Cryptococcal Meningitis

Lillian Tugume[1, †], Kenneth Ssebambulidde[1,2], John Kasibante[1], Jayne Ellis[1,3], Rachel M. Wake[4], Jane Gakuru[1], David S. Lawrence[3,5], Mahsa Abassi[6], Radha Rajasingham[6], David B. Meya[1,6,7], David R. Boulware[6,7]

[1] Infectious Diseases Institute, Makerere University, Kampala, Uganda
[2] Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA
[3] Clinical Research Department, Faculty of Infectious and Tropical Diseases London School of Hygiene and Tropical Medicine, London, UK
[4] Institute for Infection and Immunity, St George's University of London, London, UK
[5] Botswana Harvard AIDS Institute Partnership, Gaborone, Botswana
[6] Department of Medicine, University of Minnesota, Minneapolis, MN, USA
[7] These authors contributed equally: David B. Meya, David R. Boulware.

[†]Email: lilliqntugume18@gmail.com; ltugume@idi.co.ug

Acknowledgements
LT was supported by the Fogarty International Center of the National Institutes of Health under grant #D43TW009345 awarded to the Northern Pacific Global Health Fellows Program. JK and JG are supported by the Fogarty International Center and National Institute of Neurologic Disorders and Stroke (R01NS086312).

Author contributions
Introduction was written by LT, Epidemiology by LT, RR Mechanisms/Pathophysiology by LT, JK, KS, DBM; Diagnosis/Screening/Prevention by LT, RR, RMW; Management by LT, DSL, JE, DBM, JG, MA; Quality of life by LT, MA; and Outlook by LT, DRB, DBM. All authors researched data for this article, discussed content and made revisions.

Competing interests
The authors declare no competing interests.

Abstract

Cryptococcus neoformans and *Cryptococcus gattii* species complexes cause meningoencephalitis with high fatality rates and considerable morbidity, particularly in persons with deficient T cell mediated immunity, most commonly affecting people living with HIV. Whereas the global incidence of HIV-associated cryptococcal meningitis (HIV-CM) has decreased over the past decade, cryptococcosis still accounts for one in five AIDS-related deaths globally due to the persistent burden of advanced HIV disease. Moreover, mortality remains high (~50%) in low resource settings. The armamentarium to decrease cryptococcosis-associated mortality is expanding: cryptococcal antigen (CrAg) screening in the serum and pre-emptive azole therapy for cryptococcal antigenemia are well established, whereas enhanced pre-emptive combination treatment regimens to improve survival of persons with cryptococcal antigenemia are under trials. Short course (≤ 7 days) amphotericin-based regimens combined with flucytosine are currently the preferred options for induction therapy of cryptococcal meningitis. Whether short course induction regimens improve long term morbidity such as depression, reduced neurocognitive performance and physical disability among survivors is subject to further study. Here, we discuss underlying immunology, changing epidemiology, and updates on management of cryptococcal meningitis with emphasis on HIV-associated disease.

[H1] Introduction

Cryptococcal meningitis is an opportunistic mycosis caused by invasion of *Cryptococcus* into the central nervous system (CNS), and most commonly occurs in individuals with advanced HIV disease (AHD). Less commonly, cryptococcal meningitis may occur in individuals with non-HIV associated immunosuppression, or in apparently immunocompetent hosts[1-3], and incidence of non-HIV associated disease is increasing in high-income countries. *Cryptococci* are ubiquitous encapsulated yeasts of the phylum Basidiomycota and consist of several species. *Cryptococcus neoformans* and *Cryptococcus gattii* species complexes are known to cause disease in humans[4,5]. *Cryptococcus neoformans* typically causes fatal meningoencephalitis, especially amongst immunocompromised hosts. *C. gattii* is more likely to cause meningitis in apparently immunocompetent hosts except for cases in South East Asia where *C. neoformans* species predominate[2,6]. Disease phenotypes are underpinned by complex host–yeast interactions.

Cryptococcal meningitis accounts for 15-20% of AIDS-associated deaths globally[7]. Nearly two-thirds of all deaths from HIV-associated cryptococcal meningitis (HIV-CM) occur in Africa, and a 10-week mortality is as high as 50% of the incidents in routine care settings [8].

Current therapeutic protocols include the use of a combination of antifungal drugs, including amphotericin B, flucytosine and fluconazole. The polyene amphotericin B binds to ergosterol in the cytoplasmic membrane of fungi, causing the extravasation of intracellular electrolytes, carbohydrates and proteins, and is an essential anti-cryptococcal drug owing to its low resistance rate and potent fungicidal activity[9]. 5-fluorouracil, a flucytosine derivative, inhibits fungal protein synthesis[10]. Due to the rapid development of resistance, flucytosine monotherapy is not recommended. Both a fungistatic action through inhibition of ergosterol synthesis and a fungicidal activity associated with dose-dependent apoptotic responses have been described for fluconazole[11].

Poor treatment outcomes in routine settings have been partly attributed to life-threatening toxicities associated with 14-day course of amphotericin -based induction regimens. Based on two multicenter landmark trials conducted in Africa in years 2013 – 2022,[12,13] abbreviated courses ($\leq$ 7 days) of amphotericin are now recommended by the World Health Organization (WHO), and these short regimens successfully reduce case fatality to ~25% in the clinical trial context. As treatment outcomes have improved over time, screening and prevention strategies have advanced in parallel. Cryptococcal meningitis is preceded by the detection of cryptococcal antigen (CrAg) in blood, and CrAg screening, followed by pre-emptive fluconazole therapy in cases of cryptococcal antigenemia with no CNS involvement is life-saving and cost-effective for patients with AHD[14].

In this Primer, we discuss updates on the epidemiology, immunology, diagnosis, management, and prevention of cryptococcal meningitis, with emphasis on HIV-CM. We additionally discuss prospects for improving survival.

[H1] Epidemiology

The molecular age of taxonomy for cryptococci has enabled identification of strains by species complex, species, serotype, molecular type or lineage, and sequence type. *C.neoformans* and *C. gatti* species complexes are globally distributed, though *C.neoformans* is approximately eight to nine-fold more frequently isolated than *C.gattii*[15]. Five major genetically distinct lineages of *C. neoforman*s (VNI, VNII, VNIII VNIV, VNB) and six of *C.gattii* (VGI, VGII VGIII, VGIV, VGIV/VGIIIc and VGV) can be identified by multi locus sequence typing[16]. This is not inclusive of the C.*neoformans/* C.*gatti* hybrid genotypes. Of the eleven molecular types, VNI is the most prevalent worldwide, except for Australia and Papua New Guinea, where *C.gattii* is more prevalent[15]. The proportion of *C.gattii* is relatively lower in African and European isolates compared to Asia and the Americas, and is endemic in Australia[17]. *C. gatti* was initially thought to be ecologically restricted to tropical and subtropical regions but the 1999 outbreak in temperate regions across north America disproved this assumption[18]. Distribution of *C. gattii* lineages varies by region; for instance, VGII is the most common *C. gattii* lineage in the Americas and is linked to the 1999 outbreak in North America, which is thought to have originated from South America[18,19]. VGI is the most common lineage in Europe, Asia and Australia, whereas VGII and VGIII are the most prevalent in the Americas. VGIV is the most common lineage in southern African isolates, and seems to have a predilection for immunocompromised hosts including HIV associated cases[17]. Whether this distribution of *C. gatti* lineages is primarily a result of importation of pathogens from endemic areas is unclear because travel data are not consistently available. Molecular epidemiological data must be interpreted with caution because of sampling bias; for instance, Asia is over represented in published multi locus sequencing data and only a subset (approximately <15%) of isolates globally have been molecularly typed[15] . Additionally, over 80% of the published isolates are clinical isolates, which represent only a subset of the diversity in the environment[15]. Robust environmental genomic surveillance is useful for defining risk of exposure to cryptococcus and may partially explain patterns of disease.

[H2] HIV-associated Cryptococcal Meningitis

The global incidence of HIV-CM has generally decreased over the last decade but cryptococcal meningitis still accounts for an estimated 19% of all AIDS-related mortality[7,20]. This global decline is due to improved access to antiretroviral therapy (ART), and increased cryptococcal antigen (CrAg) screening and subsequent pre-emptive antifungal therapy. In Africa, the HIV-CM burden remains the highest globally, representing more than half of all incident HIV-CM cases in 2020, followed by HIV-CM burden in the Asia and Pacific regions[7] **(FIG.1).** Historically, HIV-CM occurred predominantly in ART-naïve patients who presented to care for the first time with AHD. Currently, and as a result of improved access to ART, more than half of patients diagnosed with HIV-CM have had prior exposure to ART at the time of diagnosis[21-23]. Generally, survival in patients with HIV-CM is not associated with ART status, except for cases in which ART unmasks the infection [24,25]. A Ugandan study reported a 2-fold increase in hazard of death when ART was initiated sooner than 2 weeks before cryptococcal diagnosis[26].

The incidence of cryptococcal antigenemia among people living with HIV globally is 6.5% when CD4 T cell counts are ≤100 cells/μL and approximately 2.0% when CD4 T cell counts are between 101-200 cells/μL[7,20,27]. Evidence that cryptococcal antigenemia precedes cryptococcal meningitis if untreated is mainly derived from retrospective studies. A study with 707 patients initiating ART in South Africa demonstrated that baseline

cryptococcal antigenemia could predict the development of subsequent cryptococcal meningitis within a year with 100% sensitivity and 96% specificity[28]. Testing of bio-banked samples from a Ugandan cohort of patients who subsequently developed cryptococcal meningitis found that detectable CrAg in blood preceded meningitis symptoms by a median of 22 days (range 5-234)[29].

A complication of a recovering immune system with ART after cryptococcosis is a paradoxical reaction. In HIV, this is termed paradoxical immune reconstitution inflammatory syndrome (IRIS). Over the last two decades, the incidence of cryptococcal IRIS has generally decreased from being approximately 30% between 2003-2008,[30-33] to 3-20% in years 2014 - 2022[34,13,35,36], probably owing to the improvement of anti-fungal therapy combinations and the recommendation for delayed ART initiation. The median duration between ART initiation and incidence of IRIS remains 4-8 weeks[37], while mortality following cryptococcal IRIS is 8-30%.[38].

While the global incidence of HIV CM has generally decreased, most recent global estimates indicate that the case fatality rate probably exceeds 50% outside of clinical trials. There were approximately 152,000 new HIV-CM diagnoses in 2020 resulting in approximately 112,000 deaths, with two-thirds of the reported deaths occurring in Africa[7]. Cryptococcal-related mortality is highest during the first 12 weeks following diagnosis[34,39,40]. Ten-week mortality under routine care conditions ranges from 19-50% in low- and middle-income countries (LMICs), to less than 20% in high-income countries[8,41-45]. Prognosis in clinical trials conducted in LMICs has improved in the past years (10-week mortality of 24-36%) [12,13].

Despite pre-emptive antifungal therapy, cryptococcal antigenemia remains a risk factor for death in patients with AHD. In prospective studies using fluconazole pre-emptive treatment, subsequent diagnoses of clinical cryptococcal meningitis are rare[46-48]. However, patients with AHD and cryptococcal antigenemia have a 2 to 3-fold higher risk of death within six months, than patients with AHD and equally low CD4 T cell counts but CrAg-negative blood tests[46-48]. This persistent excess mortality is most pronounced in persons with high CrAg titers. Individuals with serum or plasma CrAg titers $\geq$1:160 are three times more likely to die compared to those with CrAg titers $\leq$1:80 despite receiving fluconazole[49]. The increased mortality risk suggests that fluconazole monotherapy is a suboptimal antifungal treatment for those with high disseminated burden of infection, reflective by high CrAg titers in blood.

The strongest predictors of acute cryptococcal mortality under amphotericin-based induction therapy are the presence of altered mental state and high fungal burden at diagnosis[39]. Moreover, the rate of fungal clearance from cerebrospinal fluid (CSF) is inversely associated with all-cause mortality[50,51]. Thus, in the acute setting, the strategy of improving survival has been largely focused on maximizing clearance of the fungus with the most efficacious and safe antifungal regimens[12,13]. Contrarily, improving outcomes by additionally targeting altered mental state in HIV CM is more challenging as the pathogenesis is only partially understood.

[H2] Non-HIV Cryptococcal Meningitis

The global epidemiology of non-HIV CM is not well described, but disease incidence is generally increasing in high-income countries in association with an increase in use of immunosuppressive therapies and an expansion of multi-morbid, aging populations[52]. Risk factors among HIV-negative individuals include solid organ transplantation, autoimmune diseases, hematological malignancies, diabetes mellitus, chronic kidney and liver diseases, use of corticosteroids and other immunosuppressive drugs, and alcoholism[53]. The presence of granulocyte-macrophage colony-stimulating factor (GM-CSF)-specific autoantibodies and idiopathic CD4 lymphocytopenia have been demonstrated on further immunological testing in some individuals with non-HIV CM[54-57].

In the US, approximately 20% of patients with non-HIV CM have no identifiable underlying immunosuppressive condition, and this proportion is higher in Asia (75 to 80%) [1-3]. Whether this discrepancy is attributable to an unidentified immune deficit, increased genetic susceptibility in the host, or increased pathogenicity of *Cryptococcus* species is unclear. Some host factors have been partially associated with susceptibility of apparently immunocompetent individuals to cryptococcal meningitis , including a specific HLA class II allele (DQB1*05:02), or late onset immunodeficiency syndrome associated with the production of interferon gamma (IFNγ)-specific autoantibodies[58-60]. The likelihood of unidentified idiopathic CD4 lymphocytopenia in the studied apparently immunocompetent patients with non-HIV CM is low, as immunological testing was conducted.

Infection with *C. gattii* (especially with the VGI & VGII *C. gattii* lineages) is more common in apparently immune-competent individuals, whereas *C. neoformans* is the predominant species in immunocompromised individuals with cryptococcal meningitis[6,61]. Paradoxically, *Cryptococcus neoformans* species complex, sequence type 5 (ST 5) is the dominant species isolated from apparently immunocompetent individuals in China and Vietnam[2,3,62]. In vitro studies from a Vietnam cohort suggest that the ability to infect apparently immunocompetent hosts is related to an evolutionary advantage of *C. neoformans* ST 5 as evidenced by high phenotypic variation[63]. Further, the presence of GM-CSF-specific autoantibodies in apparently immunocompetent patients with cryptococcal meningitis is presumably rare and commonly associated with *C. gattii*[64-66].

Studies from the United States and Taiwan from years 1990–2015 suggested that in-hospital mortality from cryptococcal meningitis is slightly higher among HIV-negative individuals compared to those living with HIV[67,68]. Delayed diagnosis contributes to this excess mortality, which stems from a low index of suspicion among HIV-negative individuals[40,67]. Whether the specific underlying immune compromising condition impacts survival is non-conclusive, as absolute numbers of patients are too few to study this robustly[1,69]. Presently, any potential heterogeneity in immune status is not factored into the therapy of non-HIV-CM. Overall, susceptibility to cryptococcal meningitis, disease severity and clinical outcomes are associated with complex yeast and host interactions, and an advanced understanding of this relationship may enhance current prevention and treatment strategies.

[H1] Mechanisms/pathophysiology

C. neoformans is a ubiquitous basidiomycetous fungus isolatable from avian and non-avian sources[70]. *C.gatti* has been isolated from the soil, air, water and several tree species, especially eucalypts[71-73]. Immunopathogenesis of *C.neoformans* infections is more intensely studied than that of *C.gatti* infections, therefore the following sections focus on disease mechanisms identified in studies of *C.neoformans*. Overall, *C.gatti* and *C.neoformans* express the same major virulence determinants.

[H2] Primary infection

C. neoformans infects individuals after they inhale desiccated yeast cells or infectious propagules called basidiospores into the lungs (**FIG. 2a**)[74]. Simultaneous or consecutive inhalation of multiple *C.neoformans* strains may result in mixed infections, with hosts testing positive for multiple yeast genotypes[75]. The primary immune response that is initiated in the lungs may successfully clear the fungi. Any uncleared fungi are walled off and contained in granulomas, establishing a latent infection in immunocompetent hosts. In some individuals, immunosuppression leads to reactivation of the latent infection and distant hematogenous spread[76]. Epidemiological studies suggest that cryptococcal meningitis may occur in solid organ transplant recipients with no preceding latent stage [77,78].

[H2] Immune response to the primary infection

Alveolar macrophages and dendritic cells are the first responders to primary lung infection with *Cryptococci* [79]. The fungus is recognized by pattern recognition receptors (PRRs) including, C-type lectin receptors, Dectin-1, mannose receptors, and Toll-like receptor 2 (TLR2) and TLR4[80,81]. Fungal recognition induces intracellular signaling that culminates in eventual pathogen phagocytosis. Fungal opsonization with antibodies and C3b fragments of the complement enhances phagocytosis[82]. Following phagocytosis of the fungi, macrophages secrete cytokines including TNF, interleukin 1 beta (IL-1β), IL-6, IL-12, and GM-CSF, which all promote the expression of chemokines, including CXCL1/2, and chemokine receptors (such as CXCR2)[80,81]. These cytokines and chemokines attract additional innate immune cells, including neutrophils and monocytes, to the lung (**FIG. 2b**).

Despite successful phagocytosis of the fungi, some virulence mechanisms of *C. neoformans* block intracellular pathogen killing responses in phagocytes.[83] As a result, T cell help is required for effective clearance of phagocytosed fungi. Upon having phagocytosed the fungi, dendritic cells mature and express the costimulatory molecules CD80 and CD86 and the the chemokine receptor CCR7, which directs dendritic cell migration towards CCL21-secreting local lymph nodes[84]. Inside the lymph nodes, dendritic cells present *C. neoformans*-derived antigens to naïve T and B lymphocytes [84] (**FIG. 2b**). In addition, infected innate immune cells, including macrophages, secrete IL-12, which polarizes $CD4^+$T helper cells to a pro-inflammatory T helper 1 (Th1) cell phenotype[85]. CD4 Th1 cells in turn secrete IFNγ, which potentiates the killing capacity of phagocytic cells[85]. The clearance of *C. neoformans* coincides with the development of an adaptive immune response that enhances killing or containment of the fungi in granulomas (**FIG. 2b**)[79]. Therefore, successful containment of the fungus requires both innate and immune responses.

[H2] Host factors for disease reactivation

T cell-mediated immunity is crucial for controlling *C. neoformans* infection. Conditions associated with T cell defects predispose to disseminated cryptococcal disease (**FIG. 2c**). These include HIV with low CD4+ T cell count (usually <100 cells/μL); solid organ transplantation; use of immunosuppressive drugs, such as high dose corticosteroids, azathioprine, and cyclophosphamide; hematologic and solid malignancies; idiopathic CD4 lymphopenia; cytokine-specific autoantibodies; or genetic polymorphisms that impair immune cell function. Less commonly, chronic diseases such as diabetes mellitus, chronic lung disease, renal failure, and liver disease impair T cell function, thereby conferring susceptibility to reactivation of latent cryptococcal infection [86-88]. Pregnancy-related immunomodulation may increase risk for disseminated cryptococcosis[89].

Autoantibody-associated syndromes targeting IFNγ or GM-CSF have been implicated in cryptococcal disease[66]. The IL-12–IFNγ axis is required to ensure that phagocytic cells kill intracellular *C. neoformans*, thus patients with defective IFNγ signaling, including patients with IFNγ-specific autoantibodies are unable to clear fungi [90]. GM-CSF promotes the differentiation and functioning of alveolar macrophages including chemotaxis, phagolysosome maturation, and microbicidal activity[91]. Patients with GM-CSF-specific autoantibodies are therefore at risk to acquire cryptococcal meningitis, especially following infection with *C. gattii*[65,66].

[H2] Virulence factors and immune evasion

The polysaccharide capsule of *Cryptococci* is the main virulence factor that protects the fungus from phagocytosis, intracellular killing, and reactive oxygen species[83,92,93]. It is unsurprising that capsule size positively correlates with resistance to *in vitro* killing[93]. Additionally, glucuronoxylomannan (GXM), the most abundant capsular polysaccharide inhibits immune cell trafficking and facilitates fungal dissemination[92,94]. Chitin, a component of the fungal cell wall, is another vital virulence factor that influences capsular structure, extracellular vesicle trafficking, and protection from the surrounding environment[95]. Fungi with reduced levels of chitosan (a chitin derivative) have 'leaky melanin' and are more sensitive to cell wall inhibitors and high temperatures[96,97]. The term 'leaky melanin' refers to decreased ability of the cryptococcal cell wall to retain melanin. Melanin functions as a scavenger for reactive nitrogen and oxygen species[98,99]. Reactive nitrogen and oxygen species are effector molecules released by human immune cells and contribute to microbial killing through induction of apoptosis. Melanin also makes the fungus cell wall thicker and more resistant to phagocytosis and intracellular killing.

Several cryptococcal enzymes contribute to immune evasion, including phenol oxidase and laccase, phospholipases, and ureases (**FIG. 2a**). Phenol oxidase and laccase enable *C. neoformans* to synthesize melanin, leading to melanin accumulation in the fungal cell wall – a process also known as melanization[98,99]. Phospholipases involved in sphingolipid metabolism promote *C. neoformans* survival in the hostile intracellular environment of phagolysosomes that are characterized by oxidative, nitrosative and acidic stresses[100]. Additionally, phospholipases confer resistance to antibody and complement-mediated phagocytosis in the CNS and promote brain damage[101] The mechanism by which phospholipases protect against phagocytosis is unclear but possibly related to titan cell formation[102]. Phospholipase activity also promotes fungal adherence to the lung epithelia by disrupting surfactant and cell membranes of immune cells in the lungs[103,104]. Finally,

ureases, which promote nitrogen acquisition from the environment, help increase the pH in phagolysosomes[105,106]. Urease activity complements melanization in virulence as ammonia released by urease promotes the melanization of distant fungal cells[107].

[H2] Dissemination to the CNS.

Dissemination of the fungi from the lungs to the CNS occurs via bloodstream and at this point, fungi are detectable in blood by cryptococcal antigen testing and fungal culture. *Cryptococcus* traverses the blood–brain barrier into subarachnoid space via transcellular, paracellular, or 'trojan horse' mechanisms (**FIG. 2d**). During transcellular dissemination, the fungus adheres to and gets internalized by microvascular endothelial cells in the brain[76]. Paracellular invasion is facilitated by *C. neoformans* secreted metalloproteases, which enzymatically degrade intercellular junction adhesion molecules and the basement membrane to facilitate passage of the fungi into the subarachnoid space. In the 'trojan horse' mechanism, phagocytic immune cells mainly monocytes and macrophages traffic phagocytosed *C. neoformans* into the CNS[108].

[H2] Immune responses in the brain

Invasion of the subarachnoid space leads to the recruitment monocytes, macrophages, neutrophils, dendritic cells, and microglia to the foci of infection (**FIG. 2e**). Cytokines and chemokines are released after fungal invasion of the blood brain barrier and alter the avidity and expression of endothelial cell adhesion molecules, such as L selectin, ICAM-1, ICAM-2. Selectins and ICAM molecules bind integrin on circulating immune cells and promote extravasation of immune cells into the CSF. Early during the disease course, neutrophilic pleocytosis may be found in CSF. Neutrophils are critical in early clearance of *C. neoformans* from leptomeningeal capillaries[109].

The chemokine CCL2 attracts monocytes and macrophages to the subarachnoid space. In murine models, inflammatory monocytes have been shown to traffic phagocytosed *C. neoformans* to the subarachnoid space[108]. M1 macrophages are protective against fungal infections and acquire their pro-inflammatory phenotype in the presence of IFNγ produced by Th1 cells, natural killer cells, and CD8 T cells[110]. In the subarachnoid space of mice, however, inflammatory monocytes can be polarized towards an alternatively activated M2 phenotype which is permissive for fungal infections[111]. In fact, patients with HIV-CM who survived the initial two weeks of treatment showed a increased levels of IFNγ in the CSF at baseline compared to those who died[112]. Consistently, IFNγ adjunctive therapy has shown an increased rate of fungal clearance in two phase II randomized clinical trial in patients with HIV-CM [113,114].

Microglia, the main subset of CNS-resident macrophages, are self-replicating and are maintained by IL-34. *In vitro* studies show that microglia phagocytose *C. neoformans*, but the fungus survives and replicates in microglial phagosomes[115]. Microglia display increased phagocytosis in the presence of antibodies that specifically bind to GXM of the cryptococcal capsule, thereby inhibiting fungal growth[116] Following phagocytosis, microglia present fungal antigens to T cells and secrete inflammatory cytokines including TNF, IL-1, IL-6, and IL-12[117].

Activation of a T cell immune response is beneficial in controlling CNS cryptococcosis , but CD4 T cell mediated immune injury has been described in murine models of fungal growth in the CNS[118]. The contribution of CD4 T cell mediated immune injury in human CNS disease outcomes remains a subject of inquiry.

B cells are unnecessary for protection against *C. neoformans* in mouse models of disease dissemination.[119] In mice, vaccine-acquired immunity against the fungus depends on CD4+ T cells rather than B cells[119]. Further, capsule-specific antibodies do not neutralize *C. neoformans* in the absence of T lymphocytes and this phenomenon may explain recurrent cryptococcosis in people living with HIV who have not started treatment, interrupted their treatment or disengaged from care[116]. Nevertheless, B cells potentially have a role in regulating immune response against *C. neoformans*, as implied by the positive association between expression of the immune checkpoint programmed death ligand-1 (PD-L1) on plasma cells and survival in patients with HIV-CM[120]. Having a combination immune defect of T cell deficiency and B cell dysfunction may predispose to more severe disease or IRIS.[121]

[H2] **Cryptococcal IRIS**

Cryptococcal IRIS is a dysregulated inflammatory response to Cryptococci that occurs when the immune system begins to recover following treatment with ART.[122] 'Unmasking' IRIS is characterized by overt clinical symptoms of a previously undiagnosed subclinical infection soon after ART was started. Unmasking IRIS events commonly may present with more acute presentations or atypical manifestations in unusual anatomical locations. 'Paradoxical' IRIS refers to the worsening of a previously diagnosed and treated infection after ART was started.[122,123]

Risk factors driving the onset of paradoxical cryptococcal IRIS can generally be grouped as: pathogen factors (initial fungal burden); host immune response factors (paucity of baseline immune response,[124] GXM-specific antibody responses in the CSF,[121] immune system dysfunction with poor macrophage killing,[125,126] impaired clearance of cryptococcal yeast cells,[127] and dysregulated homeostatic, regulatory mechanisms resulting in exaggerated inflammatory responses);[41,128] and timing of ART initiation[129] (Box 1).

[H1] Diagnosis, screening and prevention

[H2] Diagnosis of primary infection

CrAg testing on CSF, serum, plasma, or whole blood, performed by lateral flow assay (LFA), latex agglutination assay, or enzyme-linked immunosorbent assay (ELISA) is the cornerstone for prompt cryptococcal diagnosis. All available diagnostic tests are outlined in Table 1. CrAg LFA by Immy, Inc (Norman, OK, USA) is currently the best performing commercially available assay that can be used as a point-of-care test: it has superior performance to other diagnostics, with sensitivity and specificity of beyond 99%, when applied on CSF samples[130-133]**.** Other CrAg LFAs have been developed, but their diagnostic performance is suboptimal.[134-136]

In HIV-negative samples or very early after cryptococcal infection, CrAg titer in CSF can be very low or at times absent, and patients may slowly develop a chronic presentation that can be mistaken for other pathology such as tuberculous meningitis or coccidioidomycosis[52,67]. Repeat CSF testing may be necessary, and we recommend always testing for CrAg also in the blood in the setting of unexplained meningitis[52,137]. Cerebral computer tomography scan and magnetic resonance imaging (MRI) are useful in detecting complications such as cerebral cryptococcomas, which are space occupying masses loaded

with *Cryptococcus* yeasts. Cryptococcomas are most frequently found in immunocompetent hosts[138,139]. Surgical resection, histopathology, and culture may be used in cases of large CNS lesions where confirmation is needed[140].

[H2] Diagnosis of relapse

CrAg testing cannot distinguish among cryptococcal meningitis relapse, paradoxical IRIS, and a new non-cryptococcal CNS diagnosis, as CSF remains positive for CrAg for months to years after initial diagnosis[141]. CSF cryptococcal culture is the only definitive diagnostic test to validate relapsed infection, yet results are obtained only 5-14 days later, which is too late to facilitate timely clinical decision making. Without access to timely confirmatory results, clinicians are forced to make empiric treatment decisions about the application of toxic anti-fungal therapy for suspected relapse versus steroid therapy for suspected paradoxical IRIS, often with detrimental consequences if incorrect [142]. In a study analyzing adults with recurrence of HIV CM symptoms, a multiplex PCR assay correctly classified 10/11 individuals in terms of relapse versus paradoxical IRIS, but the current FDA-approved PCR multiplex assay has poor sensitivity when CSF quantitative culture yields <100 colony forming units/mL and high costs[143]. A real-time quantitative PCR assay for *Cryptococcus* is under development, which offers promise that more sensitive tools to differentiate between relapse and IRIS will soon become available[144].

[H2] Prevention and screening in people with HIV

There are currently no vaccines available for preventing cryptococcosis, but preclinical studies using recombinant vaccines of a chitin deacetylase subunit (Cda2) administered with an adjuvant show potential[145]. However, the commercial viability of recombinant vaccines for HIV-CM is expected to involve significant challenges. Screening for CrAg in blood is recommended for people living with AHD[146]. In 2011, WHO Rapid Advice introduced CrAg screening and pre-emptive fluconazole treatment based on observational evidence, including evidence from a Ugandan prospective cohort demonstrating that patients with HIV and cryptococcal antigenemia who received low-dose fluconazole (400 mg daily or lower) had a survival benefit[147,148]. In addition, a 'screen-and-treat' approach was modelled as being more cost- and life-saving in comparison to the 'no screening' or 'universal prophylaxis' approaches[14]. A multisite randomized prospective trial (REMSTART) confirmed previous observational findings, showing that CrAg screening and pre-emptive treatment of patients with cryptococcal antigenemia contributed to a mortality risk reduction of around a third[47].

Prospective screening studies have identified a clinical entity described as subclinical cryptococcal meningitis, that affects approximately a third of individuals with cryptococcal antigenemia and is associated with high CrAg titers in the blood, *Cryptococcus* in the CSF, but no overt clinical features of meningitis, such as severe headache, confusion, seizures or reduced consciousness[49,149,150]. WHO guidelines, therefore, recommend lumbar punctures to exclude subclinical cryptococcal meningitis in all CrAg-positive patients irrespective of symptoms. However, some experts recommend to guide decisions about the need for lumbar puncture based on plasma CrAg titers and symptoms, recognizing that the risk of asymptomatic CNS disease is low among individuals with plasma CrAg LFA titer $\leq$1:80 and becomes very common at CrAg LFA titers $\geq$1:1280 (**FIG. 3A**)[150].

[H2] Screening for co-infections

Co-prevalent opportunistic infections are likely to contribute to poor outcomes of HIV-CM[151-153]. Interventions to optimize prevention, and/or early diagnosis and treatment of opportunistic infections are therefore critical.

Approximately 25% of hospitalized patients with HIV CM also have active tuberculosis (TB), with TB co-infection being associated with an increase of >50% in risk of mortality[154]. The considerable overlap in non-specific symptoms between TB and cryptococcosis means that without systematic screening, TB diagnoses will be missed. Accordingly, the WHO recommends universal urine testing for TB-LAM (detecting the TB antigen lipoarabinomannan) for all hospitalized patients with AHD, including those with cryptococcal meningitis[155]. Low complexity automated RT-PCR test applied on sputum samples is the first line diagnostic for pulmonary TB and should be utilized whenever a sputum sample can be obtained[156], but given the predominance of extra-pulmonary TB in AHD, and the frequent inability to produce sputum due to severe disease states associated with cryptococcal meningitis, non-sputum-based TB diagnostics are required in this high-risk population[155].

Due to profound immunocompromise, in-dwelling medical devices and prolonged hospitalization, an estimated 5-15% of patients with HIV-CM acquire a concurrent blood stream infection during hospitalization, with a high proportion of multi-drug resistant infections[157]. Blood cultures remain the gold standard diagnostic, but novel multiplex PCR diagnostics carry the potential to increase yield and decrease time to result[158]. Other than cotrimoxazole, broader antimicrobial prophylaxis including azithromycin is not currently recommended due to concerns relating to antimicrobial resistance[159]. Meningococcal and pneumococcal vaccination should be offered to all individuals with AHD [155].

Cytomegalovirus (CMV) reactivation is common in AHD. Amongst adults with HIV-CM, the prevalence of CMV viraemia is reported to be between 36-52%, and is associated with 3-fold increased risk of mortality compared to those without CMV viraemia[152,153]. CMV quantitative-PCR is the most sensitive method for detecting CMV. When utilized in non-HIV immunocompromising conditions other than HIV infection, such as stem cell transplants, to guide CMV prophylaxis/treatment, high CMV viraemia in the blood is a good predictor of CMV disease[160]. It is probable that quantitative PCR CMV screening would also facilitate risk stratification in patients with AHD, but viral-load cut-offs need to be defined. Guidelines currently highlight the importance of early ART initiation rather than anti-CMV prophylaxis due to concerns regarding the side effects, drug resistance, or cost-effectiveness of CMV-targeting drugs, as well as the lack of a proven survival benefit associated with older anti-CMV drugs, such as oral ganciclovir [161,162]. In cryptococcal meningitis however, the early ART strategy is untenable due to the risk of IRIS. Whether anti-CMV prophylaxis/treatment (for example using valganciclovir or letermovir) prior to ART may improve outcomes for this high-risk population warrants investigation[163].

Additional important co-pathogens which may contribute to poor outcomes in cryptococcal meningitis include cerebral toxoplasmosis, *pneumocystis jirovecii* pneumonia, and disseminated histoplasmosis; however, the global burden of morbidity and mortality attributable to these infections is poorly characterized because appropriate diagnostic facilities are lacking in most settings with high AHD burden[155].

[H1] Management

[H2] Management of HIV-CM

Treatment of cryptococcal meningitis is divided into three phases: induction, consolidation, and maintenance or secondary prophylaxis[164]. While guidelines about the consolidation and maintenance phases have remained largely unchanged and are managed with fluconazole, the induction phase has gone through several iterations to maximize survival. Historically, induction phase consisted of a 14-day course of an amphotericin B deoxycholate-based regimen[140]. Amphotericin B deoxycholate is notoriously toxic and prolonged, 14-day courses, are associated with severe side effects such as anemia, hypokalemia, hypomagnesemia, and nephrotoxicity[165-167]. Due to the poor tolerance of extended amphotericin B therapy and high cryptococcal mortality in routine care settings, shorter courses have been tested[8,168,169]. Two landmark randomized clinical trials conducted in Africa with patients living with HIV demonstrated that it is possible to achieve a ten-week mortality rate below 30% with shorter courses of amphotericin B. Based on the 'Advancing Cryptococcal Treatments for Africa (ACTA)' and 'AMBIsome Therapy Induction OptimisatioN (AMBITION-cm) trials, abbreviated courses ($\leq$ 7 days) of amphotericin-based induction therapy are now preferred for the management of HIV-CM [12,13]. (**Box 2**).

The ACTA trial sought to determine whether seven-day courses of amphotericin B deoxycholate based regimens were non-inferior to 14-day courses[12]. This trial additionally tested an amphotericin-sparing combination of flucytosine 100mg/kg/day and fluconazole 1200mg/day for 14 days. Results showed that the 7-day amphotericin-based regimens were non-inferior to the 14-day regimens. Significantly, seven days of amphotericin 1mg/kg/day plus flucytosine 100mg/kg/day was associated with the lowest ten-week mortality (24.2%) when compared with all other regimes, including two weeks of amphotericin plus flucytosine[12]. Additionally, the ACTA trial demonstrated that flucytosine was a superior partner antifungal to fluconazole[12]. Despite having the slowest rate of *Cryptococcus* clearance from the CSF, the amphotericin sparing arm was the second best-performing arm with 10-week mortality of 35% and was also the safest[12]. As a result, the one week of amphotericin B and flucytosine, and two weeks of fluconazole and flucytosine ACTA trial regimens became the WHO recommended first and second-line regimens for the management of HIV CM in 2018[170].

The liposomal form of amphotericin is associated with reduced drug-related toxicities compared to amphotericin B deoxycholate and has a long half-life in the CNS[171]. A single, high dose of 10mg/kg of liposomal amphotericin B was demonstrated to be non-inferior to daily dosing of liposomal amphotericin B when combined with fluconazole in the phase II AMBITION-cm trial[13]. Given the emerging evidence from the ACTA trial on the importance of flucytosine, this led to the AMBITION-cm phase III, non-inferiority trial comparing a single, 10mg/kg dose of liposomal amphotericin combined with 14 days of flucytosine (100mg/kg) and fluconazole (1200mg) to standard of care. The standard of care was 7 days of amphotericin (1mg/kg/day) and flucytosine (100mg/kg) followed by 7 days of fluconazole (1200mg). This trial demonstrated that the single-dose liposomal amphotericin arm was non-inferior to the control group in terms of survival, with equivalent CSF fungal clearance over 14 days[13]. The AMBITION-cm regimen was also associated with fewer grade 3 and 4 adverse events, including anemia, nephrotoxicity, and hypokalemia, and no

additional risk of cytopenias or transaminitis[13]. Moreover, the AMBITION-cm regimen was cost-effective in resource-limited settings and highly acceptable to participants and healthcare providers[172,173]. The AMBITION-cm regimen was subsequently adopted as the WHO-recommended regimen for the management of HIV CM in 2022[146].

Current guidelines for HIV-CM in high-income countries recommend two weeks of liposomal amphotericin B and flucytosine for induction therapy; however, this regimen represents an evolution of treatment over time and has never been tested in a randomized controlled trial and there has been no direct comparison with the AMBITION-cm regimen [164]. Harrison *et al* argue that the AMBITION-cm regimen should be adopted across all settings due to its high rates of CSF sterility at two weeks, absence of relapse cases occurring in the trial, and that the single dose regimen reduces the occurrence of drug toxicities associated with prolonged courses of amphotericin[174].

The use of corticosteroids is not recommended as a component of induction treatment of HIV CM as it was associated with increased mortality in CryptoDex, a large randomised controlled trial among patients with HIV-CM. Steroids may still be considered in particular patients that do not respond to treatment with antifungals despite a sterile CSF culture and/or upon identification of inflammatory brain lesions[175], including in some case of paradoxical IRIS. Dexamethasone should not be used as a substitute for lumbar punctures or CNS shunts for the management of raised ICP[175,176].

[H3] Pre-emptive therapy for cryptococcal antigenemia

The WHO recommends high-dose fluconazole with 800-1200 mg daily for 2 weeks for all individuals living with HIV that develop cryptococcal antigenemia without any other symptoms of cryptococcal meningitis, irrespective of titer, followed by consolidation (800 mg daily for 8 weeks) and maintenance therapy (200 mg daily) for at least a year to allow for immune reconstitution on ART (**FIG. 3B**)[146]. Southern African guidelines recommend an increased induction fluconazole dose of 1200 mg based on a general evolution over time to higher doses of fluconazole which have been well tolerated, including for the treatment of meningitis[177].

Fluconazole monotherapy may not be adequate for preventing disease progression in CrAg-positive patients, particularly those who have undiagnosed subclinical cryptococcal meningitis[178]. A study investigating cause of death in 17 asymptomatic CrAg-positive patients who died within 6 months after initiation of treatment with 800mg fluconazole, attributed 71% of deaths to cryptococcal disease as an immediate or contributing cause[48]. In response to a growing recognition that fluconazole monotherapy may be suboptimal treatment for cryptococcal antigenemia, two randomized controlled trials aim to investigate combination treatments: the ACACIA Trial (Uganda) will test liposomal amphotericin B 10 mg/kg plus fluconazole (NCT03945448)[179]; and the EFFECT Trial (South Africa and Tanzania) will test flucytosine plus fluconazole treatment (ISRCTN30579828)[180] for reducing all-cause mortality in patients with HIV and cryptococcal antigenemia. These trials will also assess if combination drug regimens should be targeted to individuals at higher risk of disease progression, as stratified based on CrAg titer.

[H3] Timing of ART

Despite widespread availability of ART worldwide, the incidence of cryptococcal meningitis remains high[7]. Before 2014, it was considered imperative to initiate ART as soon

as possible, without any concerns about the timing of ART initiation in the context of an existing opportunistic infection. Five trials have examined the impact of early or deferred ART initiation on outcomes in the setting of cryptococcal meningitis. The ACTG 5164 trial showed that ART initiation within 14 days after diagnosis of HIV-CM reduced death and AIDS progression as compared to ART initiated four weeks later, albeit with non-significant results. However, the trial included only 41 participants with cryptococcosis and was underpowered to influence guidelines[181]. Conflicting results were reported by a trial that was conducted in Zimbabwe and involved 54 patients with HIV and cryptococcosis that were treated with fluconazole monotherapy: participants randomly assigned to initiate ART within 24 hours of meningitis diagnosis had a higher risk of death compared to those who deferred ART (median of 10 weeks later)[182]. Another randomized trial in Botswana involving 27 patients with cryptococcosis showed a higher risk of IRIS among participants who were randomized to early ART, without survival benefit from deferred ART[183]. The conflicting, underpowered results of the above-mentioned trials created an equipoise, indicating that the timing of ART initiation in the setting of HIV-CM should accommodate a balanced consideration of the untoward risk of an occasionally fatal ART complication, IRIS, and the benefit of ART[181-183].

The COAT trial, which involved 177 patients with HIV-CM definitively concluded that deferring ART for 4-6 weeks after the diagnosis of meningitis conferred an absolute survival benefit of 15% as compared to initiating ART within 1-2 weeks[34]. These results were replicated in a randomized trial with 102 participants with HIV CM from China that demonstrated higher mortality with ART initiation at <4 weeks versus >4 weeks after meningitis diagnosis[35].

Based on the above findings, clinicians can now make informed decisions about ART initiation following HIV-CM diagnosis. However, it remains unclear whether ART should be interrupted when HIV-CM is diagnosed up to 14 days after ART initiation. In a cohort of 605 patients who received amphotericin-based therapy following a first-episode of HIV-CM, those who had initiated ART up to 14 days before the diagnosis of cryptococcal meningitis had a significantly higher two-week mortality rate (of 47%) compared to patients who had been on ART for 15-182 days prior to diagnosis of cryptococcal meningitis and had a two-week mortality rate of 14%[26]. Although these findings have not yet been replicated in other cohorts, a group of experts have suggested that ART interruption may lower mortality risk in patients diagnosed with HIV-CM within 14 days after ART initiation[184]. However, this warrants further investigation.

Some controversy has arisen in 2023 whereby some believe the randomized clinical trial data on ART timing after cryptococcal meningitis is not applicable to high-income settings, based on observational pooled cohort data of 630 people collected from 1996 to 2012.[185] There are substantial limitations of these observational data, not least that 70% had missing outcome data or were unjustifiably excluded from analysis.[185] The recommendation to delayed ART initiation by >4 weeks after cryptococcal meningitis remains the recommended strategy.

[H3] Cryptococcal IRIS

The occurrence or severity of cryptococcal IRIS should be prevented via early diagnosis of HIV infection, with ART initiation, CrAg screening and preemptive therapy, using appropriate antifungal therapy. ART delay per current guidelines should decrease the risk of IRIS.

When cryptococcal IRIS occurs, it is important that symptomatic management is considered. Management consists of: controlling raised ICP via therapeutic lumbar punctures;[177] excluding co-infections; therapeutic interventions to decrease inflammation, including corticosteroids[186]. Thalidomide and TNF-specific monoclonal antibodies have been used in patients with corticosteroid-refractory IRIS[187,188].

[H2] Management of non-HIV-CM

As non-HIV CM is increasingly being diagnosed, particularly in high-income countries, the paucity of randomized controlled evidence about the management of non-HIV CM, particularly a lack of more current data using what have become standard doses of antifungals, becomes increasingly apparent[189]. Guidelines of Infectious Diseases Society of America (IDSA) that were published in 2010, recommend standard two week amphotericin and flucytosine induction therapy for organ transplant recipients with non-HIV CM. In non-HIV and non-transplant patients with cryptococcal meningitis the recommendation is amphotericin and flucytosine induction therapy for at least four weeks with guidance to extend to six weeks in the presence of neurological complications and/or positive CSF culture at week two after therapy initiation, although the guideline authors do acknowledge the paucity of evidence and lack of consensus on this topic[140]. There are no data on high-dose liposomal amphotericin B regimens for non-HIV CM, and the findings of the AMBITION-cm trial may not be transferrable to patients with non-HIV CM due to the differences between pathologies, particularly in terms of host immune function and the absence of any treatment comparable to ART that can rapidly restore the immune system.

Cryptococcal post-infectious inflammatory response syndrome (PIIRS) is an excessive inflammatory reaction that may occur in patients with non-HIV CM, resulting in host damage. PIIRS is diagnosed based on several factors, including but not limited to: clinical deterioration; new lesions appearing on brain imaging; raised ICP in the presence of a sterile CSF culture; elevated CSF white cell count; and low CSF glucose levels [189]. In a single arm observational study of 15 patients with non-HIV CM and PIIRS, tapered courses of steroids dosed as one week of high-dose methylprednisolone (1g/day) followed by oral prednisone 1mg/kg/day were associated with improvements in PIIRS-related complications of cryptococcal meningitis, including vision and hearing impairment[190]. IDSA guidelines suggest 2–6 week courses of steroids at a tapering dose starting at 0.5-1.0mg/kg/day prednisolone equivalent for PIIRS.

[H2] Ancillary Support

Amphotericin B deoxycholate causes life threatening side effects, including electrolyte abnormalities (hypokalemia and hypomagnesaemia), anemia, kidney injury, thrombophlebitis, and consequent bacteremia[157,165,166,191]. Although protocols for standardized electrolyte supplementation and preemptive hydration have been effective in reducing mortality, their consistent implementation is challenging, especially in resource-

constrained settings[165,166]. Single high-dose liposomal amphotericin which is less toxic is therefore a much more desirable treatment option in such settings[13].

However, ancillary support for patients with cryptococcal meningitis has an important survival benefit and is multi-modality. Severe baseline hyponatremia (with serum sodium levels of <125 mmol/L) occurs in up to 15% of patients with cryptococcal meningitis and this complication is associated with a doubling in 2-week mortality and 30-day mortality risks[192]. The development of hyponatremia in patients with cryptococcal meningitis is likely to be a multifactorial insult, in which raised intracranial pressures (ICP), high quantitative cryptococcal cultures, and seizures, leading to either the syndrome of inappropriate antidiuretic hormone secretion (SIADH) or cerebral salt wasting (CSW). However, laboratory tests to precisely differentiate between SIADH or CSW and guide management are often inaccessible in low-resource settings. Further research is needed to develop a standardized approach to hyponatremia that does not improve with management of cryptococcal meningitis.

An objective assessment of impaired consciousness resulting in a Glasgow Coma Scale score (GCS) that is lower than 15 at diagnosis is a strong independent predictor for acute mortality, therefore management of altered mental status is likely to further improve survival. Baseline GCS <15 is associated with a five-fold increase in the probability of death[39] and is linked to increased ICP, seizures, hyponatremia, and elevated CSF lactate (> 5 mmol/L)[192-195]. Seizures are common in patients with cryptococcal meningitis, occurring in 28% of the cases, and associated with a higher 10-week mortality risk[194]. Thus, ancillary support for patients with cryptococcal meningitis should include neurologic supportive care that incorporates aggressive management of ICP, treatment of hyponatremia, and seizures. The selection and duration of antiepileptic drugs to control seizures is influenced by the individual's medical history, comorbidities, and potential drug-drug interactions.

Increased ICP (CSF opening pressure >20 cmH_2O) is common in patients with CM and associated with significant mortality and morbidity. Guidelines recommend aggressive management of raised ICP through serial lumbar punctures, lumbar drain placement, or ventriculoperitoneal shunting until ICP has normalized and symptoms have resolved (**Table 2**). While measurement of baseline opening pressure is encouraged to determine the need for subsequent therapeutic lumbar punctures, it has previously been shown that one therapeutic lumbar puncture in the first week, irrespective of baseline opening pressure, is associated with a 69% relative improvement in survival in the first 10-days[196]. Kagimu *et al* also showed that baseline opening pressure > 35 cmH_2O is associated with a higher mortality, while Bicanic *et al* did not find an association between baseline opening pressure and 2- and 10-week outcomes when applying a protocolized schedule of therapeutic lumbar punctures[13,197,198]. Therefore, we recommend that at a minimum, scheduled lumbar punctures be performed at day three post diagnosis, and prior to discharge in all persons with cryptococcal meningitis, irrespective of baseline opening pressures (**Table 2**).

[H1] Quality of Life

Mortality remains high in the first 6 months after diagnosis of cryptococcal meningitis, and there is a high prevalence of concurrent disability and neurocognitive impairment within the first 6 to 12 months after diagnosis [40,199]. Quality of life assessment among individuals having completed treatment for cryptococcal meningitis revealed that

those with a self-perceived low quality of life at week 10 after diagnosis had a higher mortality rate within the first 6 months compared to those with higher self-perceived quality of life at week 10 after diagnosis [200,201]. Data on long term (one year and beyond) quality of life are needed. Cranial nerve impairments resulting in vision loss and hearing loss possibly contribute to long term disability and low quality of life in individuals that have survived after a CM diagnosis [202-204]. Vision loss in cryptococcal meningitis can occur as a presenting symptom or a complication. Rapid onset (<3 days) is attributed to optic nerve infiltration or inflammatory arachnoiditis and may occur without papilledema or increased ICP[205]. Gradual onset (>3 days) is linked to uncontrolled raised ICP[205]. Temporary vision loss is often preceded by diplopia or decreased vision and improves with antifungal treatment and management of raised ICP, whereas irreversible loss is commonly associated with optic nerve damage from increased ICP[205]. The incidence rate of sensorineural hearing loss among patients with CM is still unknown, and hearing loss might be associated with temporal bone invasion by cryptococcus, damage of spiral ganglion cell and cochlear nerve fiber, or cryptococcal meningeal infiltration [206,207]. Sensorineural hearing loss is more common in individuals with increased ICP and accompanying visual impairment[206]. The course of hearing loss varies, ranging from progression to permanent hearing loss, stabilization with residual hearing loss, or complete improvement with antifungal therapy and ICP decompression[206].

Individuals with cryptococcal meningitis may initially present with psychiatric symptoms such as mania, depression, and early signs of neurocognitive impairment at the time of cryptococcal meningitis diagnosis[208]. Depression rates among survivors with HIV are high (67% at one month, 44% at three months after diagnosis) and associated with baseline altered mental status and increased distance from a healthcare center, indicating that delays in care and severe infection result in higher rates of depression[209]. Long-term data on neurological sequelae in cryptococcal meningitis are limited, and the existing literature exhibits methodological and analytical heterogeneity[40]. Reports of disability among survivors of cryptococcal meningitis at one year after diagnosis vary from 11% to 69.2%[40]. A longitudinal assessment of neurocognitive performance utilizing a battery of neuropsychological tests that evaluate eight cognitive domains demonstrated impaired neurocognitive performance in 89% of individuals at one month, 59% at 3 months, and 41% at 6 months from diagnosis[210]. Overall, neurocognitive performance continues to improve during the first year, with residual impairment noted in cognitive domains of motor speed, gross motor and executive function[210]. Whether the new induction regimens will impact long term outcomes, including impaired neurocognitive performance, is a subject for further study. In the interim, strengthening rehabilitation services, including physical, occupational, and cognitive rehabilitation is needed.

[H1] Outlook

The armamentarium to decrease the morbidity and mortality associated with cryptococcal meningitis has expanded over the last 2 decades. This arsenal includes CrAg LFA for prompt diagnosis, serum CrAg screening and pre-emptive antifungal therapy among persons with HIV and cryptococcal antigenemia,[211-213] therapeutic lumbar punctures,[196,198] delaying ART initiation after the diagnosis of cryptococcal meningitis in ART-naïve persons living with HIV,[34] and the current development of shorter and more efficient and cost effective[172] fungicidal regimens alongside adjunctive pre-supplementation with electrolytes[166].

Increasingly more persons with HIV-CM are ART-experienced globally, suggesting that ART alone is not sufficient to eliminate the incidence of HIV-CM[12,13,23,26,34]. Lack of knowledge about cryptococcal meningitis among the general population results in lumbar puncture refusal, and a low index of suspicion among healthcare providers leads to delays in cryptococcal diagnosis, with a negative impact on outcomes[214]. A study in rural Uganda showed that 70% of patients who died following a HIV-CM diagnosis had sought care ≥3 times before the diagnosis was made. Additionally, only 10% of patients and 40% of family members knew about cryptococcal meningitis as a comorbidity of HIV infection[215].

Among patients with non-HIV CM, atypical manifestations and nonspecific neuroradiological findings due to lack of inflammatory responses are responsible for delays in diagnosis that may contribute to fatal outcomes[216,217]. In contrast to guidelines for people living with HIV, there are no specific guidelines for routine serum CrAg screening among patients with other immune suppressive conditions. Patients without HIV are more likely to present with cryptococcal pulmonary involvement and without CNS involvement, thus lumbar punctures may not be routinely performed in the absence of neurological symptoms[218,219]. Thus, in immunosuppressed individuals, such as recipients of solid-organ transplants, the requirements and optimal strategy for CrAg screening remain to be defined.

CrAg screening and preemptive therapy for CrAg positive patients living with HIV remain important interventions to decrease the incidence of HIV-CM, especially in high burden countries. However, programmatic inadequacies, including inaccessibility to CD4 T cell count tests (which are the current entry point for CrAg screening), CrAg tests, and anti-fungal drugs, and suboptimal adherence to guidelines by healthcare providers coupled with poor retention of CrAg positive patients to ensure treatment completion remain significant bottlenecks in decreasing the incidence of HIV CM and associated mortality[220,221].

The most suitable anti-fungal regimen for preemptive therapy remains a subject of research, and a one-size fits all approach may not be optimal. Approximately 8% of 152 individuals with cryptococcal antigenemia developed breakthrough meningitis following preemptive treatment with the previously recommended 10-week fluconazole regimen[212]. Six-month outcomes of individuals with cryptococcal antigenemia might improve if pre-emptive fluconazole therapy is combined with other antifungal drugs, especially as the current fluconazole is inadequate for those with CrAg titer ≥1:160[212,222].

In addition to ART naïve individuals with HIV, country guidelines should also target ART experienced persons with suboptimal virological suppression for CrAg screening and preemptive therapy, as an increasing proportion of persons with cryptococcosis are ART-experienced[223]. Serum CrAg positive persons with neurological symptoms should routinely undergo lumbar punctures to exclude meningitis; however, even when CSF CrAg tests are negative, the mortality of patients with this clinical phenotype remains high, similar to that among patients with cryptococcal meningitis, suggesting that they could benefit from enhanced anti-fungal therapy[137,149].

With a persisting mortality rate between 25-30% in trial settings and a 15-20% contribution to HIV related mortality, the need to expand the pipeline for developing more potent antifungal drugs to treat cryptococcosis remains significant. Recent data show that a less toxic oral encochleated formulation of amphotericin, MAT2203, given for 2 weeks with flucytosine and continued for 4 more weeks with fluconazole for treatment resulted in 18-

week survival of 85-90% in patients presenting with normal baseline GCS[224]. Optimizing adjunctive antifungal and host directed therapy might still be possible. Tamoxifen and Sertraline have anti-fungal activity in vitro and in vivo but were shown to have no impact on cryptococcal clearance or mortality as adjunctive therapies[225,226]. A small study of 90 patients showed that addition of two doses of short course IFNγ to standard treatment increased the rate of fungal clearance without any increase in adverse events.[113] Another study showed a trend to improved combined mycologic and clinical success in recipients of recombinant IFNγ-1b[114]. To optimize cost-effectiveness, biomarkers for selection of patient subgroups most likely to benefit from such therapies are needed.

Novel anti-fungal therapeutics that have undergone early phase trials include fosmanogepix, and the VT-molecules. Fosmanogepix inhibits the fungal enzyme Gwt1 of the glycosylphosphatidylinositol biosynthesis pathway, thus preventing the biosynthesis of cell wall mannoproteins to compromise cell wall integrity and fungal growth[227]. VT-1598 prevents biosynthesis of ergosterol within fungal wall cell membranes by inhibiting fungal rather than mammalian CYP51[228]. ATI-2307 exhibits equivalent anti-fungal activity against cryptococcal isolates. As the minimum inhibitory concentrations of ATI-2307 are comparable to those of fluconazole, ATI-2307 could potentially be used in the context of fluconazole resistance[229]. Lastly, a new third-generation polyene SF-001, which is an amphotericin-like glycosylated polyene macrolide, [230] is under development by Sfunga therapeutics.

The inclusion of *Cryptococcus* in the 2022 WHO fungal priority pathogen list highlights the contribution of this pathogen to human disease and the need to further invest in research and development of new antifungal therapies[231]. Recently adopted point-of-care diagnostics and abbreviated liposomal amphotericin regimens should translate into timely and expanded access to diagnostics and treatment to reduce mortality. The 'End CM Deaths by 2030' strategic framework recommends the priorities to focus on in order to reduce the morbidity and mortality from cryptococcal meningitis[232].

References:

1 Brizendine, K. D., Baddley, J. W. & Pappas, P. G. Predictors of mortality and differences in clinical features among patients with Cryptococcosis according to immune status. *PLoS One* **8**, e60431, doi:10.1371/journal.pone.0060431 (2013).

2 Chau, T. T. *et al.* A prospective descriptive study of cryptococcal meningitis in HIV uninfected patients in Vietnam - high prevalence of Cryptococcus neoformans var grubii in the absence of underlying disease. *BMC Infect Dis* **10**, 199, doi:10.1186/1471-2334-10-199 (2010).

3 Chen, J. *et al.* Cryptococcus neoformans strains and infection in apparently immunocompetent patients, China. *Emerg Infect Dis* **14**, 755-762, doi:10.3201/eid1405.071312 (2008).

4 Freij, J. B. *et al.* Conservation of Intracellular Pathogenic Strategy among Distantly Related Cryptococcal Species. *Infect Immun* **86**, e00946-00917, doi:10.1128/IAI.00946-17 (2018).

5 Casadevall, A. & Pirofski, L. A. The damage-response framework of microbial pathogenesis. *Nature reviews. Microbiology* **1**, 17-24, doi:10.1038/nrmicro732 (2003).

6 Baddley, J. W. *et al.* MSG07: An International Cohort Study Comparing Epidemiology and Outcomes of Patients With Cryptococcus neoformans or Cryptococcus gattii Infections. *Clin Infect Dis* **73**, 1133-1141, doi:10.1093/cid/ciab268 (2021).

7 Rajasingham, R. *et al.* The global burden of HIV-associated cryptococcal infection in adults in 2020: a modelling analysis. *The Lancet infectious diseases* **22**, 1748-1755, doi:10.1016/S1473-3099(22)00499-6 (2022).

8 Patel, R. K. K. *et al.* High Mortality in HIV-Associated Cryptococcal Meningitis Patients Treated With Amphotericin B-Based Therapy Under Routine Care Conditions in Africa. *Open forum infectious diseases* **5**, ofy267, doi:10.1093/ofid/ofy267 (2018).

9 Finkelstein, A. & Holz, R. Aqueous pores created in thin lipid membranes by the polyene antibiotics nystatin and amphotericin B. *Membranes* **2**, 377-408 (1973).

10 Vermes, A., Guchelaar, H. J. & Dankert, J. Flucytosine: a review of its pharmacology, clinical indications, pharmacokinetics, toxicity and drug interactions. *J Antimicrob Chemother* **46**, 171-179, doi:10.1093/jac/46.2.171 (2000).

11 Lee, W. & Lee, D. G. A novel mechanism of fluconazole: fungicidal activity through dose-dependent apoptotic responses in Candida albicans. *Microbiology (Reading)* **164**, 194-204, doi:10.1099/mic.0.000589 (2018).

12 Molloy, S. F. *et al.* Antifungal Combinations for Treatment of Cryptococcal Meningitis in Africa. *N Engl J Med* **378**, 1004-1017, doi:10.1056/NEJMoa1710922 (2018).

13 Jarvis, J. N. *et al.* Single-Dose Liposomal Amphotericin B Treatment for Cryptococcal Meningitis. *N Engl J Med* **386**, 1109-1120, doi:10.1056/NEJMoa2111904 (2022).

14 Jarvis, J. N. *et al.* Cost effectiveness of cryptococcal antigen screening as a strategy to prevent HIV-associated cryptococcal meningitis in South Africa. *PLoS One* **8**, e69288, doi:10.1371/journal.pone.0069288 (2013).

15 Cogliati, M. Global Molecular Epidemiology of Cryptococcus neoformans and Cryptococcus gattii: An Atlas of the Molecular Types. *Scientifica (Cairo)* **2013**, 675213, doi:10.1155/2013/675213 (2013).

16 Montoya, M. C., Magwene, P. M. & Perfect, J. R. Associations between Cryptococcus Genotypes, Phenotypes, and Clinical Parameters of Human Disease: A Review. *J Fungi (Basel)* **7**, doi:10.3390/jof7040260 (2021).

17 Chen, S. C., Meyer, W. & Sorrell, T. C. Cryptococcus gattii infections. *Clin Microbiol Rev* **27**, 980-1024, doi:10.1128/CMR.00126-13 (2014).

18 Kidd, S. E. *et al.* A rare genotype of Cryptococcus gattii caused the cryptococcosis outbreak on Vancouver Island (British Columbia, Canada). *Proc Natl Acad Sci U S A* **101**, 17258-17263, doi:10.1073/pnas.0402981101 (2004).

19 Fraser, J. A. *et al.* Same-sex mating and the origin of the Vancouver Island Cryptococcus gattii outbreak. *Nature* **437**, 1360-1364, doi:10.1038/nature04220 (2005).
20 Rajasingham, R. *et al.* Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *The Lancet infectious diseases* **17**, 873-881, doi:10.1016/S1473-3099(17)30243-8 (2017).
21 Ellis, J. *et al.* The changing epidemiology of HIV-associated adult meningitis, Uganda 2015-2017. *Open forum infectious diseases* **6**, ofz419, doi:10.1093/ofid/ofz419 (2019).
22 Flynn, A. G. *et al.* Evolving Failures in the Delivery of Human Immunodeficiency Virus Care: Lessons From a Ugandan Meningitis Cohort 2006-2016. *Open Forum Infect Dis* **4**, ofx077, doi:10.1093/ofid/ofx077 (2017).
23 Okwir, M. *et al.* High Burden of Cryptococcal Meningitis Among Antiretroviral Therapy-Experienced Human Immunodeficiency Virus-Infected Patients in Northern Uganda in the Era of "Test and Treat": Implications for Cryptococcal Screening Programs. *Open forum infectious diseases* **9**, ofac004, doi:10.1093/ofid/ofac004 (2022).
24 Chang, B. H. *et al.* 2361. Timing of antiretroviral therapy prior to diagnosis of cryptococcal meningitis. *Open forum infectious diseases* (2022).
25 Kalata, N. *et al.* Short-term Mortality Outcomes of HIV-Associated Cryptococcal Meningitis in Antiretroviral Therapy-Naive and -Experienced Patients in Sub-Saharan Africa. *Open forum infectious diseases* **8**, ofab397, doi:10.1093/ofid/ofab397 (2021).
26 Rhein, J. *et al.* Detrimental Outcomes of Unmasking Cryptococcal Meningitis With Recent ART Initiation. *Open forum infectious diseases* **5**, ofy122, doi:10.1093/ofid/ofy122 (2018).
27 Ford, N. *et al.* CD4 Cell Count Threshold for Cryptococcal Antigen Screening of HIV-Infected Individuals: A Systematic Review and Meta-analysis. *Clin Infect Dis* **66**, S152-S159, doi:10.1093/cid/cix1143 (2018).
28 Jarvis, J. N. *et al.* Screening for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa. *Clin Infect Dis* **48**, 856-862, doi:10.1086/597262 (2009).
29 French, N. *et al.* Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. *AIDS* **16**, 1031-1038, doi:10.1097/00002030-200205030-00009 (2002).
30 Sungkanuparph, S. *et al.* Cryptococcal immune reconstitution inflammatory syndrome after antiretroviral therapy in AIDS patients with cryptococcal meningitis: a prospective multicenter study. *Clin Infect Dis* **49**, 931-934, doi:10.1086/605497 (2009).
31 Shelburne, S. A., 3rd *et al.* The role of immune reconstitution inflammatory syndrome in AIDS-related Cryptococcus neoformans disease in the era of highly active antiretroviral therapy. *Clin Infect Dis* **40**, 1049-1052, doi:10.1086/428618 (2005).
32 Kambugu, A. *et al.* Outcomes of cryptococcal meningitis in Uganda before and after the availability of highly active antiretroviral therapy. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **46 11**, 1694-1701 (2008).
33 Lortholary, O. *et al.* Incidence and risk factors of immune reconstitution inflammatory syndrome complicating HIV-associated cryptococcosis in France. *AIDS* **19**, 1043-1049, doi:10.1097/01.aids.0000174450.70874.30 (2005).
34 Boulware, D. R. *et al.* Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis. *N Engl J Med* **370**, 2487-2498, doi:10.1056/NEJMoa1312884 (2014).
35 Zhao, T. *et al.* The Effect of Early vs. Deferred Antiretroviral Therapy Initiation in HIV-Infected Patients With Cryptococcal Meningitis: A Multicenter Prospective Randomized Controlled Analysis in China. *Front Med (Lausanne)* **8**, 779181, doi:10.3389/fmed.2021.779181 (2021).
36 Sereti, I. *et al.* Prospective international study of incidence and predictors of immune reconstitution inflammatory syndrome and death in people with HIV and severe lymphopenia. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* (2020).

37 Han, X. *et al.* A nomogram for predicting paradoxical immune reconstitution inflammatory syndrome associated with cryptococcal meningitis among HIV-infected individuals in China. *AIDS Research and Therapy* **19** (2022).
38 Brienze, V. M. S., André, J. C., Liso, E. & Louis, I. V.-S. Cryptococcal Immune Reconstitution Inflammatory Syndrome: From Blood and Cerebrospinal Fluid Biomarkers to Treatment Approaches. *Life* **11** (2020).
39 Jarvis, J. N. *et al.* Determinants of mortality in a combined cohort of 501 patients with HIV-associated Cryptococcal meningitis: implications for improving outcomes. *Clin Infect Dis* **58**, 736-745, doi:10.1093/cid/cit794 (2014).
40 Pasquier, E. *et al.* Long-term Mortality and Disability in Cryptococcal Meningitis: A Systematic Literature Review. *Clin Infect Dis* **66**, 1122-1132, doi:10.1093/cid/cix870 (2018).
41 Boulware, D. R. *et al.* Clinical features and serum biomarkers in HIV immune reconstitution inflammatory syndrome after cryptococcal meningitis: a prospective cohort study. *PLoS Med* **7**, e1000384, doi:10.1371/journal.pmed.1000384 (2010).
42 Chaiwarith, R., Vongsanim, S. & Supparatpinyo, K. Cryptococcal meningitis in HIV-infected patients at Chiang Mai University Hospital: a retrospective study. *Southeast Asian Journal of Tropical Medicine and Public Health* **45**, 636 (2014).
43 Day, J. N. *et al.* Combination antifungal therapy for cryptococcal meningitis. *N Engl J Med* **368**, 1291-1302, doi:10.1056/NEJMoa1110404 (2013).
44 Lortholary, O. *et al.* Long-term outcome of AIDS-associated cryptococcosis in the era of combination antiretroviral therapy. *AIDS* **20**, 2183-2191, doi:10.1097/01.aids.0000252060.80704.68 (2006).
45 Vidal, J. E. *et al.* HIV-Associated Cryptococcal Meningitis Patients Treated with Amphotericin B Deoxycholate Plus Flucytosine under Routine Care Conditions in a Referral Center in Sao Paulo, Brazil. *Mycopathologia* **186**, 93-102, doi:10.1007/s11046-020-00512-2 (2021).
46 Longley, N. *et al.* Cryptococcal Antigen Screening in Patients Initiating ART in South Africa: A Prospective Cohort Study. *Clin Infect Dis* **62**, 581-587, doi:10.1093/cid/civ936 (2016).
47 Mfinanga, S. *et al.* Cryptococcal meningitis screening and community-based early adherence support in people with advanced HIV infection starting antiretroviral therapy in Tanzania and Zambia: an open-label, randomised controlled trial. *Lancet* **385**, 2173-2182, doi:10.1016/S0140-6736(15)60164-7 (2015).
48 Wake, R. M. *et al.* Cryptococcal-related Mortality Despite Fluconazole Preemptive Treatment in a Cryptococcal Antigen Screen-and-Treat Program. *Clin Infect Dis* **70**, 1683-1690, doi:10.1093/cid/ciz485 (2020).
49 Rajasingham, R. *et al.* Cryptococcal Meningitis Diagnostics and Screening in the Era of Point-of-Care Laboratory Testing. *J Clin Microbiol* **57**, e01238-01218, doi:10.1128/JCM.01238-18 (2019).
50 Bicanic, T. *et al.* Independent association between rate of clearance of infection and clinical outcome of HIV-associated cryptococcal meningitis: analysis of a combined cohort of 262 patients. *Clin Infect Dis* **49**, 702-709, doi:10.1086/604716 (2009).
51 Pullen, M. F. *et al.* Cerebrospinal Fluid Early Fungicidal Activity as a Surrogate Endpoint for Cryptococcal Meningitis Survival in Clinical Trials. *Clin Infect Dis* **71**, e45-e49, doi:10.1093/cid/ciaa016 (2020).
52 Coussement, J. *et al.* Current epidemiology and clinical features of Cryptococcus infection in patients without HIV infection: a multicentre study in 46 hospitals from Australia and New Zealand. *Clin Infect Dis*, doi:10.1093/cid/ciad321 (2023).
53 Pappas, P. G. *et al.* Cryptococcosis in human immunodeficiency virus-negative patients in the era of effective azole therapy. *Clin Infect Dis* **33**, 690-699, doi:10.1086/322597 (2001).
54 Rosen, L. B. *et al.* Anti-GM-CSF autoantibodies in patients with cryptococcal meningitis. *J Immunol* **190**, 3959-3966, doi:10.4049/jimmunol.1202526 (2013).

55 Kuo, C. Y. *et al.* Disseminated Cryptococcosis Due to Anti-Granulocyte-Macrophage Colony-Stimulating Factor Autoantibodies in the Absence of Pulmonary Alveolar Proteinosis. *J Clin Immunol* **37**, 143-152, doi:10.1007/s10875-016-0364-4 (2017).
56 Arango-Franco, C. A. *et al.* Anti-GM-CSF Neutralizing Autoantibodies in Colombian Patients with Disseminated Cryptococcosis. *J Clin Immunol* **43**, 921-932, doi:10.1007/s10875-023-01451-5 (2023).
57 Ahmad, D. S., Esmadi, M. & Steinmann, W. C. Idiopathic CD4 Lymphocytopenia: Spectrum of opportunistic infections, malignancies, and autoimmune diseases. *Avicenna J Med* **3**, 37-47, doi:10.4103/2231-0770.114121 (2013).
58 Browne, S. K. *et al.* Adult-onset immunodeficiency in Thailand and Taiwan. *N Engl J Med* **367**, 725-734, doi:10.1056/NEJMoa1111160 (2012).
59 Liu, J. *et al.* Analysis of the association of HLA subtypes with cryptococcal meningitis in HIV-negative immunocompetent patients. *Future Microbiol* **17**, 1231-1240, doi:10.2217/fmb-2021-0247 (2022).
60 Wu, U. I. *et al.* Incorrect diagnoses in patients with neutralizing anti-interferon-gamma-autoantibodies. *Clin Microbiol Infect* **26**, 1684.e1681-1684.e1686, doi:10.1016/j.cmi.2020.02.030 (2020).
61 Su, X. H. *et al.* Comparison of features and outcomes between HIV-negative patients with Cryptococcus gattii meningitis and Cryptococcus neoformans meningitis in South China. *Mycoses* **65**, 887-896, doi:10.1111/myc.13491 (2022).
62 Day, J. N. *et al.* Comparative genomics of Cryptococcus neoformans var. grubii associated with meningitis in HIV infected and uninfected patients in Vietnam. *PLoS Negl Trop Dis* **11**, e0005628, doi:10.1371/journal.pntd.0005628 (2017).
63 Thanh, L. T. *et al.* Assessing the virulence of Cryptococcus neoformans causing meningitis in HIV infected and uninfected patients in Vietnam. *Med Mycol* **58**, 1149-1161, doi:10.1093/mmy/myaa013 (2020).
64 Yang, D. H. *et al.* Cryptococcus gattii Species Complex as an Opportunistic Pathogen: Underlying Medical Conditions Associated with the Infection. *mBio* **12**, e0270821, doi:10.1128/mBio.02708-21 (2021).
65 Saijo, T. *et al.* Anti-granulocyte-macrophage colony-stimulating factor autoantibodies are a risk factor for central nervous system infection by Cryptococcus gattii in otherwise immunocompetent patients. *mBio* **5**, e00912-00914, doi:10.1128/mBio.00912-14 (2014).
66 Barcenas-Morales, G., Cortes-Acevedo, P. & Doffinger, R. Anticytokine autoantibodies leading to infection: early recognition, diagnosis and treatment options. *Curr Opin Infect Dis* **32**, 330-336, doi:10.1097/QCO.0000000000000561 (2019).
67 Liao, C. H. *et al.* Different presentations and outcomes between HIV-infected and HIV-uninfected patients with Cryptococcal meningitis. *J Microbiol Immunol Infect* **45**, 296-304, doi:10.1016/j.jmii.2011.12.005 (2012).
68 Pyrgos, V., Seitz, A. E., Steiner, C. A., Prevots, D. R. & Williamson, P. R. Epidemiology of cryptococcal meningitis in the US: 1997-2009. *PLoS One* **8**, e56269, doi:10.1371/journal.pone.0056269 (2013).
69 Marr, K. A. *et al.* A Multicenter, Longitudinal Cohort Study of Cryptococcosis in Human Immunodeficiency Virus-negative People in the United States. *Clin Infect Dis* **70**, 252-261, doi:10.1093/cid/ciz193 (2020).
70 Levitz, S. M. The ecology of Cryptococcus neoformans and the epidemiology of cryptococcosis. *Rev Infect Dis* **13**, 1163-1169, doi:10.1093/clinids/13.6.1163 (1991).
71 Bartlett, K. H., Kidd, S. E. & Kronstad, J. W. The emergence of Cryptococcus gattii in British Columbia and the Pacific Northwest. *Curr Infect Dis Rep* **10**, 58-65, doi:10.1007/s11908-008-0011-1 (2008).
72 Datta, K. *et al.* Spread of Cryptococcus gattii into Pacific Northwest region of the United States. *Emerg Infect Dis* **15**, 1185-1191, doi:10.3201/eid1508.081384 (2009).

73 Gugnani, H. C. *et al.* Isolation of Cryptococcus gattii and Cryptococcus neoformans var. grubii from the flowers and bark of Eucalyptus trees in India. *Med Mycol* **43**, 565-569, doi:10.1080/13693780500160785 (2005).
74 Kozubowski, L. & Heitman, J. Profiling a killer, the development of Cryptococcus neoformans. *FEMS Microbiol Rev* **36**, 78-94, doi:10.1111/j.1574-6976.2011.00286.x (2012).
75 Desnos-Ollivier, M. *et al.* Mixed infections and In Vivo evolution in the human fungal pathogen Cryptococcus neoformans. *mBio* **1**, doi:10.1128/mBio.00091-10 (2010).
76 Chang, Y. C. *et al.* Cryptococcal yeast cells invade the central nervous system via transcellular penetration of the blood-brain barrier. *Infect Immun* **72**, 4985-4995, doi:10.1128/IAI.72.9.4985-4995.2004 (2004).
77 George, I. A., Santos, C. A. Q., Olsen, M. A. & Powderly, W. G. Epidemiology of Cryptococcosis and Cryptococcal Meningitis in a Large Retrospective Cohort of Patients After Solid Organ Transplantation. *Open forum infectious diseases* **4**, ofx004, doi:10.1093/ofid/ofx004 (2017).
78 Kapoor, A. *et al.* Cryptococcal meningitis in renal transplant patients associated with environmental exposure. *Transpl Infect Dis* **1**, 213-217, doi:10.1034/j.1399-3062.1999.010309.x (1999).
79 Osterholzer, J. J. *et al.* Role of dendritic cells and alveolar macrophages in regulating early host defense against pulmonary infection with Cryptococcus neoformans. *Infect Immun* **77**, 3749-3758, doi:10.1128/IAI.00454-09 (2009).
80 Biondo, C. *et al.* MyD88 and TLR2, but not TLR4, are required for host defense against Cryptococcus neoformans. *Eur J Immunol* **35**, 870-878, doi:10.1002/eji.200425799 (2005).
81 Cross, C. E. & Bancroft, G. J. Ingestion of acapsular Cryptococcus neoformans occurs via mannose and beta-glucan receptors, resulting in cytokine production and increased phagocytosis of the encapsulated form. *Infect Immun* **63**, 2604-2611, doi:10.1128/iai.63.7.2604-2611.1995 (1995).
82 Kozel, T. R., Pfrommer, G. S., Guerlain, A. S., Highison, B. A. & Highison, G. J. Strain variation in phagocytosis of Cryptococcus neoformans: dissociation of susceptibility to phagocytosis from activation and binding of opsonic fragments of C3. *Infect Immun* **56**, 2794-2800, doi:10.1128/iai.56.11.2794-2800.1988 (1988).
83 Casadevall, A. *et al.* The capsule of Cryptococcus neoformans. *Virulence* **10**, 822-831, doi:10.1080/21505594.2018.1431087 (2019).
84 Wozniak, K. L. Interactions of Cryptococcus with Dendritic Cells. *J Fungi (Basel)* **4**, doi:10.3390/jof4010036 (2018).
85 Chen, G. H. *et al.* The gamma interferon receptor is required for the protective pulmonary inflammatory response to Cryptococcus neoformans. *Infect Immun* **73**, 1788-1796, doi:10.1128/IAI.73.3.1788-1796.2005 (2005).
86 George, I. A., Spec, A., Powderly, W. G. & Santos, C. A. Q. Comparative Epidemiology and Outcomes of Human Immunodeficiency virus (HIV), Non-HIV Non-transplant, and Solid Organ Transplant Associated Cryptococcosis: A Population-Based Study. *Clin Infect Dis* **66**, 608-611, doi:10.1093/cid/cix867 (2018).
87 Kiertiburanakul, S., Wirojtananugoon, S., Pracharktam, R. & Sungkanuparph, S. Cryptococcosis in human immunodeficiency virus-negative patients. *Int J Infect Dis* **10**, 72-78, doi:10.1016/j.ijid.2004.12.004 (2006).
88 Lin, Y. Y., Shiau, S. & Fang, C. T. Risk factors for invasive Cryptococcus neoformans diseases: a case-control study. *PloS one* **10**, e0119090, doi:10.1371/journal.pone.0119090 (2015).
89 Costa, M. L., Souza, J. P., Oliveira Neto, A. F. & Pinto, E. S. J. L. Cryptococcal meningitis in HIV negative pregnant women: case report and review of literature. *Rev Inst Med Trop Sao Paulo* **51**, 289-294, doi:10.1590/s0036-46652009000500010 (2009).

90 Wipasa, J. *et al.* Characterization of anti-interferon-gamma antibodies in HIV-negative immunodeficient patients infected with unusual intracellular microorganisms. *Exp Biol Med (Maywood)* **243**, 621-626, doi:10.1177/1535370218764086 (2018).
91 Golde, D. W., Territo, M., Finley, T. N. & Cline, M. J. Defective lung macrophages in pulmonary alveolar proteinosis. *Ann Intern Med* **85**, 304-309, doi:10.7326/0003-4819-85-3-304 (1976).
92 McClelland, E. E., Bernhardt, P. & Casadevall, A. Estimating the relative contributions of virulence factors for pathogenic microbes. *Infect Immun* **74**, 1500-1504, doi:10.1128/IAI.74.3.1500-1504.2006 (2006).
93 Zaragoza, O. *et al.* Capsule enlargement in Cryptococcus neoformans confers resistance to oxidative stress suggesting a mechanism for intracellular survival. *Cell Microbiol* **10**, 2043-2057, doi:10.1111/j.1462-5822.2008.01186.x (2008).
94 Denham, S. T. *et al.* Regulated Release of Cryptococcal Polysaccharide Drives Virulence and Suppresses Immune Cell Infiltration into the Central Nervous System. *Infect Immun* **86**, doi:10.1128/IAI.00662-17 (2018).
95 Rodrigues, J. *et al.* Lack of chitin synthase genes impacts capsular architecture and cellular physiology in Cryptococcus neoformans. *Cell Surf* **2**, 14-23, doi:10.1016/j.tcsw.2018.05.002 (2018).
96 Baker, L. G., Specht, C. A., Donlin, M. J. & Lodge, J. K. Chitosan, the deacetylated form of chitin, is necessary for cell wall integrity in Cryptococcus neoformans. *Eukaryot Cell* **6**, 855-867, doi:10.1128/EC.00399-06 (2007).
97 Banks, I. R. *et al.* A chitin synthase and its regulator protein are critical for chitosan production and growth of the fungal pathogen Cryptococcus neoformans. *Eukaryot Cell* **4**, 1902-1912, doi:10.1128/EC.4.11.1902-1912.2005 (2005).
98 Kwon-Chung, K. J., Polacheck, I. & Popkin, T. J. Melanin-lacking mutants of Cryptococcus neoformans and their virulence for mice. *J Bacteriol* **150**, 1414-1421, doi:10.1128/jb.150.3.1414-1421.1982 (1982).
99 Williamson, P. R. Laccase and melanin in the pathogenesis of Cryptococcus neoformans. *Front Biosci* **2**, e99-107, doi:10.2741/a231 (1997).
100 Shea, J. M., Kechichian, T. B., Luberto, C. & Del Poeta, M. The cryptococcal enzyme inositol phosphosphingolipid-phospholipase C confers resistance to the antifungal effects of macrophages and promotes fungal dissemination to the central nervous system. *Infect Immun* **74**, 5977-5988, doi:10.1128/IAI.00768-06 (2006).
101 Hamed, M. F. *et al.* Phospholipase B Is Critical for Cryptococcus neoformans Survival in the Central Nervous System. *mBio* **14**, e0264022, doi:10.1128/mbio.02640-22 (2023).
102 Evans, R. J. *et al.* Cryptococcal phospholipase B1 is required for intracellular proliferation and control of titan cell morphology during macrophage infection. *Infect Immun* **83**, 1296-1304, doi:10.1128/iai.03104-14 (2015).
103 Ganendren, R., Carter, E., Sorrell, T., Widmer, F. & Wright, L. Phospholipase B activity enhances adhesion of Cryptococcus neoformans to a human lung epithelial cell line. *Microbes Infect* **8**, 1006-1015, doi:10.1016/j.micinf.2005.10.018 (2006).
104 Santangelo, R. T. *et al.* Biochemical and functional characterisation of secreted phospholipase activities from Cryptococcus neoformans in their naturally occurring state. *J Med Microbiol* **48**, 731-740, doi:10.1099/00222615-48-8-731 (1999).
105 Fu, M. S. *et al.* Cryptococcus neoformans urease affects the outcome of intracellular pathogenesis by modulating phagolysosomal pH. *PLoS Pathog* **14**, e1007144, doi:10.1371/journal.ppat.1007144 (2018).
106 Singh, A. *et al.* Factors required for activation of urease as a virulence determinant in Cryptococcus neoformans. *mBio* **4**, e00220-00213, doi:10.1128/mBio.00220-13 (2013).

107 Baker, R. P. & Casadevall, A. Reciprocal modulation of ammonia and melanin production has implications for cryptococcal virulence. *Nat Commun* **14**, 849, doi:10.1038/s41467-023-36552-7 (2023).
108 Charlier, C. *et al.* Evidence of a role for monocytes in dissemination and brain invasion by Cryptococcus neoformans. *Infect Immun* **77**, 120-127, doi:10.1128/IAI.01065-08 (2009).
109 Sun, D. & Shi, M. Neutrophil swarming toward Cryptococcus neoformans is mediated by complement and leukotriene B4. *Biochem Biophys Res Commun* **477**, 945-951, doi:10.1016/j.bbrc.2016.07.005 (2016).
110 Leopold Wager, C. M., Hole, C. R., Wozniak, K. L., Olszewski, M. A. & Wormley, F. L., Jr. STAT1 signaling is essential for protection against Cryptococcus neoformans infection in mice. *J Immunol* **193**, 4060-4071, doi:10.4049/jimmunol.1400318 (2014).
111 Heung, L. J. & Hohl, T. M. Inflammatory monocytes are detrimental to the host immune response during acute infection with Cryptococcus neoformans. *PLoS Pathog* **15**, e1007627, doi:10.1371/journal.ppat.1007627 (2019).
112 Jarvis, J. N. *et al.* The phenotype of the Cryptococcus-specific CD4+ memory T-cell response is associated with disease severity and outcome in HIV-associated cryptococcal meningitis. *J Infect Dis* **207**, 1817-1828, doi:10.1093/infdis/jit099 (2013).
113 Jarvis, J. N. *et al.* Adjunctive interferon-gamma immunotherapy for the treatment of HIV-associated cryptococcal meningitis: a randomized controlled trial. *AIDS* **26**, 1105-1113, doi:10.1097/QAD.0b013e3283536a93 (2012).
114 Pappas, P. G. *et al.* Recombinant interferon- gamma 1b as adjunctive therapy for AIDS-related acute cryptococcal meningitis. *J Infect Dis* **189**, 2185-2191, doi:10.1086/420829 (2004).
115 Lee, S. C., Kress, Y., Zhao, M. L., Dickson, D. W. & Casadevall, A. Cryptococcus neoformans survive and replicate in human microglia. *Lab Invest* **73**, 871-879 (1995).
116 Lee, S. C., Kress, Y., Dickson, D. W. & Casadevall, A. Human microglia mediate anti-Cryptococcus neoformans activity in the presence of specific antibody. *J Neuroimmunol* **62**, 43-52, doi:10.1016/0165-5728(95)00097-l (1995).
117 Koutsouras, G. W., Ramos, R. L. & Martinez, L. R. Role of microglia in fungal infections of the central nervous system. *Virulence* **8**, 705-718, doi:10.1080/21505594.2016.1261789 (2017).
118 Neal, L. M. *et al.* CD4(+) T Cells Orchestrate Lethal Immune Pathology despite Fungal Clearance during Cryptococcus neoformans Meningoencephalitis. *mBio* **8**, e01415-01417, doi:10.1128/mBio.01415-17 (2017).
119 Aguirre, K. M. & Johnson, L. L. A role for B cells in resistance to Cryptococcus neoformans in mice. *Infect Immun* **65**, 525-530, doi:10.1128/iai.65.2.525-530.1997 (1997).
120 Okurut, S. *et al.* B Cell Compartmentalization in Blood and Cerebrospinal Fluid of HIV-Infected Ugandans with Cryptococcal Meningitis. *Infect Immun* **88**, doi:10.1128/IAI.00779-19 (2020).
121 Yoon, H. A. *et al.* Association Between Plasma Antibody Responses and Risk for Cryptococcus-Associated Immune Reconstitution Inflammatory Syndrome. *J Infect Dis* **219**, 420-428, doi:10.1093/infdis/jiy447 (2019).
122 Haddow, L. J. *et al.* Cryptococcal immune reconstitution inflammatory syndrome in HIV-1-infected individuals: proposed clinical case definitions. *The Lancet infectious diseases* **10**, 791-802, doi:10.1016/S1473-3099(10)70170-5 (2010).
123 Bonham, S., Meya, D. B., Bohjanen, P. R. & Boulware, D. R. Biomarkers of HIV Immune Reconstitution Inflammatory Syndrome. *Biomark Med* **2**, 349-361, doi:10.2217/17520363.2.4.349 (2008).
124 Boulware, D. R. *et al.* Paucity of initial cerebrospinal fluid inflammation in cryptococcal meningitis is associated with subsequent immune reconstitution inflammatory syndrome. *J Infect Dis* **202**, 962-970, doi:10.1086/655785 (2010).

125 Scriven, J. E. *et al.* The CSF Immune Response in HIV-1-Associated Cryptococcal Meningitis: Macrophage Activation, Correlates of Disease Severity, and Effect of Antiretroviral Therapy. *J Acquir Immune Defic Syndr* **75**, 299-307, doi:10.1097/QAI.0000000000001382 (2017).
126 Scriven, J. E. *et al.* Early ART After Cryptococcal Meningitis Is Associated With Cerebrospinal Fluid Pleocytosis and Macrophage Activation in a Multisite Randomized Trial. *J Infect Dis* **212**, 769-778, doi:10.1093/infdis/jiv067 (2015).
127 Chang, C. C. *et al.* Clinical and mycological predictors of cryptococcosis-associated immune reconstitution inflammatory syndrome. *AIDS* **27**, 2089-2099, doi:10.1097/QAD.0b013e3283614a8d (2013).
128 Boulware, D. R. *et al.* Higher levels of CRP, D-dimer, IL-6, and hyaluronic acid before initiation of antiretroviral therapy (ART) are associated with increased risk of AIDS or death. *J Infect Dis* **203**, 1637-1646, doi:10.1093/infdis/jir134 (2011).
129 Boulware, D. R. *et al.* Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis. *N Engl J Med* **370**, 2487-2498, doi:10.1056/NEJMoa1312884 (2014).
130 Boulware, D. R. *et al.* Multisite validation of cryptococcal antigen lateral flow assay and quantification by laser thermal contrast. *Emerg Infect Dis* **20**, 45-53, doi:10.3201/eid2001.130906 (2014).
131 Hansen, J. *et al.* Large-scale evaluation of the immuno-mycologics lateral flow and enzyme-linked immunoassays for detection of cryptococcal antigen in serum and cerebrospinal fluid. *Clinical and vaccine immunology : CVI* **20**, 52-55, doi:10.1128/CVI.00536-12 (2013).
132 Percival, A., Thorkildson, P. & Kozel, T. R. Monoclonal antibodies specific for immunorecessive epitopes of glucuronoxylomannan, the major capsular polysaccharide of Cryptococcus neoformans, reduce serotype bias in an immunoassay for cryptococcal antigen. *Clinical and vaccine immunology : CVI* **18**, 1292-1296, doi:10.1128/cvi.05052-11 (2011).
133 Bridge, S. *et al.* Evaluation of the BioFire(R) FilmArray(R) Meningitis/Encephalitis panel in an adult and pediatric Ugandan population. *Journal de mycologie medicale* **31**, 101170, doi:10.1016/j.mycmed.2021.101170 (2021).
134 Skipper, C. *et al.* Evaluation of Serum Cryptococcal Antigen Testing Using Two Novel Semiquantitative Lateral Flow Assays in Persons with Cryptococcal Antigenemia. *Journal of clinical microbiology* **58**, e02046-02019, doi:10.1128/JCM.02046-19 (2020).
135 Kwizera, R. *et al.* Evaluation of the Dynamiker Cryptococcal Antigen Lateral Flow Assay for the Diagnosis of HIV-Associated Cryptococcosis. *Journal of clinical microbiology* **59**, doi:10.1128/JCM.02421-20 (2021).
136 Mpoza, E. *et al.* Evaluation of a point-of-care immunoassay test kit 'StrongStep' for cryptococcal antigen detection. *PLoS One* **13**, e0190652, doi:10.1371/journal.pone.0190652 (2018).
137 Ssebambulidde, K. *et al.* Symptomatic Cryptococcal Antigenemia Presenting as Early Cryptococcal Meningitis With Negative Cerebral Spinal Fluid Analysis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **68 12**, 2094-2098 (2018).
138 Anjum, S. H. *et al.* Neuroimaging of Cryptococcal Meningitis in Patients without Human Immunodeficiency Virus: Data from a Multi-Center Cohort Study. *Journal of Fungi* **9**, 594 (2023).
139 Chen, S. *et al.* Epidemiology and host- and variety-dependent characteristics of infection due to Cryptococcus neoformans in Australia and New Zealand. Australasian Cryptococcal Study Group. *Clin Infect Dis* **31**, 499-508, doi:10.1086/313992 (2000).
140 Perfect, J. R. *et al.* Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the infectious diseases society of america. *Clin Infect Dis* **50**, 291-322, doi:10.1086/649858 (2010).

141 Bahr, N. C. *et al.* Recurrence of Symptoms Following Cryptococcal Meningitis: Characterizing a Diagnostic Conundrum With Multiple Etiologies. *Clin Infect Dis* **76**, 1080-1087, doi:10.1093/cid/ciac853 (2023).
142 Musubire, A. K., Boulware, D. R., Meya, D. B. & Rhein, J. Diagnosis and Management of Cryptococcal Relapse. *Journal of AIDS & clinical research* **Suppl 3**, doi:10.4172/2155-6113.s3-003 (2013).
143 Rhein, J. *et al.* Diagnostic performance of a multiplex PCR assay for meningitis in an HIV-infected population in Uganda. *Diagnostic microbiology and infectious disease* **84**, 268-273, doi:10.1016/j.diagmicrobio.2015.11.017 (2016).
144 Mbangiwa, T. *et al.* S1.4d Cryptococcus qPCR assays: the future for routine mycology labs and clinical trials dealing with cryptococcosis. *Medical Mycology* **60**, doi:10.1093/mmy/myac072.S1.4d (2022).
145 Del Poeta, M., Wormley, F. L., Jr. & Lin, X. Host populations, challenges, and commercialization of cryptococcal vaccines. *PLoS Pathog* **19**, e1011115, doi:10.1371/journal.ppat.1011115 (2023).
146 World Health Organization. *Guidelines for diagnosing, preventing and managing cryptococcal disease among adults, adolescents and children living with HIV*, <https://www.who.int/publications/i/item/9789240052178> (2022).
147 Meya, D. B. *et al.* Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4+ cell count < or = 100 cells/microL who start HIV therapy in resource-limited settings. *Clin Infect Dis* **51**, 448-455, doi:10.1086/655143 (2010).
148 World Health Organization. Rapid advice: Diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children. (2011). <www.who.int/hiv/pub/cryptococcal_disease2011>.
149 Wake, R. M. *et al.* High Cryptococcal Antigen Titers in Blood Are Predictive of Subclinical Cryptococcal Meningitis Among Human Immunodeficiency Virus-Infected Patients. *Clin Infect Dis* **66**, 686-692, doi:10.1093/cid/cix872 (2018).
150 Rajasingham, R. & Boulware, D. R. Cryptococcal Antigen Screening and Preemptive Treatment-How Can We Improve Survival? *Clin Infect Dis* **70**, 1691-1694, doi:10.1093/cid/ciz488 (2020).
151 Rutakingirwa, M. K. *et al.* Tuberculosis in HIV-Associated Cryptococcal Meningitis is Associated with an Increased Risk of Death. *Journal of clinical medicine* **9**, doi:10.3390/jcm9030781 (2020).
152 Skipper, C. *et al.* Cytomegalovirus Viremia Associated With Increased Mortality in Cryptococcal Meningitis in Sub-Saharan Africa. *Clin Infect Dis* **71**, 525-531, doi:10.1093/cid/ciz864 (2020).
153 Skipper, C. P. *et al.* Cytomegalovirus viremia as a risk factor for mortality in HIV-associated cryptococcal and tuberculous meningitis. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases* **122**, 785-792, doi:10.1016/j.ijid.2022.07.035 (2022).
154 Rutakingirwa, M. K. *et al.* Tuberculosis in HIV-Associated Cryptococcal Meningitis is Associated with an Increased Risk of Death. *Journal of clinical medicine* **9**, 781, doi:10.3390/jcm9030781 (2020).
155 World Health Organization. *Guideline for managing advanced HIV disease and the timing for initiating antiretroviral therapy*, <http://www.who.int/hiv/pub/guidelines/advanced-HIV-disease/en/> (2017).
156 World Health Organization. *WHO consolidated guidelines on tuberculosis: module 3: diagnosis: rapid diagnostics for tuberculosis detection, 2021 update*, <https://www.who.int/publications/i/item/9789240029415> (2021).

157 Rajasingham, R. *et al.* Nosocomial drug-resistant bacteremia in 2 cohorts with cryptococcal meningitis, Africa. *Emerg Infect Dis* **20**, 722-724, doi:10.3201/eid2004.131277 (2014).
158 Moore, C. C. *et al.* Etiology of Sepsis in Uganda Using a Quantitative Polymerase Chain Reaction-based TaqMan Array Card. *Clin Infect Dis* **68**, 266-272, doi:10.1093/cid/ciy472 (2019).
159 Hakim, J. *et al.* Enhanced Prophylaxis plus Antiretroviral Therapy for Advanced HIV Infection in Africa. *N Engl J Med* **377**, 233-245, doi:10.1056/NEJMoa1615822 (2017).
160 Einsele, H. *et al.* Risk factors for treatment failures in patients receiving PCR-based preemptive therapy for CMV infection. *Bone marrow transplantation* **25**, 757-763, doi:10.1038/sj.bmt.1702226 (2000).
161 Rose, D. N. & Sacks, H. S. Cost-effectiveness of cytomegalovirus (CMV) disease prevention in patients with AIDS: oral ganciclovir and CMV polymerase chain reaction testing. *AIDS* **11**, 883-887, doi:10.1097/00002030-199707000-00007 (1997).
162 Wohl, D. A. *et al.* Low rate of CMV end-organ disease in HIV-infected patients despite low CD4+ cell counts and CMV viremia: results of ACTG protocol A5030. *HIV clinical trials* **10**, 143-152, doi:10.1310/hct1003-143 (2009).
163 Skipper, C. P. & Schleiss, M. R. Cytomegalovirus viremia and advanced HIV disease: is there an argument for anti-CMV treatment? *Expert review of anti-infective therapy* **21**, 227-233, doi:10.1080/14787210.2023.2172400 (2023).
164 Panel on Guidelines for the Prevention and Treatment of Opportunistic Infections in Adults and Adolescents with HIV. *Guidelines for the Prevention and Treatment of Opportunistic Infections in HIV-infected Adults and Adolescents: Recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America.*, <https://clinicalinfo.hiv.gov/en/guidelines/adult-and-adolescent-opportunistic-infection/cryptococcosis> (2022).
165 Bicanic, T. *et al.* Toxicity of Amphotericin B Deoxycholate-Based Induction Therapy in Patients with HIV-Associated Cryptococcal Meningitis. *Antimicrob Agents Chemother* **59**, 7224-7231, doi:10.1128/AAC.01698-15 (2015).
166 Bahr, N. C. *et al.* Standardized electrolyte supplementation and fluid management improves survival during amphotericin therapy for cryptococcal meningitis in resource-limited settings. *Open forum infectious diseases* **1**, ofu070, doi:10.1093/ofid/ofu070 (2014).
167 Schutz, C. *et al.* Acute Kidney Injury and Urinary Biomarkers in Human Immunodeficiency Virus-Associated Cryptococcal Meningitis. *Open forum infectious diseases* **4**, ofx127, doi:10.1093/ofid/ofx127 (2017).
168 Muzoora, C. K. *et al.* Short course amphotericin B with high dose fluconazole for HIV-associated cryptococcal meningitis. *J Infect* **64**, 76-81, doi:10.1016/j.jinf.2011.10.014 (2012).
169 Rajasingham, R., Rolfes, M. A., Birkenkamp, K. E., Meya, D. B. & Boulware, D. R. Cryptococcal meningitis treatment strategies in resource-limited settings: a cost-effectiveness analysis. *PLoS Med* **9**, e1001316, doi:10.1371/journal.pmed.1001316 (2012).
170 World Health Organization. *Guidelines for the diagnosis, prevention, and management of cryptococcal disease in HIV-infected adults, adolescents and children*, <https://apps.who.int/iris/bitstream/handle/10665/260399/9789241550277-eng.pdf> (2018).
171 Stone, N. R., Bicanic, T., Salim, R. & Hope, W. Liposomal Amphotericin B (AmBisome((R))): A Review of the Pharmacokinetics, Pharmacodynamics, Clinical Experience and Future Directions. *Drugs* **76**, 485-500, doi:10.1007/s40265-016-0538-7 (2016).
172 Lawrence, D. S. *et al.* Cost-effectiveness of single, high-dose, liposomal amphotericin regimen for HIV-associated cryptococcal meningitis in five countries in sub-Saharan Africa: an economic analysis of the AMBITION-cm trial. *The Lancet. Global health* **10**, e1845-e1854, doi:10.1016/S2214-109X(22)00450-8 (2022).

173 Lawrence, D. S. *et al.* The acceptability of the AMBITION-cm treatment regimen for HIV-associated cryptococcal meningitis: Findings from a qualitative methods study of participants and researchers in Botswana and Uganda. *PLoS neglected tropical diseases* **16**, e0010825, doi:10.1371/journal.pntd.0010825 (2022).
174 Harrison, T. S. *et al.* How Applicable Is the Single-Dose AMBITION Regimen for Human Immunodeficiency Virus-Associated Cryptococcal Meningitis to High-Income Settings? *Clin Infect Dis* **76**, 944-949, doi:10.1093/cid/ciac792 (2023).
175 Panackal, A. A., Marr, K. A. & Williamson, P. R. Dexamethasone in Cryptococcal Meningitis. *N Engl J Med* **375**, 188, doi:10.1056/NEJMc1605205 (2016).
176 Beardsley, J. *et al.* Adjunctive Dexamethasone in HIV-Associated Cryptococcal Meningitis. *N Engl J Med* **374**, 542-554, doi:10.1056/NEJMoa1509024 (2016).
177 Govender, N. P. *et al.* Southern African HIV Clinicians Society guideline for the prevention, diagnosis and management of cryptococcal disease among HIV-infected persons: 2019 update. *South Afr J HIV Med* **20**, 1030, doi:10.4102/sajhivmed.v20i1.1030 (2019).
178 Beyene, T. *et al.* Inadequacy of High-Dose Fluconazole Monotherapy Among Cerebrospinal Fluid Cryptococcal Antigen (CrAg)-Positive Human Immunodeficiency Virus-Infected Persons in an Ethiopian CrAg Screening Program. *Clin Infect Dis* **65**, 2126-2129, doi:10.1093/cid/cix613 (2017).
179 US National Library of Medicine. ClinicalTrials.gov.
180 ISRCTN registry. Isrctn.com.
181 Zolopa, A. *et al.* Early antiretroviral therapy reduces AIDS progression/death in individuals with acute opportunistic infections: a multicenter randomized strategy trial. *PLoS One* **4**, e5575, doi:10.1371/journal.pone.0005575 (2009).
182 Makadzange, A. T. *et al.* Early versus delayed initiation of antiretroviral therapy for concurrent HIV infection and cryptococcal meningitis in sub-saharan Africa. *Clin Infect Dis* **50**, 1532-1538, doi:10.1086/652652 (2010).
183 Bisson, G. P. *et al.* Early versus delayed antiretroviral therapy and cerebrospinal fluid fungal clearance in adults with HIV and cryptococcal meningitis. *Clin Infect Dis* **56**, 1165-1173, doi:10.1093/cid/cit019 (2013).
184 Alufandika, M. *et al.* A pragmatic approach to managing antiretroviral therapy-experienced patients diagnosed with HIV-associated cryptococcal meningitis: impact of antiretroviral therapy adherence and duration. *AIDS* **34**, 1425-1428, doi:10.1097/QAD.0000000000002556 (2020).
185 Boulware, D. R. & Jarvis, J. N. Timing of Antiretroviral Therapy in Cryptococcal Meningitis: What We Can (and Cannot) Learn From Observational Data. *Clin Infect Dis* **77**, 74-76, doi:10.1093/cid/ciad123 (2023).
186 Beardsley, J. *et al.* Do Intracerebral Cytokine Responses Explain the Harmful Effects of Dexamethasone in Human Immunodeficiency Virus–associated Cryptococcal Meningitis? *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America* **68**, 1494 - 1501 (2018).
187 Brunel, A. S. *et al.* Thalidomide for steroid-dependent immune reconstitution inflammatory syndromes during AIDS. *AIDS* **26**, 2110-2112, doi:10.1097/QAD.0b013e328358daea (2012).
188 Gaube, G. *et al.* Treatment with adalimumab for severe immune reconstitution inflammatory syndrome in an HIV-infected patient presenting with cryptococcal meningitis. *Med Mal Infect* **46**, 154-156, doi:10.1016/j.medmal.2015.12.005 (2016).
189 Ssebambulidde, K. *et al.* Treatment recommendations for non-HIV associated cryptococcal meningoencephalitis including management of post-infectious inflammatory response syndrome. *Front Neurol* **13**, 994396, doi:10.3389/fneur.2022.994396 (2022).
190 Anjum, S. *et al.* Outcomes in Previously Healthy Cryptococcal Meningoencephalitis Patients Treated With Pulse Taper Corticosteroids for Post-infectious Inflammatory Syndrome. *Clin Infect Dis* **73**, e2789-e2798, doi:10.1093/cid/ciaa1901 (2021).

191 Tugume, L. *et al.* Prognostic implications of baseline anaemia and changes in haemoglobin concentrations with amphotericin B therapy for cryptococcal meningitis. *HIV medicine* **18**, 13-20, doi:10.1111/hiv.12387 (2017).
192 Tugume, L. *et al.* Association of Hyponatremia on Mortality in Cryptococcal Meningitis: A Prospective Cohort. *Open forum infectious diseases* **9**, ofac301, doi:10.1093/ofid/ofac301 (2022).
193 Lofgren, S. *et al.* Differences in Immunologic Factors Among Patients Presenting with Altered Mental Status During Cryptococcal Meningitis. *The Journal of infectious diseases* **215**, 693-697, doi:10.1093/infdis/jix033 (2017).
194 Pastick, K. A. *et al.* Seizures in Human Immunodeficiency Virus-Associated Cryptococcal Meningitis: Predictors and Outcomes. *Open forum infectious diseases* **6**, ofz478, doi:10.1093/ofid/ofz478 (2019).
195 Abassi, M. *et al.* Cerebrospinal Fluid Lactate as a Prognostic Marker of Disease Severity and Mortality in Cryptococcal Meningitis. *Clin Infect Dis* **73**, e3077-e3082, doi:10.1093/cid/ciaa1749 (2021).
196 Rolfes, M. A. *et al.* The effect of therapeutic lumbar punctures on acute mortality from cryptococcal meningitis. *Clin Infect Dis* **59**, 1607-1614, doi:10.1093/cid/ciu596 (2014).
197 Bicanic, T. *et al.* Relationship of cerebrospinal fluid pressure, fungal burden and outcome in patients with cryptococcal meningitis undergoing serial lumbar punctures. *AIDS* **23**, 701-706, doi:10.1097/QAD.0b013e32832605fe (2009).
198 Kagimu, E. *et al.* Therapeutic Lumbar Punctures in Human Immunodeficiency Virus-Associated Cryptococcal Meningitis: Should Opening Pressure Direct Management? *Open forum infectious diseases* **9**, ofac416, doi:10.1093/ofid/ofac416 (2022).
199 Kitonsa, J. *et al.* Factors affecting mortality among HIV positive patients two years after completing recommended therapy for Cryptococcal meningitis in Uganda. *PloS one* **14**, e0210287, doi:10.1371/journal.pone.0210287 (2019).
200 Kitonsa, J. *et al.* Quality of life and associated factors among HIV positive patients after completion of treatment for Cryptococcal meningitis. *PLoS Negl Trop Dis* **15**, e0008983, doi:10.1371/journal.pntd.0008983 (2021).
201 Dean, O. *et al.* Quality of life of HIV-negative, previously healthy individuals following cryptococcal meningoencephalitis. *Sci Rep* **11**, 3673, doi:10.1038/s41598-021-83176-2 (2021).
202 Krishnamoorthy, A., Joel, A. & Abhilash, K. P. Cryptococcal Meningitis with Multiple Cranial Nerves Palsies: A Review of Literature. *J Glob Infect Dis* **7**, 123-124, doi:10.4103/0974-777X.161739 (2015).
203 Mohan, S., Ahmed, S. I., Alao, O. A. & Schliep, T. C. A case of AIDS associated cryptococcal meningitis with multiple cranial nerve neuropathies. *Clin Neurol Neurosurg* **108**, 610-613, doi:10.1016/j.clineuro.2006.01.005 (2006).
204 Rival, G. *et al.* Bilateral Cranial Nerve VI Palsies in Cryptococcal Meningitis, HIV, and Syphilis: A Case Report. *Clin Pract Cases Emerg Med* **5**, 515-518, doi:10.5811/cpcem.2021.8.53347 (2021).
205 Duggan, J. & Walls, H. M. Ocular complications of cryptococcal meningitis in patients with HIV: report of two cases and review of the literature. *J Int Assoc Physicians AIDS Care (Chic)* **11**, 283-288, doi:10.1177/1545109712448537 (2012).
206 Neo, W. L., Durisala, N. & Ho, E. C. Reversible hearing loss following cryptococcal meningitis: case study. *J Laryngol Otol* **130**, 691-695, doi:10.1017/S002221511600801X (2016).
207 Wang, H. C. *et al.* The prognosis of hearing impairment complicating HIV-negative cryptococcal meningitis. *Neurology* **65**, 320-322, doi:10.1212/01.wnl.0000168904.41046.2b (2005).

208 Holikatti, P. C. & Kar, N. Psychiatric Manifestations in a Patient with HIV-Associated Neurocognitive Symptoms and Cryptococcal Meningitis. *Indian journal of psychological medicine* **34**, 381-382, doi:10.4103/0253-7176.108226 (2012).
209 Lofgren, S. M. *et al.* The effect of sertraline on depression and associations with persistent depression in survivors of HIV-related cryptococcal meningitis. *Wellcome Open Research* **6**, doi:10.12688/wellcomeopenres.16363.1 (2021).
210 Carlson, R. D. *et al.* Predictors of neurocognitive outcomes on antiretroviral therapy after cryptococcal meningitis: a prospective cohort study. *Metabolic brain disease* **29**, 269-279, doi:10.1007/s11011-013-9476-1 (2014).
211 Levin, A. E. *et al.* Outpatient Cryptococcal Antigen Screening Is Associated With Favorable Baseline Characteristics and Improved Survival in Persons With Cryptococcal Meningitis in Uganda. *Clin Infect Dis* **76**, e759-e765, doi:10.1093/cid/ciac599 (2023).
212 Meya, D. B. *et al.* Reflexive Laboratory-Based Cryptococcal Antigen Screening and Preemptive Fluconazole Therapy for Cryptococcal Antigenemia in HIV-Infected Individuals With CD4 <100 Cells/microL: A Stepped-Wedge, Cluster-Randomized Trial. *Journal of acquired immune deficiency syndromes* **80**, 182-189, doi:10.1097/QAI.0000000000001894 (2019).
213 Enock, K. *et al.* Evaluation of the initial 12 months of a routine cryptococcal antigen screening program in reduction of HIV-associated cryptococcal meningitis in Uganda. *BMC health services research* **22**, 301, doi:10.1186/s12913-022-07624-z (2022).
214 Lawrence, D. S. *et al.* Decision making in a clinical trial for a life-threatening illness: Therapeutic expectation, not misconception. *Soc Sci Med* **305**, 115082, doi:10.1016/j.socscimed.2022.115082 (2022).
215 Link, A. *et al.* Delays in Cryptococcal Meningitis Diagnosis and Care: A Mixed Methods Study in Rural Uganda. *Ann Glob Health* **88**, 22, doi:10.5334/aogh.3524 (2022).
216 Yaich, S. *et al.* Early onset of paucisymptomatic cryptococcal meningitis in a kidney transplant patient: a case report and review of the literature. *Transplant Proc* **43**, 663-665, doi:10.1016/j.transproceed.2011.01.076 (2011).
217 Bosnić, D. *et al.* [Cryptococcal meningitis as a diagnostic problem in a patient with SLE--case report]. *Lijec Vjesn* **130**, 136-140 (2008).
218 Jongwutiwes, U., Sungkanuparph, S. & Kiertiburanakul, S. Comparison of clinical features and survival between cryptococcosis in human immunodeficiency virus (HIV)-positive and HIV-negative patients. *Jpn J Infect Dis* **61**, 111-115 (2008).
219 Lin, T. Y. *et al.* Cryptococcal disease in patients with or without human immunodeficiency virus: clinical presentation and monitoring of serum cryptococcal antigen titers. *J Microbiol Immunol Infect* **42**, 220-226 (2009).
220 Srishyla, D. *et al.* Determinants of cryptococcal antigen (CrAg) screening uptake in Kampala, Uganda: An assessment of health center characteristics. *Med Mycol* **60**, doi:10.1093/mmy/myac013 (2022).
221 Namuju, O. C. *et al.* Adherence of health workers to guidelines for screening and management of cryptococcal meningitis in Uganda. *PloS one* **18**, e0284165, doi:10.1371/journal.pone.0284165 (2023).
222 Wake, R. M., Molloy, S. F., Jarvis, J. N., Harrison, T. S. & Govender, N. P. Cryptococcal Antigenemia in Advanced Human Immunodeficiency Virus Disease: Pathophysiology, Epidemiology, and Clinical Implications. *Clin Infect Dis* **76**, 764-770, doi:10.1093/cid/ciac675 (2023).
223 Mpoza, E. *et al.* Cryptococcal Antigenemia in Human Immunodeficiency Virus Antiretroviral Therapy-Experienced Ugandans With Virologic Failure. *Clin Infect Dis* **71**, 1726-1731, doi:10.1093/cid/ciz1069 (2020).
224 Boulware, D. R. *et al.* Oral Lipid Nanocrystal Amphotericin B for Cryptococcal Meningitis: A Randomized Clinical Trial. *Clin Infect Dis*, doi:10.1093/cid/ciad440 (2023).

225 Ngan, N. T. T. *et al.* An open label randomized controlled trial of tamoxifen combined with amphotericin B and fluconazole for cryptococcal meningitis. *Elife* **10**, doi:10.7554/eLife.68929 (2021).
226 Rhein, J. *et al.* Adjunctive sertraline for HIV-associated cryptococcal meningitis: a randomised, placebo-controlled, double-blind phase 3 trial. *Lancet Infect Dis* **19**, 843-851, doi:10.1016/S1473-3099(19)30127-6 (2019).
227 Shaw, K. J. *et al.* In Vitro and In Vivo Evaluation of APX001A/APX001 and Other Gwt1 Inhibitors against Cryptococcus. *Antimicrob Agents Chemother* **62**, doi:10.1128/AAC.00523-18 (2018).
228 Wiederhold, N. P. *et al.* Fungal-specific Cyp51 inhibitor VT-1598 demonstrates in vitro activity against Candida and Cryptococcus species, endemic fungi, including Coccidioides species, Aspergillus species and Rhizopus arrhizus. *J Antimicrob Chemother* **73**, 404-408, doi:10.1093/jac/dkx410 (2018).
229 Gerlach, E. S. *et al.* ATI-2307 Exhibits Equivalent Antifungal Activity in Cryptococcus neoformans Clinical Isolates With High and Low Fluconazole IC(50). *Front Cell Infect Microbiol* **11**, 695240, doi:10.3389/fcimb.2021.695240 (2021).
230 Guo, X. *et al.* Sterol Sponge Mechanism Is Conserved for Glycosylated Polyene Macrolides. *ACS Cent Sci* **7**, 781-791, doi:10.1021/acscentsci.1c00148 (2021).
231 World Health Organization. WHO fungal priority pathogens list to guide research, development and public health action., (World Health Organization, Geneva, 2022).
232 Ending Cryptococcal Meningitis Deaths by 2030 – Strategic Framework., (South Africa, 2021).
233 Faini, D. *et al.* Laboratory-Reflex Cryptococcal Antigen Screening Is Associated With a Survival Benefit in Tanzania. *J Acquir Immune Defic Syndr* **80**, 205-213, doi:10.1097/QAI.0000000000001899 (2019).
234 Nalintya, E. *et al.* A Prospective Evaluation of a Multisite Cryptococcal Screening and Treatment Program in HIV Clinics in Uganda. *Journal of acquired immune deficiency syndromes* **78**, 231-238, doi:10.1097/QAI.0000000000001669 (2018).
235 Rolfes, M. A. *et al.* Cerebrospinal Fluid Culture Positivity and Clinical Outcomes After Amphotericin-Based Induction Therapy for Cryptococcal Meningitis. *Open forum infectious diseases* **2**, ofv157, doi:10.1093/ofid/ofv157 (2015).
236 Grant, P. M. *et al.* Risk factor analyses for immune reconstitution inflammatory syndrome in a randomized study of early vs. deferred ART during an opportunistic infection. *PLoS ONE* **5**, e11416, doi:10.1371/journal.pone.0011416 (2010).
237 Bicanic, T. *et al.* Immune reconstitution inflammatory syndrome in HIV-associated cryptococcal meningitis: a prospective study. *J Acquir Immune Defic Syndr* **51**, 130-134, doi:10.1097/QAI.0b013e3181a56f2e (2009).
238 Meya, D. B. *et al.* Cellular immune activation in cerebrospinal fluid from ugandans with cryptococcal meningitis and immune reconstitution inflammatory syndrome. *J Infect Dis* **211**, 1597-1606, doi:10.1093/infdis/jiu664 (2015).

Figure 1: Global map for estimated incidence of HIV associated cryptococcal meningitis in 2020

Data from reference 7 plotted on the global map. Regional estimates of incidence of HIV-associated cryptococcal meningitis (HIV-CM) are based on UNAIDS estimates of the number of people living with HIV in years 2019-2020, and the number of individuals with HIV at risk for cryptococcosis (that is with CD4 T cell counts of less than 200 cells/μL), and on the prevalence of cryptococcal antigenemia in those with a CD4 T cell count of less than 200 cells/μL by country and by region based on published literature. Number of cryptococcal antigen (CrAg)-positive people in each country and region was then estimated by multiplying the number of individuals at risk for cryptococcal infection by the prevalence of cryptococcal antigenemia. Progression from cryptococcal antigenemia to meningitis was then estimated based on published literature[7].

Figure 2: Host-response to C. neoformans infection.

Following inhalation of Cryptococci via inhalation into the lung (a), the primary immune response in the lungs is marked by phagocytosis of the yeast cells by lung-resident macrophages (b): initially the alveolar macrophages and later the parenchymal resident macrophages. Dendritic cells also phagocytose yeast cells and process them to prime T cells while also producing cytokines, such as tumor necrosis factor (TNF), GM-CSF, interleukin-1β (IL-1β), IL-6, and IL-12, which favors a CD4 T helper 1 (Th1) phenotype. CD4 Th1 T cells provide help to resident macrophages by secreting interferon gamma (IFNγ) and tumor necrosis factor (TNF). Th1 cell-derived cytokines enhance the killing capacity of macrophages for intracellular cryptococci. In healthy individuals, the primary immune response can lead to activated phagocytic activity with clearance of the fungi by innate immune cells or walling off fungi in granulomas . With immunosuppression in previously exposed individuals, the integrity of granulomas is compromised (c) leading to dissemination of fungi to other body organs including the brain via the blood stream and blood-brain barrier (d). Immune response in the brain is characterized by mobilization of innate immune cells including microglial cells, monocytes, and alternatively activated macrophages (M2) which are ineffective at killing phagocytosed fungi (**e**).

Figure 3.

Cryptococcal antigen screening in HIV CM. a. Association of cryptococcal antigen (CrAg) titers in the plasma of individuals living with HIV reporting meningitis symptoms (headache) and CrAg detection in the cerebrospinal fluid (CSF).[150] Data are based on multiple cohorts from Ethiopia, South Africa, Tanzania and Uganda that instituted lumbar punctures among individuals with CrAg-positive plasma tests, irrespective of presence of meningitis symptoms, as recommended by 2018 and 2022 World Health Organization guidelines[149,178,233,234]. Mortality is significantly lower in asymptomatic persons and increases with CrAg titer. Survival by titer is summarized elsewhere[150]. 'Symptomatic' refers to reporting headache. b. HIV-CM management following CrAg screening and pre-emptive treatment. Recommended timing of anti-retroviral therapy (ART) initiation (2 weeks) in CrAg+ is based on expert opinion.

Box 1. Risk factors for cryptococcal immune reconstitution inflammatory syndrome following ART

[bH1] Microbiologic

- Higher fungal burden or antigen titre at diagnosis. [30,31,41,113]
- Positive residual culture at the end of induction therapy when starting fluconazole 400mg for consolidation therapy.[113,127] Excess risk was not observed when using fluconazole 800mg for consolidation therapy.[235]

[bH1] Immunologic

- Lower pre-ART CD4 T cell count. [113,236]
- A robust immunologic response to ART with >4 fold CD4 T cell increase. [37,237]
- Elevated values of C-reactive protein (CRP) in the blood.[36,37,41]
- Low levels of immunoglobulin M (IgM), Laminarin-binding IgM, glucuronoxylomannan (GXM)-specific IgM in the plasma at diagnosis.[121]
- Paucity of CSF inflammation, denoted by white cells (<25 cells/µL) and total protein (<50 mg/dl) in the cerebrospinal fluid (CSF). [124]
- Trafficking of inflammatory monocytes, activated CD4 T cells, natural killer (NK) cells into CNS compartment.[238]
    - Low levels of interferon gamma (IFNγ), tumor necrosis factor (TNFα), interleukin 2 (IL-2), IL-6, IL-8, and IL-17 in the CSF.[113,124,127]

[bH1] Therapeutic

- ART initiation within 4 weeks of therapy for HIV-CM. [34,37,181-183]

Box 2. The 2022 World Health Organization treatment guidelines for HIV-CM [146,170]

[bH1] Induction phase

- A single high dose (10 mg/kg) of liposomal amphotericin B with 14 days of flucytosine (100 mg/kg per day divided into four doses per day) and fluconazole (1200 mg/daily for adults; 12 mg/kg per day for children and adolescents up to a maximum of 800 mg daily).

[bH2] Alternative therapy for induction phase

- **If liposomal amphotericin B is not available:** A seven-day course of amphotericin B deoxycholate (1 mg/kg per day) and flucytosine (100 mg/kg per day, divided into four doses per day) followed by seven days of fluconazole (1200 mg daily for adults and 12 mg/kg per day for children and adolescents up to a maximum of 800 mg daily).
- **If no amphotericin** B deoxycholate **is available:** 14 days of fluconazole (1200 mg daily, 12 mg/kg per day for children and adolescents) and flucytosine (100 mg/kg per day, divided into four doses per day).
- **If flucytosine is not available:** 14 days of liposomal amphotericin B (3–4 mg/kg per day) and fluconazole (1200 mg daily, 12 mg/kg per day for children and adolescents up to a maximum of 800 mg daily).
- **If liposomal amphotericin B and flucytosine are not available:** 14 days of amphotericin B deoxycholate (1 mg/kg per day) and fluconazole (1200 mg daily, 12 mg/kg per day for children and adolescents up to a maximum of 800 mg daily).

[bH1] Consolidation phase

- Fluconazole (800 mg daily for adults or 6–12 mg/kg per day for children and adolescents up to a maximum of 800 mg daily) is recommended for the consolidation phase (for eight weeks following the induction phase to 10 weeks).

- Start ART between week 4-6 from initiation of antifungal treatment.

[bH1] Maintenance phase

- Fluconazole (200 mg daily for adults or 6 mg/kg per day for adolescents and children) until immune reconstitution (CD4 T cell counts> 200/µL) and suppression of viral loads on ART.

Table 1: Diagnostic tests for Cryptococcal meningitis[130,133]

Assay	Sensitivity	Specificity	Pros	Cons
CSF fungal culture[+]	82.4-94.2%	100%	Can be used for diagnosis of cryptococcal meningitis relapse.	Sensitivity dependent on CSF volume cultured
CrAg lateral flow assay [+]	99.3%	99.1%	Rapid results (within 10 minutes); low cost; storage at room temperature; ease of use	Inability to differentiate cryptococcal meningitis relapse from paradoxical IRIS
CrAg latex agglutination[+]	97.0-97.8%	85.9-100%	Turnaround time shorter than fungal culture	Requires refrigeration of reagents, laboratory infrastructure and expertise
India ink microscopy[+]	86.1%	97.3%	Rapid results; low cost; storage at room temperature; ease of use	Sensitivity highly dependent on fungal burden; false positives from non-viable yeasts
Multiplex PCR[++]	82%	98%	Turnaround time shorter than fungal culture	Sensitivity dependent on fungal burden; low sensitivity at < 100 CFU/mL CSF

CSF, celebrospinal fluid; CrAg, cryptococcal antigen; PCR, polymerase chain reaction*
[++]BioFire® FilmArray® Meningitis/Encephalitis panel. [+] Reference test: A composite gold standard defined as CSF culture-positive or a culture-negative sample with ≥2 positive test results (India ink microscopy, CRAG latex, or CRAG LFA) and without an alternative etiologic

explanation[130]. ⁺⁺ Reference standard test: CrAg-LFA in patients presenting with the first episode of cryptococcal meningitis, and fungal culture in patients with a previous history of cryptococcosis[133].

Table 2. Guideline and Consensus Recommendation for the Management of Raised Intracranial Pressure in HIV and non-HIV Associated Cryptococcal Meningitis

	IDSA	Southern African	WHO	Consensus*
Baseline LP	Measure baseline OP. If OP >25 cmH_2O remove CSF until OP <20 cmH_2O or reduced by 50%.	Measure baseline OP. If OP >25 cmH_2O remove 10-30 mL of CSF until OP to <20 cmH_2O or reduced by 50%.	Measure baseline OP. If OP is > 25 cmH_2O remove 20-30 mL of CSF until OP <20 cmH_2O or reduced by 50%.	Measure baseline OP. If OP >25 cmH_2O remove CSF until OP has normalized <20 cmH_2O. If unable to measure OP, recommend large volume CSF removal (20-25 mL).
Therapeutic LP	If persistently raised ICP >25 cmH_2O and/or symptoms of raised ICP, repeat LP daily until CSF pressure and symptoms remain resolved for 2 days. Consider lumbar drain, ventriculostomy, or VP shunt if continued raised ICP.	Repeat LP whenever there are signs or symptoms of raised ICP. If persistently raised ICP and failure to respond to daily LPs for more than 1 week, consider lumbar drain or shunting procedures.	If baseline OP >25 cmH_2O and/or symptoms of raised ICP, repeat LPs daily until OP is normal and symptoms resolved for 2 days. Remove ~20-30 mL CSF with each LP. Early repeat of LP (day 3) with measurement of OP to assess for raised ICP in the absence of symptoms of raised ICP. Consider lumbar drain or ventricular	If baseline OP> 25 cmH_2O and/or symptoms of raised ICP, perform large volume CSF removal daily until OP normalize, and symptoms resolve. If unable to measure baseline OP, repeat at a minimum 2 subsequent LPs, one at day 3 and ideally day 7, or prior to discharge. Consider lumbar drain or VP shunt if continued raised ICP.

			shunts if continued raised ICP.	
Adjunctive therapies	Mannitol, Acetazolamide and corticosteroids should not be used to control raised ICP.	N/A	Mannitol, Acetazolamide, Furosemide, or corticosteroids should not be used to control raised ICP.	Mannitol, Acetazolamide and corticosteroids should not be used to control raised ICP.

Abbreviations: LP, lumbar puncture; OP, opening pressure; ICP, intracranial pressures; CSF, cerebrospinal fluid; VP, ventriculoperitoneal shunt; IDSA, Infectious Diseases Society of America; WHO, World Health Organization

*Consensus recommendation is based the expert opinion of panel attendees at the 11th International Conference on Cryptococcus & Cryptococcosis session on Therapeutic Lumbar Punctures in Cryptococcal Meningitis in Kampala, Uganda in January 2024.