whether the originator company plans to develop the drug for cryptococcal treatment or are willing to licence it for external development.

Key trial

ENACT: encochleated oral amphotericin for cryptococcal meningitis trial

International registry identifier: NCT04031833 and NCT05541107

Type: Phase 1/2 trial and Phase 3 trial.

Description: This study is designed as two sequential Phase 1/2 trials and a Phase 3 trial. The first is a phase I open label trial to evaluate the safety and tolerability of MAT2203 (an encochleated oral amphotericin). The maximum tolerated and non-toxic daily dose will then be moved forward into a multi-day safety trial. The Phase 2 trial will investigate toxicity and early fungicidal activity of MAT2203 with 5-FC, followed by a planned Phase 3 all-cause mortality endpoint study.

Sample size and population: 178 participants Phase 1/2, 270 Phase 3, adult inpatients with HIV older than 18 years

Study sites: Uganda

Interventions: Phase 1A consists of a single ascending dose study with nine participants to test three doses to determine the maximum tolerated dose. In Phase 1B multiple day dosing, nine subjects will receive the Phase 1A 100% tolerated MAT2203 dose for seven days. Phase 2 studies will test the safety and tolerability and microbiological efficacy of MAT2203 among people living with HIV with cryptococcal meningitis compared with standard intravenous amphotericin B. The planned Phase 3 trial has three arms: (1) two days of intravenous amphotericin B (amphotericin B) + 5-FC followed by oral MAT2203 + 5-FC for 12 days of induction therapy followed by MAT2203 + fluconazole for four weeks of consolidation therapy; (2) two days intravenous amphotericin B + 5-FC followed by oral MAT2203 + 5-FC for 12 days of induction therapy followed by 4 weeks of standard of care consolidation therapy; (3) standard-of-care induction therapy (intravenous amphotericin B + 5-FC) followed by four weeks of standard of care consolidation therapy (fluconazole).

Primary outcome: CSF early fungicidal activity during two weeks of induction therapy. All-cause mortality at two weeks in Phase 3.

Status: Recruiting. Phase 1 and 2 completed, with the results published in mid-2023 *(65)*. The study showed that oral amphotericin had an acceptable safety profile. with a lower rate of grade 3 or 4 laboratory events than occurred with conventional amphotericin B deoxycholate. Mean fungal clearance was only slightly lower with the all-oral MAT2203 arm than trial arms containing intravenous amphotericin B, but there was wide between-patient variability with the oral arm, with mean early fungicidal activity influenced by a small number of outlying participants with very rapid fungal clearance. The Phase 3 study is not currently funded and at present does not look likely to proceed (see notes below).

Key trial

5-FC HIV-Crypto:

International registry identifier: Phase 1 clinical trial in fasting conditions – PACTR 202201760181404; Phase 1 clinical trial in fed conditions: PACTR202211836109661; Phase 2: to be registered upon EC/HA approval. Details available at: https://dndi.org/research-development/portfolio/5fc-cryptococcal-meningitis

Type: Phase 1 (fasting conditions): comparative four-period crossover study to determine the relative bioavailability of an immediate-release formulation and three sustained-release formulations for healthy volunteers in fasting conditions; Phase 1 (fed conditions): comparative two-period crossover study to determine the relative bioavailability of an immediate-release formulation and a sustained-release formulations for healthy volunteers in fed conditions; Phase 2 study: a 10-week study to evaluate the comparative bioavailability, efficacy and safety of sustained-release 5-FC versus immediate-release 5-FC for adults with cryptococcal meningitis.

Description: consortium funded by the European and Developing Countries Clinical Trials Partnership for developing and testing a sustained-release prototype formulation of 5-FC.

Sample size and population: first Phase 1 (fasting): 42 enrolled and 35 completers. Second phase 1 (fed): 36 enrolled and 35 completers. Phase 2 trial, 72 participants. Adults older than 18 years.

Study sites: Malawi, South Africa and United Republic of Tanzania.

Interventions: sustained-release 5-FC (pellet formulation for suspension) versus standard-release 5-FC used as part of the preferred WHO regimen for cryptococcal meningitis.

Primary outcome: pharmacokinetic parameters (Phase 1 studies); pharmacokinetic parameters and early fungicidal activity (Phase 2 study).

Status: Phase 1 (concluded and reporting); Phase 2: regulatory approval (Q1 2024 planned initiation)

The status of the planned phase-3 ENACT Trial is currently unclear. The company manufacturing the novel amphotericin B formulation has had difficulty in supplying the drug for the Phase 3 trial because of manufacturing and drug stability issues, and economic and commercial considerations have led to a focus on other fungal pathogens in high-income settings. It is unclear whether these issues will be resolved and if they are, when. Further practical issues that may ultimately limit the utility of oral encochleated amphotericin B treatment are the need for a cold chain for drug storage and the requirement for up to six times a day dosing and associated gastrointestinal intolerance.

Summary points

- Novel semiquantitative CrAg tests may enable risk-stratification and differentiated care pathways for individuals with cryptococcal antigenaemia identified through CrAg screening programmes and those with cryptococcal meningitis. Data from secondary analysis of the AMBITION-cm study, plus prospective data from the EFFECT Trial and ENCORE Trial, will provide further data in late 2025 or early 2026 to guide the use of these assays.
- Two prospective randomized controlled trials examining enhanced antifungal treatment for individuals with cryptococcal antigenaemia identified through CrAg screening are underway. Both will report in 2026, with the results likely to lead to guideline changes.
- Ongoing randomized controlled trials are testing novel encochleated formulations of amphotericin B and sustained-release 5-FC for HIV-associated cryptococcal meningitis treatment. The status of the Phase 3 stage of the encochleated amphotericin B trial is uncertain, with Phase 2 results alone insufficient to influence guidelines. The sustained-release 5-FC trial is scheduled to complete recruitment and report in 2025. Submission for registration and WHO prequalification is expected in 2025. A clinical study evaluating the population pharmacokinetics of sustained release 5-FC among individuals with asymptomatic cryptococcal antigenaemia is also planned as a substudy of EFFECT/IMPRINT consortium activities.

Implications

Updates to CrAg screening could potentially incorporate risk assessment and stratification based on semiquantitative antigen testing, plus enhanced antifungal treatments may be required, based on evidence from trials that are projected to end and report at some stage in 2026. Major updates to cryptococcal meningitis treatment are unlikely to be justified in the medium term (2–3years), with the results of the 5-FC HIV-Crypto Phase 2 trial expected in 2025, which may indicate adjustments to 5-FC dose and frequency if successful. Oral amphotericin B formulations have a low likelihood of affecting international guidelines and policies given the uncertainties about stability of the formulation, high cost and limited feasibility, even if efficacy is shown to be adequate. However, if the ENACT trial does proceed, Phase 3 results should be reviewed when available, likely in mid- to late 2026. It is suggested that the cryptococcal guidelines be updated in 2027 (five years after the 2022 update).

4. Severe bacterial infections

Severe bacterial infections are one of the three leading causes of mortality among individuals with advanced HIV disease *(11)*. Because of lack of access to effective diagnostics and laboratory facilities in many of the regions where advanced HIV disease is prevalent and the absence of detailed data on the causes of death among people living with HIV, the epidemiology of severe bacterial infections in the context of advanced HIV disease is poorly understood. WHO undertook a scoping consultation on severe bacterial infections among people with advanced HIV disease in 2021 *(17)*; meeting participants highlighted the need to develop accurate estimates of the burden of severe bacterial infections and antimicrobial resistance among people living with HIV to help understand how bacterial infections are implicated in advanced HIV disease mortality. The scoping consultation report recommended examining the issue of severe bacterial infections in terms of patient subpopulations – outpatients and hospitalized patients – since each of these populations has a different set of needs and different risks of mortality and morbidity – and managing hospitalized people with advanced HIV disease with sepsis in low- and middle-income countries was identified as an important focus for implementation research and guidance.

As a result of the weak evidence base, current WHO guidelines make very limited recommendations regarding prevention, diagnosis and management of severe bacterial infections in the context of advanced HIV disease. Co-trimoxazole prophylaxis is advised for all people living with HIV and CD4 counts below 350 cells/µL or clinical stage 3 or 4 disease or at any CD4 count in settings with high prevalence of malaria or severe bacterial infections, in part because of its role in reducing the risk of severe bacterial infections. A meta-analysis of studies performed in the context of ART supports these recommendations *(66)*. The other prophylactic intervention that has been evaluated for preventing severe bacterial infections is azithromycin *(67)*, until recently used in high-income countries as prophylaxis for *Mycobacterium avium* complex infections, with current data coming from a single trial (REALITY) *(67)*. The REALITY trial tested a package of interventions incorporating continuous co-trimoxazole, 12 weeks of isoniazid-pyridoxine, 12 weeks of fluconazole and a single dose of albendazole in addition to five days of azithromycin. The Guideline Development Group for the current advanced HIV disease guidelines did not consider that current evidence supports including the additional broad-spectrum antibiotic regimen tested in REALITY (500 mg of azithromycin once a day for five days) within the advanced HIV disease package given the unclear benefits of this specific component of the REALITY package and concerns about the potential for developing antimicrobial resistance. Antibiotic resistance concerns need to be weighed against the extremely high risk of mortality from severe bacterial infections among people with advanced HIV disease; azithromycin has been used widely in mass drug administration programmes for neglected tropical diseases with only limited emergence of resistance *(68,69)* and no loss of efficacy *(70)* and is also widely prescribed for respiratory tract infections. In the context of targeted use in individuals identified as having advanced HIV disease, the mortality benefits may well outweigh the hypothetical risks of developing drug resistance. Further research is clearly needed in this area; however, the research pipeline is currently limited.

Research pipeline

The lack of diagnostic microbiology capacity is being addressed by a variety of capacity-strengthening initiatives *(71,72)*, and WHO has established methods for quantifying the burden of antimicrobial resistance. These investments are anticipated to make leveraging existing data or undertaking prospective surveillance of severe bacterial infections less expensive and more rapid in all patient groups, including those with advanced HIV disease. A single randomized controlled trial is examining the role of azithromycin prophylaxis in advanced HIV disease. The REVIVE Trial (see key trial details below) plans to enrol 8000 participants, focusing on outpatients without evidence of a severe opportunistic infection or acute illness. The outcomes are hospitalization and all-cause mortality over the first 24 weeks after randomization, and no disaggregation for sepsis or severe bacterial infections is currently proposed. No ongoing large-scale trials were identified through the current research landscape and mapping exercise investigating the inpatient management of severe bacterial infections among people with advanced HIV disease. A study in South Africa is determining the incidence and causes of culture-confirmed bacterial bloodstream infections in the context of advanced HIV disease at the population level and is due to report in 2028 (personal communication: Nelesh Govender, National Institute of Communicable Diseases of South Africa, Johannesburg, September 2023). Diagnostic development and evaluation for bacterial infections and sepsis in low- and middle-income countries more broadly is a large and evolving field that is outside the scope of this briefing document.

Key trial

REVIVE: reducing mortality in adults with advanced HIV disease

International registry identifier: NCT05580666

Type: Phase 3 trial.

Description: A double-blinded, placebo-controlled, multicentre trial to evaluate the effectiveness of azithromycin prophylaxis on mortality in advanced HIV disease.

Sample size and population: 8000 participants, adults 18 years and older with advanced HIV disease but without severe illness requiring immediate or continued hospitalization.

Study sites: Multiple African study sites.

Interventions: Experimental: oral azithromycin 250 mg once daily for one month. Placebo comparator: oral matching placebo, once daily.

Primary outcome: All-cause mortality over the first 24 weeks after randomization.

Status: Recruiting. Overall results probably 2026 or 2027.

Summary points

- The epidemiology and impact of severe bacterial infections in advanced HIV disease are poorly understood, and research to determine the disease burden and causes is urgently required.
- A single randomized controlled trial started in 2023 examining the role of azithromycin prophylaxis in advanced HIV disease. The trial is projected to report in 2027.
- There are no ongoing studies of the prevention and management of severe bacterial infections among inpatients with advanced HIV disease.

Implications

The findings of the REVIVE Trial will have important implications for advanced HIV disease policies but will not be available for another four to five years. It may provide evidence to support outpatient prophylaxis for severe bacterial infections in advanced HIV disease, pending the results of the clinical trial completion.

5. *Pneumocystis* pneumonia

Pneumocystis pneumonia is a life-threatening opportunistic infection caused by the fungus *Pneumocystis* jirovecii. The epidemiology in low- and middle-income countries is poorly defined, largely because of the lack of effective and accessible diagnostics. Recent systematic reviews suggest that *Pneumocystis* pneumonia accounts for about 20% of all hospital admissions among people living with HIV presenting with respiratory symptoms, and HIV-associated *Pneumocystis* pneumonia outcomes are poor in sub-Saharan Africa, with case-fatality ranging from 15% to 30% in pooled studies *(73,74)*. Current advanced HIV disease guidelines recommend co-trimoxazole prophylaxis for all people living with HIV and CD4 counts below 350 cells/µL or clinical stage 3 or 4 disease.

Research pipeline

Almost no late-stage clinical research on *Pneumocystis* pneumonia in the context of HIV is currently ongoing. Research is primarily laboratory-based and translational research aiming to develop rapid near-patient diagnostics that do not require invasive sampling. Currently, definitive *Pneumocystis* pneumonia diagnosis relies on bronchoscopy or induced sputum, with direct microscopic examination or polymerase chain reaction (PCR). Several groups are investigating novel PCR assays to better determine colonization versus infection targeting mitochondrial genes (personal communication: Alexandre Alanio, Institut Pasteur, Paris, France, May 2023) and determine transmissibility by quantifying the ratio of tropic to cystic forms (personal communication: Jay Kolls, Tulane University, New Orleans, LA, USA). Neither of these research avenues is likely to yield novel diagnostic or screening assays applicable to HIV-associated *Pneumocystis* pneumonia in low- and middle-income countries. Quantitative PCR (qPCR) on sputum *(75,76)* or oral wash samples *(77)* and serum BD-glucan *(78)* are promising non-invasive methods for diagnosing *Pneumocystis* pneumonia, but threshold values have not been defined in settings with a high burden of HIV and TB *(79)*. Novel diagnostic approaches, including developing antibody-based LFAs targeting fungal antigens for more rapid detection of *Pneumocystis* pneumonia, are also needed. Research priorities include evaluating new and existing non-invasive diagnostics and ultimately integrating them into a more accurate clinical prediction tool for *Pneumocystis* pneumonia in a population with a high burden of HIV and TB *(80)*.

A study funded by the United Kingdom National Institute for Health and Care Research (IMPRINT *(81)*) based in Cape Town, South Africa is evaluating the performance of non-invasive diagnostic tests for *Pneumocystis* pneumonia, including evaluating the diagnostic accuracy and threshold values of a qPCR assay on induced sputum and oral wash samples, plus serum BD-glucan, using bronchoalveolar lavage immunofluorescent antibody as a reference standard. The work is exploring clinical and laboratory parameters associated with *Pneumocystis* pneumonia to inform the design of a larger study to develop and validate a clinical prediction tool. The study is also undertaking work to develop monoclonal antibodies specific to *Pneumocystis* for use in a rapid LFA. The study runs from 2022 to 2026, with results anticipated in 2026.

Lack of access to diagnosis results in empirical therapy for most people based on characteristic clinical and radiological findings. Treatment for HIV-associated *Pneumocystis* pneumonia has not changed significantly in more than two decades. The current treatment recommendations for high-dose co-trimoxazole are based on very limited data *(82,83)*. Pharmacokinetic data suggest that the current dosing and treatment duration may exceed optimal levels, leading to avoidable drug-related toxicity *(84–90)*, and a Phase 2 study is planned to examine the efficacy of lower co-trimoxazole dosing in HIV-associated *Pneumocystis* pneumonia (see below). Several echinocandin and echinocandin-like drugs have anti-*Pneumocystis* pneumonia activity, including rezafungin (long-acting echinocandin) and ibrexafungerp (triterpenoid), and have been used in specific situations. Rezafungin has shown promising activity in animal models of *Pneumocystis* pneumonia for both treatment and prophylaxis *(91)*. A Phase 2 treatment study examining rezafungin plus co-trimoxazole is currently in the late planning stages, and study recruitment is hoped to commence later this year (personal communication: Sean Wasserman, St George's, University of London, United Kingdom, September 2023).

LOW-TMP: low-dose trimethoprim-sulfamethoxazole for the treatment of *Pneumocystis jirovecii* pneumonia

International registry identifier: NCT04851015

Type: Phase 3 trial.

Description: A recent meta-analysis of observational studies showed that reduced treatment doses of co-trimoxazole for *Pneumocystis* pneumonia reduce drug-related adverse events without mortality differences. This Phase 3 randomized, placebo-controlled trial will compare the efficacy and safety of low-dose (10 mg/kg per day of co-trimoxazole) with that of the standard of care (15 mg/kg per day) among people living with HIV with *Pneumocystis* pneumonia for the primary outcome of death, new mechanical ventilation and change of treatment.

Sample size and population: 300 adults 18 years and older

Study sites: Canada

Interventions: Reduced-dose co-trimoxazole (10 mg/kg per day) versus standard dose (15 mg/kg per day) for 21 days.

Primary outcomes: Composite of death, new mechanical ventilation or treatment change for presumed inefficacy or severe adverse events.

Status: Not yet recruiting.

Rezafungin for treatment of *Pneumocystis* pneumonia among adults with HIV

International registry identifier: NCT05835479

Type: Phase 2 trial.

Description: This study aims to generate clinical data on the efficacy safety, and tolerability of rezafungin combined with seven days of co-trimoxazole for treating adults living with HIV for *Pneumocystis* pneumonia. Participants who are diagnosed with definitive, presumptive or clinically suspected *Pneumocystis* pneumonia will be randomized in a 1:1 ratio to either the rezafungin and co-trimoxazole group or co-trimoxazole monotherapy group. Randomization will be stratified by disease severity into two categories (mild or moderate to severe).

Sample size and population: 50 people living with HIV with *Pneumocystis* pneumonia 18 years and older.

Study sites: Brazil and South Africa.

Interventions: Rezafungin: weekly intravenous infusion with a loading dose of 400 mg over one hour (±10 minutes) on day 1 followed by maintenance doses of 200 mg over one hour (±10 minutes) on day 8 and day 15. From day 1 to day 7, co-trimoxazole will also be given with trimethoprim 15–20 mg/kg per day and sulfamethoxazole 75–100 mg/kg per day. Active comparator: co-trimoxazole. From day 1 to day 21, co-trimoxazole will be given with trimethoprim 15–20 mg/kg per day and sulfamethoxazole 75–100 mg/kg per day.

Primary outcomes: Therapeutic failure on day 8, defined if any one of the following is met and confirmed by an independent, blinded data review committee: clinical deterioration; requirement for alternative primary therapy for *Pneumocystis* pneumonia or intensification of corticosteroid therapy due to lack of efficacy; or death from any cause.

Status: Recruitment not yet started. Planned for late 2023.

Summary points

- Studies of novel diagnostic strategies for *Pneumocystis* pneumonia are underway but at an early stage and will report in 2026 at the earliest. Guidance on diagnostic algorithms is required based on context-specific resources.
- Very limited data support current co-trimoxazole dosing and duration recommendations, and pharmacokinetic studies are needed. A Phase 2 study of lower-dose co-trimoxazole for *Pneumocystis* pneumonia treatment is planned.
- The long-acting echinocandin, rezafungin, shows potential for *Pneumocystis* pneumonia prevention and treatment in combination with co-trimoxazole. Phase 2 studies are planned. If these are successful, definitive Phase 3 trials would be needed to change guidelines and will take several years to perform.

Implications

No guideline-changing results are likely in the near term. Diagnostic and screening guidelines may need to be updated in 2026 or 2027 depending on the results of ongoing diagnostic evaluations.

6. Toxoplasmosis

The estimated pooled prevalence of global *Toxoplasma gondii* infection based on serological testing among people living with HIV is 36% (95% CI 31–41%), including 45% (95% CI 32–58%) in sub-Saharan Africa, where country prevalence ranges from 3.1% to 93.3% *(92)*. However, the epidemiology of HIV-associated cerebral toxoplasmosis in low- and middle-income countries is extremely poorly defined. Limited data from selected cohorts suggests that, in parts of western Africa, cerebral *Toxoplasma* is a more frequent cause of CNS infection than *Cryptococcus* among individuals with advanced HIV disease *(93)*; it has been reported as a common opportunistic infection in Brazil *(94)* and Asia *(95)*. Although the prevalence of HIV-associated cerebral toxoplasmosis appears to vary by geographical region, with much lower rates reported in southern Africa *(96)*, available data suggest that it continues to be a common global opportunistic disease among individuals with advanced HIV disease in low- and middle-income countries, with a worldwide systematic review and meta-analysis of hospital admissions among people living with HIV reporting that cerebral toxoplasmosis causes 15% of deaths *(97)*. The need for cross-sectional imaging (computed tomography or magnetic resonance imaging) to make an accurate diagnosis of cerebral toxoplasmosis means that accurate incidence estimates are difficult to generate in most low-resource settings. Rapid point-of-care *Toxoplasma* immunoglobulin G serology could support the diagnostic and empirical treatment for people with CNS lesions *(98)*, and non-invasive diagnostic approaches are being explored; a recent proof-of-concept study using nanoparticle concentration in urine samples showed potential for diagnosing cerebral toxoplasmosis *(99)*. Diagnostics including CSF PCR assays are being validated in ongoing studies in Botswana (personal communication, James Milburn, London School of Hygiene and Tropical Medicine, United Kingdom), and updated prevalence estimates from western Africa are being generated through the DREAMM Project *(100)*. Treatment in most low- and middle-income countries is based on high-dose co-trimoxazole. A recent systematic review and meta-analysis concluded that co-trimoxazole appears to be as effective and safer than the pyrimethamine-containing regimens for *Toxoplasma* encephalitis *(101)*. In addition, co-trimoxazole presents potential advantages over pyrimethamine-containing regimens such as the convenience of the lower pill burden and dosing frequency; availability of intravenous formulations and several generic formulations; prevention of *Pneumocystis* pneumonia, some bacterial infections and malaria; and the convenience of use simplifying the early initiation of ART *(102)*. Co-trimoxazole is also active as prophylaxis against *Toxoplasma* encephalitis and is recommended as part of the current advanced HIV disease package.

Research pipeline

Limited additional data regarding disease prevalence in advanced HIV disease are anticipated from western, southern and eastern Africa through the DREAMM and Botswana National Meningitis Survey studies, with results anticipated in early 2024.

Summary points

- Limited incidence and prevalence data are available.
- Research is needed to better define disease incidence and clinical outcomes.
- Diagnosis is challenging because of the lack of cross-sectional imaging and access to biopsies and histology services.
- Guidance on diagnostic algorithms and syndromic approaches to CNS infections in HIV would be useful.

Implications

No guideline-changing results are likely in the near term, although existing evidence from the systematic review showing that co-trimoxazole appears to be as effective and safer than the pyrimethamine-containing regimens for *Toxoplasma* encephalitis has not yet been reflected in some existing country-level guidelines. Guidance on an approach to CNS infections in the context of HIV would be useful but may be outside the scope of the WHO public health approach.

7. Cytomegalovirus

Cytomegalovirus is a common infection in the context of advanced HIV disease. Cytomegalovirus infection may be associated with severe end-organ disease, including retinitis, colitis, oesophagitis, pneumonitis, myelitis and encephalitis. The incidence of cytomegalovirus end-organ disease such as retinitis has declined with ART roll-out but continues to occur among severely immunosuppressed individuals. Cytomegalovirus viraemia remains common among individuals with advanced HIV disease, present among up to 50% of all individuals presenting with opportunistic infections such as cryptococcal meningitis and TB meningitis *(103,104)* and up to 63% of hospitalized people living with HIV and severe immunosuppression *(105)*. Extensive evidence from both the pre-ART and ART eras also indicates that cytomegalovirus viraemia, even in the absence of overt end-organ disease, is associated with excess mortality *(106–109)*. However, cytomegalovirus is a human herpesvirus that causes lifelong latent infection and may reactivate among people who are immunocompromised, and it is unknown whether cytomegalovirus contributes directly to excess mortality in advanced HIV disease or just indicates immune suppression. Studies to determine whether targeted use of cytomegalovirus antiviral agents would reduce mortality have been proposed *(110)*, with the availability of newer and safer orally bioavailable cytomegalovirus antiviral agents such as letermovir making treatment in low- and middle-income countries potentially more feasible.

Research pipeline

Discussions regarding randomized controlled trials of cytomegalovirus treatment using letermovir are ongoing, but no studies have yet been funded. Applications for Phase 2 studies of valganciclovir have been submitted to funders; however, no definitive plans are yet in place (personal communication: Sean Wasserman, St George's, University of London, United Kingdom, September 2023).

Summary points

- Cytomegalovirus infection is highly prevalent, with cytomegalovirus viraemia frequently detected among individuals with advanced HIV disease.
- The impact of cytomegalovirus viraemia on outcomes for people with advanced HIV disease without evidence of end-organ disease is unclear.
- Randomized controlled trials examining cytomegalovirus treatment in advanced HIV disease using existing drugs such as valganciclovir and novel drugs such as letermovir are in very early stages of conceptualization.

Implications

No guideline-changing results are likely in the near term.

8. Talaromycosis

In South-East Asia, talaromycosis – caused by the thermally dimorphic fungus *Talaromyces marneffe* (previously known as *Penicillium marneffeii*) is among the leading cause of HIV-related opportunistic infections and deaths, accounting for 4–16% of HIV-related admissions. In highly endemic countries, the disease burden surpasses that of TB and cryptococcal meningitis, with mortality ranging from 15% to 50% *(111–113)*. Despite ART roll-out, Asia and the Pacific has one of the highest prevalences of advanced HIV disease in the world, with 50% of newly diagnosed individuals presenting with CD4 counts <200 cells/µL. About 17 300 culture-confirmed talaromycosis cases and 4900 deaths occur annually, with numbers projected to increase by 35% by 2025 because of the rising incidence of advanced HIV disease in China and Asia and the Pacific *(10,114)*. Despite this substantial disease burden in the largest and most rapidly growing populations in the world, talaromycosis is poorly researched, and diagnostic and treatment modalities remain severely limited. Critical barriers to reducing talaromycosis mortality include the inability to make early diagnosis and a lack of effective and practical treatment options. WHO does not currently have guidelines for managing talaromycosis.

Research pipeline

Several of the recent advances made in HIV-associated cryptococcal meningitis may be translatable to talaromycosis, notably the potential for novel sensitive antigen-detection tests to identify preclinical or asymptomatic infection among people with advanced HIV disease, enabling pre-emptive treatment before severe disease develops and the likely utility of short-course liposomal amphotericin B therapy and 5-FC combination therapy for disseminated disease. Ongoing research likely to affect future policies and guidelines in the areas of diagnostics, screening and treatment is considered below.

Diagnostics

Promising non-culture assays are being evaluated for rapid diagnosis, including qPCR and enzyme immunoassays for use on blood samples. Ribosome targets have been identified for PCR showing high specificity (100%) but limited sensitivity (70% and 80%) in whole-blood samples *(115–118)*. A major limitation is that qPCR assays require sophisticated laboratory infrastructure that is not accessible in many low-resourced settings.

Overcoming many of these limitations, several novel Mp1p enzyme immunoassays have been developed and validated that are highly specific (98%) and more sensitive than blood culture (85% versus 72%) *(119,120)*. In addition, the Mp1p enzyme immunoassays can detect disease from plasma, sera and urine up to 16 weeks before culture positivity *(119)*, supporting its role in screening for early disease (see screening below). One of these assays is now available as a commercial enzyme immunoassay with validation data regarding the clinical performance of the assay from Viet Nam anticipated in early 2024.

Two point-of-care or near-patient diagnostics are also under development for talaromycosis. Prospective validation studies are ongoing, with anticipated completion in 2024, and the LFA test is anticipated to be brought to market by 2025 (personal communication: J.T. Harrison, May 2023). A second immunochromatographic test strip-based assay is under development by a Thai group based on the yeast phase-specific monoclonal antibody 4D1 and *Galanthus nivalis* agglutinin *(121)*. Large-scale prospective evaluation study results are anticipated in 2024.

Screening

Talaromycosis has an indolent course over weeks to months with non-specific constitutional symptoms indistinguishable from TB and other opportunistic infections, and many people die before a diagnosis is made by conventional methods *(122)*. The LFA test described above potentially paves the way to follow the cryptococcal meningitis model of shifting the disease management paradigm from treating late-stage disease to proactively screening and pre-emptively providing antifungal therapy to prevent disease development and associated mortality.

One multicentre prospective study is underway aiming to generate data on the utility of antigen screening using the Mp1p enzyme immunoassays for early diagnosis of talaromycosis. The study is anticipated to complete enrolment of 900 symptomatic hospitalized people with advanced HIV disease and 500 outpatients with advanced HIV disease not suspected of having active infection by the end of 2023, with completion of follow-up and data report in 2024 for the hospitalized cohort and in 2025 for the outpatient cohort (see key study below). A second component of the prospective observational study (through the IMPRINT project) is prospectively evaluating the utility of triple fungal screening in advanced HIV disease for CrAg, talaromycosis using Mp1p enzyme immunoassays and histoplasmosis enzyme immunoassays, with results anticipated in 2024.

Making an early diagnosis of talaromycosis using a novel antigen test

International registry identifier: NCT04033120

Type: Prospective diagnostic and screening study

Description: This study aims to determine the diagnostic and prognostic values and the clinical impact of *Talaromyces marneffei* antigenaemia among people with advanced HIV disease using a novel enzyme immunoassay detecting Talaromyces marneffei–specific cell wall mannoprotein Mp1p. The primary objective is to screen for *Talaromyces marneffei* antigenaemia and determine its diagnostic and prognostic performance among symptomatic and asymptomatic people living with HIV with a CD4 count ≤100 cells/μL and/or WHO stage 3 or 4 disease.

Sample size and population: 1400 (900 inpatients, 500 outpatients) people living with HIV 18 years or older

Study sites: Hanoi and Ho Chi Minh City, Viet Nam

Interventions: *Talaromyces marneffei* antigenaemia testing

Primary outcome: Incidence of microscopy and/or culture-confirmed talaromycosis. Secondary outcomes include how the presence of *Talaromyces marneffei* antigenaemia affects clinical outcomes, including developing culture-confirmed talaromycosis, incidence of stage 3 and 4 AIDS events, subsequent hospitalization and death over 6–12 months of follow-up. The diagnostic and prognostic values of the Mp1p enzyme immunoassays when performed in sera and urine samples will be determined based on culture reference standard.

Status: Recruiting. Due for completion in 2024 for the hospitalized cohort and in 2025 for the outpatient cohort.

Treatment

Current treatment for talaromycosis is amphotericin B induction therapy followed by itraconazole consolidation and maintenance therapy, based on robust randomized controlled trial data *(122)*. The potential utility of single high-dose liposomal amphotericin B for talaromycosis, as now recommended for cryptococcal meningitis, will soon be explored through a Phase 2 trial in Viet Nam (Personal communication: Thuy Le, Duke University, Durham, NC, USA, April 2023). Evidence from in vitro testing suggests that 5-FC would be a highly effective fungicidal partner drug of amphotericin B for talaromycosis, providing a strong rationale for testing amphotericin B and 5-FC in combination therapy, analogous to the AMBITION-cm regimen for cryptococcal meningitis. A factorial trial design is therefore being developed, incorporating single high-dose liposomal amphotericin B and 5-FC as interventions. Funding is currently being sought for the study.

Summary points

- Novel antigen tests have been developed for talaromycosis that have better sensitivity than blood culture and good specificity.
- Two lateral flow and immunochromographic point-of-care tests are in late-stage development and expected to become commercially available in 2025.
- Studies evaluating the utility of screening via antigen testing among individuals (inpatients and outpatients) with advanced HIV disease are underway and expected to report in 2024 or 2025.
- A Phase 3 study exploring the utility of single high-dose liposomal amphotericin B with or without 5-FC is planned to start once funding is in place.

Implications

Several ongoing research studies are likely to provide evidence to inform updated guidelines in the next two years. Data on novel point-of-care diagnostics should be available in 2024 and on antigen screening in 2024 or 2025. A regional guideline can be considered in 2024 or 2025, for possible inclusion in consolidated guidelines as part of HIV-associated fungal infections in 2025 or 2026.

9. Histoplasmosis

Disseminated histoplasmosis has a high prevalence in the Americas, where it is one of the leading causes of AIDS-related deaths *(123)*. Mortality rates are high even with treatment. Lack of simple diagnostic tests limits knowledge of the disease burden, and histoplasmosis is likely to be markedly underdiagnosed in many low-resource endemic areas in Africa and South-East Asia owing to a non-specific clinical syndrome that overlaps with other diseases, especially TB, and the many limitations of the currently available diagnostic methods (culture and histopathology) *(124)*. Coinfection with TB is also frequent, especially among people with low CD4 counts *(124)*.

Current WHO guidelines recommend that disseminated histoplasmosis be diagnosed by antigen detection among people living with HIV *(14)*. Histoplasmosis diagnostics have also been included in the WHO Essential Diagnostics List *(125)*. Antigen detection assays are likely to have significant benefits over culture-based diagnosis. In a recent systematic review and meta-analysis, *Histoplasma* antigen detection assays had a pooled sensitivity of 95% and a pooled specificity of 97% *(124)*. In a recent multicentre study carried out in Brazil, adding urinary *Histoplasma* antigen testing increased the diagnosis of disseminated histoplasmosis by 54% versus classical mycological methods *(126)*. New molecular assays for histoplasmosis have also improved sensitivity versus standard culture and histology methods. By amplifying whole nucleic acids in clinical samples and by targeting the specific multi-copy mitochondrial ribosomal small subunit RNA gene, a novel PCR assay detected 98% of proven cases of histoplasmosis in 907 consecutive people with suspected disease in France *(127)*.

Research is ongoing to better define the epidemiology of histoplasmosis in South-East Asia and sub-Saharan Africa. Several research studies are underway to determine optimal diagnostic and screening strategies, with modelling data suggesting that this may be a cost-effective strategy *(128)*, and to improve treatment. At present, significant research questions remain regarding diagnostic algorithms, the utility of screening and optimal treatments.

Research pipeline

Diagnostics

Two point-of-care LFAs are available for histoplasmosis. In addition, a third novel LFA is in the final stages of laboratory testing and optimization and work is currently ongoing to extend the test shelf life and develop procedures to avoid the need for any pretreatment steps. It is anticipated to be ready for piloting and validation studies in late 2023 (personal communication: J.T. Harrison, May 2023). There are some production problems with one of the currently available tests that have limited its uptake (personal communication: Tom Chiller, United States Centers for Disease Control and Prevention, April 2023). Test performance of the other currently available tests could be improved *(129)*.

Screening

Pilot studies of screening people living with HIV for histoplasmosis in Guatemala have recently been reported. Between 2017 and 2018, 2127 adults newly diagnosed with HIV were screened for histoplasmosis regardless of CD4 count. The frequency of histoplasmosis detection was 7.9%. Mortality rates within six months were high among those with an opportunistic infection, at about 30%, although they declined during implementation of screening *(130)*. Similar prevalence rates have been reported from Nigeria, although from a slightly differing population comprising people living with HIV with CD4 cell counts <200 cells/µL or WHO stage 3 or 4 disease who also had more than two clinical features of disseminated histoplasmosis. Of 988 participants, 76 (7.7%) were positive for *Histoplasma* antigen using a commercially available enzyme immunoassay *(131)*. Screening is ongoing in Guatemala, and further data will be available from studies in the Democratic Republic of the Congo, Guinea, Mozambique, South Africa and Viet Nam in 2024 or 2025 through the IMPRINT study. In Brazil, histoplasmosis accounts for up to 45% of causes of fever among people living with HIV admitted to the hospital in some country areas *(132)*. The United States Centers for Disease Control and Prevention and the Pan American Health Organization are collaborating on implementing and evaluating several other *Histoplasma* screening projects in Argentina, Brazil, Dominican Republic, Ecuador, Guyana, Paraguay and Trinidad and Tobago.

Treatment

Current treatment recommendations are based on liposomal amphotericin B treatment at conventional dose (3 mg/kg) for two weeks. Similar to cryptococcal meningitis, the strategy of treatment using a single high dose of liposomal amphotericin B is being explored in HIV-associated histoplasmosis. A Phase 2 trial has recently been completed (see key trial below) with promising preliminary results, suggesting that the approach is safe and encouraging initial efficacy data *(133)*. A Phase 3 trial is now planned; the protocol has been written, and current projections are for initiation in 2023 and completion in 2026.

Key trial

Liposomal amphotericin B Phase 3: efficacy and safety of high-dose liposomal amphotericin B for disseminated histoplasmosis in AIDS

International registry identifier: NCT05814432

Type: Phase 3 trial.

Description: Disseminated histoplasmosis is one of the major AIDS-defining infections responsible for high mortality rates among people living with HIV. Liposomal amphotericin B is considered the therapy of choice for AIDS-associated histoplasmosis. However, many people in Latin America are still treated with high doses of amphotericin B deoxycholate for long periods. These regimens are associated with toxicity and thus reduced efficacy. Therefore, a better treatment strategy is necessary to improve the activity of this amphotericin B treatment. This proposal seeks to determine the efficacy and safety of two liposomal amphotericin B regimens as induction therapy for disseminated histoplasmosis among people with advanced HIV disease: 10 mg/kg single dose versus 3 mg/kg for two weeks.

Sample size and population: 280 (140 per arm) adult inpatients

Study sites: Brazil

Interventions: (1) single intravenous dose of 10 mg/kg liposomal amphotericin B on day 1; (2) intravenous dose of 3 mg/kg liposomal amphotericin B for two weeks.

Primary outcome: Overall mortality after two weeks of induction therapy. Secondary endpoints will include clinical response, toxicities grades III and intravenous and 50% decrease in *Histoplasma* urinary antigen concentrations (day 14); need for an additional antifungal course of liposomal amphotericin B and mortality at week 10. An ordinal scale will be used to define outcome (Desirability of Outcome Ranking).

Status: Planned to start in late 2023, with anticipated completion in 2026.

An earlier-stage treatment study run by the Mycoses Study Group in the United States is examining the pharmacokinetics, safety, efficacy, tolerability and health economics of an alternative formulation of oral itraconazole, SUBA-itraconazole, in endemic mycoses, with 40 people with histoplasmosis included in the study, although the HIV status of participants is not specified. These findings may have implications in the medium to long term for treatment of HIV-associated disseminated histoplasmosis.

Summary points

- Histoplasmosis is recognized as a major opportunistic infection in Latin America; studies are ongoing to determine disease burden in Africa and South-East Asia.
- New point-of-care LFAs for urine *Histoplasma* antigen detection are likely to be commercially available within the next year.
- Data on the utility of screening for Histoplasma antigen are available from Guatemala, and further data will be available from other countries in the Americas and other regions, including South-East Asia, within the next few years. Significant questions remain regarding how to target diagnostics and screening, particularly given the relatively low disease prevalence and the less than 100% test specificity.
- Simplified treatment using a single high dose of liposomal amphotericin B has been tested in a Phase 2 trial with promising results, and a Phase 3 trial will be starting soon, with results projected to be available in 2026.

Implications

New evidence on epidemiology, diagnosis, screening and treatment is projected to be available in late 2025 and will almost certainly have implications for WHO guidelines. A guideline update focused on histoplasmosis may be required in late 2025.

10. Other conditions and interventions associated with advanced HIV disease

People living with advanced HIV disease are at risk of numerous other infections, either occurring at a lower frequency than those covered above, such as non-tuberculous mycobacterial infections and emergomycosis, or of lower severity, such as oesphageal candidiasis; people living with advanced HIV disease are also at increased risk of malignancies, including Kaposi's sarcoma, lymphoma and cervical cancer. Kaposi's sarcoma in particular is a common cause of cancer in eastern and southern Africa, often presenting at an advanced stage, and carries high mortality. People living with advanced HIV disease may also be at particular risk from emerging and re-emerging infections such as COVID-19 and mpox *(12,13,134)*.

Current WHO advanced HIV disease guidelines set forth a public health approach to covering leading causes of mortality, and do not directly cover any of these diseases; as such, they are not included in this research pipeline document. Detailed guidance for diagnosing and managing rarer infections and malignancies are likely to be beyond the scope of WHO public health guidelines, with the possible exceptions of Kaposi's sarcoma and cervical cancer, both of which have

relatively high incidence in the context of advanced HIV disease. Consideration should be given to including specific guidance around early detection, diagnosis and management of Kaposi's sarcoma and cervical cancer screening in future advanced HIV disease guidance. Emerging and re-emerging infections such as mpox pose key questions relating to advanced HIV disease when they emerge, including whether individuals living with advanced HIV disease are more susceptible to infection, whether the presentation, severity and outcomes of infections differ among those with advanced HIV disease and whether differential management approaches are required, including prevention strategies, treatments and ART use. Specific guidance on an overall approach to emerging and re-emerging infections in the context of advanced HIV disease could be considered.

Management of Kaposi's sarcoma is included in 2014 WHO guidelines *(20)*. Since then, there is new evidence regarding both diagnosis and treatment for Kaposi's sarcoma. Because of the reliance on histopathology to make a definitive diagnosis and the lack of histopathology capacity in many low- and middle-income countries, recent work has focused on alternative diagnostic strategies, including PCR and detection of Kaposi's sarcoma-associated herpesvirus based on loop-mediated isothermal amplification *(135)* and artificial intelligence–assisted reading of digital photographs *(136)*. For treating people living with HIV with advanced Kaposi's sarcoma, clinical trials in both high-income countries and low- and middle-income countries have shown superior clinical response for paclitaxel versus etoposide or bleomycin–vincristine *(137,138)*, and data from a small study in the United States of America has shown similar efficacy for paclitaxel and pegylated liposomal doxorubicin but fewer adverse events with pegylated liposomal doxorubicin *(139)*. A study in Mozambique showed the safety, tolerability and effectiveness of using pegylated liposomal doxorubicin as first-line therapy in low- and middle-income countries *(140)*. Cost–effectiveness analyses in African settings have also demonstrated that these newer therapies are likely to be highly cost effective. Paclitaxel would substantially increase life expectancy and be cost-effective compared with bleomycin–vincristine for advanced Kaposi's sarcoma, and pegylated liposomal doxorubicin would further improve survival and be cost-effective if the price could be reduced by 44% *(141)*.

In addition to managing specific infections, current WHO advanced HIV disease guidelines recommend intensified treatment adherence support and task sharing to nurses and other mid-level health-care workers for the clinical management of people with advanced HIV disease. Very few data are available to support these recommendations in the specific context of advanced HIV disease, and this remains an evidence gap that requires innovative research to inform policy and practice *(142)*. The REMSTART study, described in the cryptococcal disease section above *(59)*, demonstrated that a combined intervention of a short period of community support to supplement clinic-based services plus serum cryptococcal antigen screening led to an 28% mortality reduction in outpatients initiating ART with CD4 counts less than 200 cells/μL versus standard of care. The individual effects of the two components of this combined intervention could not be disaggregated, but it does provide indirect evidence for enhanced adherence support in this context.

Many people with advanced HIV disease are managed as inpatients in hospitals. For example, almost all cryptococcal meningitis, disseminated talaromycosis and histoplasmosis treatments occur in inpatient settings, and many individuals with disseminated TB, *Pneumocystis* pneumonia and severe bacterial infections will be hospitalized. WHO recently released a policy brief on providing care to people with advanced HIV disease who are seriously ill *(19)*. Critical knowledge gaps exist in managing inpatients with advanced HIV disease, highly vulnerable transitions into and out of inpatient care to and from community facilities and preventing the high rates of post-discharge mortality *(143,144)*. Identifying strategies to manage referral more effectively to inpatient facilities when needed, to improve inpatient management and determine whether there is a role for presumptive treatment (such as for TB, *Pneumocystis jirovecii* pneumonia, bacterial infections and cryptococcal disease) and to ensure safe discharge and linkage back to outpatient care could lead to significant improvements in outcomes and are research priorities.

11. Knowledge gaps

This landscaping exercise and subsequent expert group consultation held in September 2023 was not designed or performed as a formal research agenda–setting exercise; nevertheless, several critical research gaps were highlighted during the course of the research landscape mapping exercise and expert consultation. It was noted that a lack of established monitoring systems and the absence of reliable burden-of-disease data and data regarding proximal causes of death in advanced HIV disease globally are major challenges for effective planning and programme management. The Gates Foundation is supporting updated adult mortality epidemiology using Child Health and Mortality Prevention Surveillance (CHAMPS) techniques *(145)*, including minimally invasive tissue sampling and determination of cause of death protocols, with preliminary results expected by July 2025. Additional research data and reliable routine monitoring and evaluation systems for advanced HIV disease are urgently needed. The following list of research gaps and priorities is not intended as an exhaustive summary but includes the major areas identified by the consultation panel (Table 3).

CD4 counts and identification of advanced HIV disease

As access to CD4 counting technologies declines and given the cyclical nature of engagement and disengagement from HIV care, the optimal methods of targeting CD4 count testing need to be determined, with guidance on when CD4 testing should be performed to identify the large proportion of individuals with advanced HIV disease who present following treatment interruption. Determining whether there are very-high-risk groups of individuals who could be considered as having advanced HIV disease even in the absence of CD4 count testing, such as based on previous nadir CD4 counts and a period of disengagement from care, could be considered.

How to operationalize point-of-care CD4 testing

The introduction of novel lateral flow–based CD4 tests has provided new options for CD4 testing in HIV programmes; however, there are critical questions regarding how best to use such tests. The test performance characteristics different from those of conventional flow-cytometry CD4 testing and may affect the feasibility and cost–effectiveness of testing guidelines. Feasibility studies are needed to determine how point-of-care CD4 testing using LFAs can be implemented in routine care, how challenges with relatively short shelf life and concerns around the potential reductions in test specificity as products reach their expiry date, whether digital readers could assist with test interpretation and how clinic workflows and cadres of personnel can be best deployed and used to incorporate point-of-care testing. Studies examining the cost–effectiveness of point-of-care CD4 testing and the effects on subsequent interventions related to advanced HIV disease are needed to determine the likely effects of misidentifying individuals as having advanced HIV disease who have CD4 counts more than 200 cells/μL because of the lower specificity of point-of-care CD4 LFA-based tests.

Burden of severe bacterial infection and antimicrobial resistance in advanced HIV disease

The epidemiology and impact of severe bacterial infections and antimicrobial resistance in advanced HIV disease are poorly understood, and research to determine the disease burden and causes is urgently required.

Including newer TB diagnostic tests into TB screening algorithms in advanced HIV disease

Understanding how newer molecular rapid diagnostics, non-sputum testing and next-generation LAM assays are incorporated into advanced HIV disease screening algorithms requires further research.

Pneumocystis pneumonia

The current evidence base for *Pneumocystis* pneumonia diagnosis and treatment is weak, based on small studies primarily from the pre-ART era. Research is needed to robustly define co-trimoxazole dose and duration and develop and test alternative treatment regimens, including novel antifungals and host-directed therapies, to develop new diagnostics and diagnostic algorithms and to accurately determine disease burden (including through postmortem studies).

Toxoplasmosis

Data on the global prevalence of toxoplasmosis in advanced HIV disease are lacking. Because of the lack of access to cross-sectional imaging and biopsy and histology services in most areas where toxoplasmosis related to advanced HIV disease is likely to be prevalent, regionally appropriate syndrome-based management algorithms for CNS infections should be developed.

Talaromycosis

Current treatment options for talaromycosis remain limited to just amphotericin B deoxycholate and itraconazole. Safer and more effective treatment strategies are needed. The clinical utility of screening for asymptomatic talaromycosis using antigen tests remains to be defined.

Histoplasmosis

The prevalence and public health importance of Histoplasma infection for individuals living with advanced HIV disease remains unknown in most of Africa and Asia. The targets and thresholds for diagnosis and screening, and the clinical utility of screening, need to be defined.

Improving inpatient care and linkage between outpatient and inpatient services for people with advanced HIV disease

Development of inpatient care bundles and pathways for people hospitalized with advanced HIV disease and improved linkage between clinics and hospitals could reduce the high mortality of these people.

Qualitative research

Qualitative research to determine why individuals are still presenting with advanced HIV disease despite widespread access to ART, why current treatment services are failing to meet the needs of these people and how services could be improved to enhance engagement and retention in care is critically important to prevent the development of advanced HIV disease and related morbidity and mortality.

Studies in advanced HIV disease including infants and children

Undetected HIV and advanced HIV disease among children younger than five years is a significant and persistent challenge *(146)*. Many studies, including those for advanced HIV disease–related conditions for which children are at particularly high risk, do not recruit children for various reasons, and both dosing and drug suitability are then not clear for this age group.

Table 3. Summary

Research area	Key findings	Key studies	Timelines
CD4 testing	Updates to the CD4 testing recommendations for identifying advanced HIV disease will be required to account for scaling back conventional laboratory-based CD4 testing, restricted availability of near-patient rapid CD4 testing devices and the introduction of the point of care LFA advanced HIV disease test.	Service evaluations of point of care advanced HIV disease testing in countries supported by the Clinton Health Access Initiative including Nigeria, plus studies in South Africa and Lesotho FIND multicountry evaluation of point of care LFA advanced HIV disease performance ENCORE: an enhanced package of care to reduce mortality in advanced HIV disease	Evidence to guide updates is already available from the FIND evaluation and ENCORE studies, with further data projected by 2024 **To be included in a scoping review for the update of the advanced HIV disease guidelines in 2024.**
Cryptococcal meningitis	Updates to CrAg screening guidelines to incorporate risk assessment and stratification based on semiquantitative antigen testing plus enhanced antifungal treatments may be required Updates to cryptococcal meningitis treatment guidelines are unlikely to be required in the medium term, but minor changes may be needed	EFFECT: treatment of cryptococcal antigen–positive patients identified through screening using fluconazole plus 5-FC vs fluconazole alone. ACACIA: single-dose liposomal amphotericin for asymptomatic cryptococcal antigenemia ENACT: encochleated oral amphotericin for cryptococcal meningitis trial 5-FC HIV-Crypto	Guideline updates are likely required based on evidence from trials that are projected to end and report in 2026 Evidence from trials that are projected to end and report in late 2026 may lead to modifications to guidelines **To be included in scoping for the update of the cryptococcal guidelines in 2027** (five years after the 2022 update)
Severe bacterial infections	The epidemiology and impact of severe bacterial infections in advanced HIV disease are poorly understood, and research and support for laboratory microbiology infrastructure to determine disease burden and causes is urgently required	REVIVE: reducing mortality in adults with advanced HIV disease	Results projected in 2027 or 2028. **Potentially included for scoping of the guidelines to develop recommendations on prophylaxis for severe bacterial infections in advanced HIV disease, pending the results of clinical trial completion in 2028**

Table 3. Summary (continued)

Research area	Key findings	Key studies	Timelines
TB	Advanced HIV disease guidelines will require updates to ensure that TB-focused recommendations incorporate updates from TB diagnostic and TB preventive therapy guideline development groups and data from TB screening studies due to report in 2023 Two trials are investigating enhanced TB treatment and steroids for disseminated TB in the context of advanced HIV disease	CASTLE: computer-aided screening for tuberculosis in low-resource environments DATURA: determination of adequate tuberculosis regimen in patients hospitalized with HIV-associated severe immune suppression. NEW-STRAT TB: testing new strategies for patients hospitalised with HIV-associated disseminated tuberculosis	Results available in 2024 **To be included in a scoping review for the update of the advanced HIV disease guidelines in 2024** Results available in 2026 **Potentially included for scoping of guidelines to develop recommendations on TB treatment in advanced HIV disease on completion of trial in 2026 or 2027 if the results are positive**
Pneumocystis pneumonia	No guideline changing results are likely in the near term. Diagnostic and screening guidelines may need to be updated in 2026 or 2027 depending on the results of ongoing diagnostic evaluations	Planned diagnostic development studies underway Planned Phase 2 treatment trial of rezafungin and low-dose co-trimoxazole	2027 or later **Potentially included for scoping of guidelines to develop an interim Pneumocystis pneumonia recommendation in 2025 or 2026**
Toxoplasmosis	Limited incidence and prevalence data available. Research is needed to better define disease incidence and clinical outcomes. No guideline-changing results are likely in the near term	Epidemiology studies to better define disease burden underway	No guideline-changing research results likely in the next five years
Cytomegalovirus	Randomized controlled trials examining cytomegalovirus therapy in advanced HIV disease and the use of novel drugs such as letermovir are in very early stages of conceptualization. No guideline-changing results are likely in the near term	None	No guideline-changing research results likely in next five years

Table 3. Summary (continued)

Research area	Key findings	Key studies	Timelines
Talaromycosis	Novel antigen tests have been developed for talaromycosis that have reasonable sensitivity and good specificity. Two lateral flow and immunochromographic point-of-care tests are in late-stage development and expected to become commercially available in 2024 Studies evaluating the utility of screening via antigen testing for individuals with low CD4 counts are underway and expected to report in 2025 or 2026 A Phase 2 study exploring the utility of single high-dose liposomal amphotericin B ± 5-FC is planned to start in 2023	Making an early diagnosis of talaromycosis using a novel antigen test	Several ongoing research studies are likely to provide evidence to inform updated guidelines in the next 1–3 years. Data on novel point-of-care diagnostics should be available in 2024 and on antigen screening in 2025 or 2026 The results of treatment studies are projected for 2027 **Potentially included for scoping of guidelines to develop region-specific recommendations in 2024 or 2025**
Histoplasmosis	New point-of-care LFA tests for detecting urinary Histoplasma antigen are likely to be commercially available within the next year. Data on the utility of screening for Histoplasma antigen are available from Guatemala and other countries in the Americas, and further data will be available from other regions, including Africa and South-East Asia, within the next 3–5 years Simplified treatment using a single high dose of liposomal amphotericin B has been tested in a Phase 2 trial with promising results, and a Phase 3 trial will start soon, with results projected to be available in 2025	Randomized trial of liposomal amphotericin B for histoplasmosis in AIDS patients	New evidence on epidemiology, diagnosis, screening and treatment is projected to be available in late 2025 and will almost certainly have implications for WHO guidelines **Potentially included for scoping of guidelines to develop recommendations on histoplasmosis in late 2025 or early 2026**
Other conditions related to advanced HIV disease	Kaposi's sarcoma: new data on both diagnosis and treatment have become available since the last WHO guidance published in 2014 Emerging and re-emerging infections: the recent global experience with COVID-19 and mpox demonstrate that a standardized approach to managing emerging and re-emerging infections in advanced HIV disease could be beneficial		The 2014 Kaposi's sarcoma guidance needs to be updated

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Annex 1. TB meningitis trials

INTENSE-TBM: intensified tuberculosis treatment to reduce the mortality of patients with tuberculous meningitis

ClinicalTrials.gov Identifier: NCT04145258

Type: Phase 3 trial

Description: INTENSE-TBM is a randomized controlled, Phase 3, multicentre, two-by-two factorial plan superiority trial assessing the efficacy of two interventions to reduce mortality from tuberculous meningitis among adolescents and adults with or without HIV infection in sub-Saharan Africa: intensified tuberculous meningitis treatment with high-dose rifampicin and linezolid versus WHO standard tuberculous meningitis treatment. Aspirin versus not receiving aspirin. The trial will be open label for anti-TB treatment and placebo-controlled for aspirin treatment.

Sample size: 768

Study sites: Côte d'Ivoire, Madagascar, Uganda and South Africa

Interventions: randomization 1: rifampicin 35 mg/kg for eight weeks plus linezolid 1200 mg for four weeks then 600 mg for four weeks plus standard H, Z, E dosing for eight weeks, standard continuation phase versus WHO standard regimen. Randomization 2: aspirin 100 mg orally for eight weeks versus placebo.

Primary outcome: Mortality to 40 weeks.

Status: Started in 2021. Behind schedule. Possibly 2024 finish.

HARVEST trial: improving outcomes from TB meningitis with high-dose oral rifampicin

International registry identifier: ISRCTN15668391 (https://doi.org/10.1186/ISRCTN15668391)

Type: Phase 3 trial.

Description: HARVEST is a double-blinded parallel group randomized placebo-controlled Phase 3 trial of high-dose oral rifampicin (35 mg/kg) alongside other regular first-line anti-TB drugs to improve survival and nervous system outcomes from tuberculous meningitis versus standard-of-care TB treatment.

Sample size: 500

Study sites: Indonesia, South Africa and Uganda

Interventions: Rifampicin 35 mg/kg for eight weeks, standard isoniazid, pyrazinamide and ethambutol for eight weeks, standard continuation phase versus WHO standard regimen

Primary outcome: six-month survival.

Status: Recruiting. Expected to be completed by June 2025.

ALTER Trial: adjunctive linezolid for the treatment of tuberculous meningitis (ALTER)

ClinicalTrials.gov Identifier: NCT04021121

Type: Phase 2 trial (pharmacokinetic).

Description: A phase 2 randomized open-label factorial trial of high- versus standard-dose rifampicin with or without linezolid for the first four weeks of treatment for tuberculous meningitis at Masaka Regional Referral Hospital in Uganda. Initial randomization will be to high-dose (35 mg/kg per day) versus standard-dose (10 mg/kg per day) oral rifampicin for the first four weeks of intensive therapy. Participants will then undergo a second randomization to linezolid 1200 mg daily versus no linezolid for the first four weeks of therapy.

Sample size: 60

Study sites: Uganda

Interventions: rifampicin 10 mg/kg; rifampicin 10 mg/kg plus linezolid 1200 mg for four weeks; rifampicin 35 mg/kg; rifampicin 35 mg/kg plus linezolid 1200 mg for four weeks. All with standard doses of isoniazid, pyrazinamide and ethambutol. After four weeks, linezolid will be discontinued in the linezolid-containing arms and high-dose rifampicin will return to the standard dose for the remainder of treatment.

Primary outcome: pharmacokinetic parameters.

Status: 2021–2023.

SIMPLE Trial: pharmacokinetic study of linezolid for TB meningitis

ClinicalTrials.gov Identifier: NCT03537495

Type: Phase 2 trial (pharmacokinetic).

Description: a parallel-arm Phase 2 pharmacokinetic study testing high-dose rifampicin with two different doses of linezolid.

Sample size: 36

Study sites: Indonesia

Interventions: rifampicin 35 mg/kg; rifampicin 35 mg/kg plus linezolid 600 mg 2 for weeks; rifampicin 35 mg/kg plus linezolid 1200 mg for 2 weeks. All given with isoniazid, pyrazinamide and ethambutol according to international guidelines.

Primary outcome: pharmacokinetic parameters.

Status: completed in 2023.

ACT HIV Trial: adjunctive corticosteroids for tuberculous meningitis in HIV-infected adults

ClinicalTrials.gov Identifier: NCT03092817

Type: Phase 3 trial.

Description: A randomized, double-blind, placebo-controlled trial of adjunctive dexamethasone in the initial (6–8 weeks) treatment of tuberculous meningitis in Vietnamese adults.

Sample size: 520

Study sites: Indonesia and Viet Nam

Interventions: Standard anti-TB drugs plus dexamethasone for 6–8 weeks versus standard anti-TB drugs plus placebo.

Primary outcome: 12-month survival.

Status: Published in October 2023: https://www.nejm.org/doi/full/10.1056/NEJMoa2216218.

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