INFECTIONS OF THE CNS

Cryptococcal meningitis: epidemiology, immunology, diagnosis and therapy

Peter R. Williamson[1], Joseph N. Jarvis[2], Anil A. Panackal[1], Matthew C. Fisher[3], Síle F. Molloy[4], Angela Loyse[4] and Thomas S. Harrison[4]

Abstract | HIV-associated cryptococcal meningitis is by far the most common cause of adult meningitis in many areas of the world that have high HIV seroprevalence. In most areas in Sub-Saharan Africa, the incidence of cryptococcal meningitis is not decreasing despite availability of antiretroviral therapy, because of issues of adherence and retention in HIV care. In addition, cryptococcal meningitis in HIV-seronegative individuals is a substantial problem: the risk of cryptococcal infection is increased in transplant recipients and other individuals with defects in cell-mediated immunity, and cryptococcosis is also reported in the apparently immunocompetent. Despite therapy, mortality rates in these groups are high. Over the past 5 years, advances have been made in rapid point-of-care diagnosis and early detection of cryptococcal antigen in the blood. These advances have enabled development of screening and pre-emptive treatment strategies aimed at preventing the development of clinical infection in patients with late-stage HIV infection. Progress in optimizing antifungal combinations has been aided by evaluation of the clearance rate of infection by using serial quantitative cultures of cerebrospinal fluid (CSF). Measurement and management of raised CSF pressure, a common complication, is a vital component of care. In addition, we now better understand protective immune responses in HIV-associated cases, immunogenetic predisposition to infection, and the role of immune-mediated pathology in patients with non-HIV associated infection and in the context of HIV-associated immune reconstitution reactions.

[1]*Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA.*
[2]*Botswana-University of Pennsylvania Partnership, P.O. Box AC157AC, Gaborone, Botswana.*
[3]*Department of Infectious Disease Epidemiology, Imperial College School of Public Health, St Mary's Campus, Norfolk Place, London W2 1PG, UK.*
[4]*Institute of Infection and Immunity, St Georges University of London, Cranmer Terrace, London, SW17 ORE, UK.*

Correspondence to T.S.H.
tharriso@sgul.ac.uk

doi:10.1038/nrneurol.2016.167
Published online 25 Nov 2016

Cryptococcal meningitis (CM) is the most common cause of adult meningitis in large parts of the world with high rates of HIV infection[1–3]. In addition, it occurs in an increasing number of patients with other forms of natural and iatrogenic immunosuppression, and in the apparently immunocompetent, particularly in the Far East[4]. In the USA, deaths from non-HIV-related CM now account for approximately one quarter of CM-related hospitalizations and one third of CM-related deaths[5]. Furthermore, an outbreak of cryptococcal disease in Pacific Northwest North America caused by a novel lineage[6] highlights the threat posed by *Cryptococcus* spp. (REF. 7) (BOX 1).

Cryptococcal meningitis is a subacute meningoencephalitis. The organism is acquired by inhalation, but can then disseminate, probably in many cases after a latent period of being contained within lung lymph nodes[8]. *Cryptococcus* spp. are currently the only notable human fungal pathogens from the large group of basidiomycete fungi. The species in this group — *C. neoformans* and *C. gattii* — share a number of properties that have made fungal organisms such a growing threat not only to human health, but also to animal and plant health; these properties include high virulence, generalism, long-lived environmental stages, and the potential for wide dispersal and rapid evolutionary change[9].

Herein, we review recent advances in the epidemiology, diagnosis, and clinical management of CM, including the optimization of antifungal therapy and the management of neurological complications. We discuss adjunctive immunosuppressive and immunoaugmentation therapies specific to patient and clinical characteristics, based on advances in our understanding of susceptibility to infection, immunopathological mechanisms, and protective immune responses. Moreover, we outline strategies for the prevention of HIV-associated CM through screening for subclinical infection, and pre-emptive therapy.

Key points

- In most clinical centres in Africa, despite access to antiretroviral therapies, cases of HIV-associated cryptococcal meningitis (CM) are not decreasing owing to challenges with retention and adherence to HIV care
- CM in HIV-negative individuals is relatively rare, but carries a mortality at least as high as in HIV-associated disease; therefore, CM must be considered in all cases of lymphocytic meningitis — even in the apparently immunocompetent
- A point-of-care, lateral flow 'dipstick' test to detect cryptococcal antigen in the blood or cerebrospinal fluid (CSF) is a significant advance: it is highly specific, sensitive, and easy to use
- Amphotericin B (in conventional or liposomal formulation) combined with flucytosine remains the induction therapy of choice, and is associated with a survival advantage over amphotericin B alone
- Measurement of CSF opening pressure and appropriate management of raised CSF pressure can reduce mortality
- Any future attempts at adjunctive immunotherapies will need to be closely guided by the specific immune status of the host at the time of any intervention

Epidemiology

HIV-associated cryptococcal infection

In a landmark 2009 study, the Centers for Disease Control and Prevention (CDC) estimated the global burden of HIV-associated CM on the basis of available incidence data in HIV-infected cohorts in different regions. According to the CDC, the incidence of CM was estimated at close to one million cases per year, with at least 100,000 and perhaps as many as 500,000 deaths per year in Sub-Saharan Africa alone[10]. Other data, suggesting that in some populations 10–15% of AIDS-related deaths (which peaked at 2.3 million per year in 2005 according WHO figures) are caused by cryptococcal disease[11], also put the number of CM-associated deaths in the hundreds of thousands. An updated analysis by Boulware and colleagues, some of whom were from the CDC, has used a different approach, based on the number of patients at risk (those with $CD4^+$ T cell counts <100 cells/μl and not on effective antiretroviral therapy (ART)), the prevalence of cryptococcal antigenaemia and risk of CM progression. This analysis indicates a lower number of CM-related deaths, but nevertheless estimates CM to cause 140,000 deaths per year, of which 102,000 are in Africa, and suggests that CM accounts for 17% of AIDS-related mortality[12].

In Europe and North America, the numbers of cryptococcal cases fell dramatically after introduction of effective ART, with hospitalizations falling by half according to one US study[5]. In South Africa, where cryptococcal surveillance has been carried out since the early 2000s, incidence has modestly reduced from a peak between 2005 and 2009 (REF. 13).

Importantly, however, there is as yet no evidence of a decrease in cases in many high-incidence African countries despite increased access to ART[14]. In Botswana, which has a relatively well-resourced and functioning ART programme, nationwide surveillance shows that the numbers of cryptococcal cases have actually increased since 2011, probably driven by the numbers of patients defaulting from care[15]. Although total numbers of CM cases remain relatively static, the management of CM is complicated by the fact that in many centres, half of all patients with CM now present with a history of ART use, but with persisting low $CD4^+$ T cell counts due to loss to follow-up, non-adherence, and/or the development of ART resistance (Harrison, T. *et al.* unpublished enrolment data from the ACTA trial, ISRCTN 45035509)[16,17].

Infection in HIV-negative individuals

In many low-income and middle-income countries, the incidence of HIV-associated CM dwarfs that of non-HIV CM. Nevertheless, cryptococcal meningitis is a significant problem in transplant recipients and other patients with defects in cell-mediated immunity, with high rates of death despite therapy[5]. In a US series of over 300 HIV-negative patients with cryptococcal infection, half had CNS involvement and of these, 25% had received steroid therapy, 24% had chronic liver, kidney or lung disease, 16% had a malignancy, and 15% had received solid organ transplants[18]. Haematopoietic malignancies are associated with an increased risk of CM, although stem cell transplant patients are typically not at increased risk because of widespread use of azole prophylaxis in this population. In addition, there is a well-recognized but poorly understood association with sarcoidosis, as well as other autoimmune diseases such as ankylosing spondylitis, dermatomyositis, systemic lupus erythematosus, and autoimmune hepatitis, although some of these associations could be attributed to steroid therapy[19]. Interestingly, even in the developed world, mycobacterial disease is an associated comorbidity[5], possibly because of shared susceptibility to these two intracellular pathogens because this relationship is seen in HIV-related cases as well[20].

Of note, in the US series, 30% of the patients had no apparent underlying condition. *C. neoformans* cases occur in apparently immunocompetent patients across the world, with large numbers reported in the Far East[4]. In addition, meningitis caused by *C. gattii* occurs throughout the tropics, including Australasia[21] and South America, in apparently immunocompetent patients, and notably in the outbreak in Pacific Northwest of North America that has been ongoing since 1999 (REF. 6).

Immunodeficiency syndromes. CM in previously healthy individuals is relatively rare, with approximately 3,000 cases reported annually in the USA, which would put the incidence at approximately one in 100,000 individuals per year. This incidence rate suggests that these apparently 'normal hosts', could in fact harbour rare primary immune defects or uncommon autoimmune diseases.

As shown in BOX 2, a number of immune deficiencies have been associated with cryptococcal meningitis. Idiopathic $CD4^+$ lymphopenia (ICL) is the best-known risk factor. In a recent meta-analysis of ICL cases, cryptococcosis was the most common infection, seen in 27% of reported cases[22]. ICL is a heterogeneous disease of unknown cause, although several potentially related monogenic mutations have been reported, including a recent association between defects in T-cell receptor signalling and a mutation in the *UNC119* gene[23].

Idiopathic $CD4^+$ lymphopenia
Repeated presence of a $CD4^+$ T lymphocyte count of < 300 cells/ml without a predisposing cause.

Hyperimmunoglobulin E recurrent infection syndrome
This syndrome, also known as Job syndrome, is caused by mutations in the signal transducer and activator of transcription (*STAT3*). Patients typically have eosinophilia, eczema, and recurrent skin and pulmonary infections.

Pulmonary alveolar proteinosis, which is characterized by the presence of autoantibodies against granulocyte-macrophage colony stimulating factor (GM-CSF), has been associated with intracellular infections, including cryptococcosis[24]. Some apparently immunocompetent patients with CM, including some with *C. gattii* infection in the Pacific Northwest, have been reported to have GM-CSF antibodies, which inhibit macrophage signalling by blocking STAT5 phosphorylation[25,26]. Autoantibodies against IFN-γ have also been associated with cryptococcal disease[27].

Cryptococcosis has also been associated with syndromes caused by monogenic mutations, including an autosomal dominant sporadic monocytopenia caused by mutations in *GATA2* zinc finger transcription factor, which is essential for lymphatic angiogenesis[28–30], hyperimmunoglobulin E recurrent infection syndrome[31,32], as well as X-linked hyper-IgM immunodeficiency, which has been associated with a number of mutations[33,34]. In addition, although CM in previously healthy patients is a rare disease, common genetic polymorphisms in, for example, FCy receptor IIB have been associated with disease in this population, and could represent disease modifier genes[35].

Clinical features, pathology, radiology

Cryptococcal meningitis is a subacute meningoencephalitis. The organism is acquired by inhalation, but can then disseminate, probably in many cases after a latent period of being contained within lung lymph nodes[8]. Whilst involvement of almost all organs and tissues has been reported, *Cryptococcus* spp. have a strong predilection for the CNS. This neurotropism has been linked to a number of cryptococcal-specific virulence factors that facilitate penetration of the blood–brain barrier, such as specific metalloproteinases and ureases; enzymes that cause neuroimmunomodulation, such as a dopamine-utilizing laccase; and mechanisms that facilitate survival within the nutrient-deprived environment of the brain, such as autophagy and high-affinity sugar transporters[36].

Thus, patients with CM present with neurological symptoms, most typically headache and altered mental status, as well as with fever, nausea and vomiting. The median duration from symptom onset to presentation is 2 weeks in patients with HIV infection and 6–12 weeks in non-HIV CM cases. Many patients develop visual symptoms, such as diplopia and, later in the disease, reduced acuity secondary to high CSF pressure (see below), and/or involvement of the optic nerve and tracts[37]. Without treatment, the disease progresses and symptoms extend to confusion, seizures, reduced level of consciousness, and eventually coma.

Many patients also have concomitant lung involvement, although in HIV-associated CM, this is often overlooked or misdiagnosed as tuberculosis[38]. Indeed, although meningoencephalitis dominates the clinical presentation in HIV-infected patients, disseminated infection is probably common. In patients without HIV infection, patients exhibit marked heterogeneity, and clinical presentation can be influenced by host immune responses in particular, and fungal species or lineage differences. For example, organ transplant recipients undergoing intensive immune conditioning can have shorter-lasting presentations with limited inflammatory sequelae, or present with immune reconstitution inflammatory syndrome (IRIS)-like characteristics after reductions of immunosuppression[39]. Previously healthy HIV-negative patients infected with either *C. neoformans* or *C. gattii* typically have a more chronic course

Box 1 | **Ecology, evolution and virulence of *Cryptococcus***

Cryptococcus is a genus within the Tremellales, an order of fungi that are commonly found growing on rotting wood as saprophytes and are called 'jelly fungi' because of their gelatinous fruiting bodies. Over 30 species of *Cryptococcus* are known; two of these, *C. neoformans* and *C. gattii*, cause the majority of human infections. Both species can be easily extracted from the environment: they can be isolated from the bark of a wide variety of tree species and from other organic matter, notably, bird faeces.

Cryptococci are sapronoses[138], a class of accidental parasites which primarily infect immunocompromized individuals, and which gain no evolutionary advantage from infecting the human host[139]. The virulence of *Cryptococcus*, therefore, is most likely owed to adaptations that allow it to survive in the environment[140]. An array of recognized 'dual-use' virulence factors are known[141], including a thick polysaccharide capsule[142], which in the environment defends against parasitic amoeba; however in the human, the capsule allows the fungus to survive macrophage assault and to disseminate as an intracellular parasite.

Genetic analysis has shown that *C. neoformans* and *C. gattii* have had independent evolutionary histories for an estimated 30–40 million years[143]. This separation has resulted in subtle variation in their virulence, with *C. neoformans* being the predominating infection in immunocompromized individuals (that is, individuals with HIV infection or AIDS), whereas *C. gattii* is a rarer infection and is seen in putatively immunocompetent individuals. Both species are highly diverse and have a number of phylogenetically distinct lineages that are likely to represent cryptic species; the two species are currently undergoing taxonomic revision and could be divided into seven species[144].

Genomic analysis of *C. gattii* has described four main major lineages (var. *gattii* (VG) I-IV)[145]. Population genetic analyses suggest that the hypervirulent VGII lineage originates from South America[146]. *C. neoformans* is similarly diverse, with two recognized cryptic species, each containing evolutionary distinct lineages: *C. neoformans* var. *grubii* (lineages var. *neoformans* (VN) I, II, and NB), which appears to have a centre of diversity in Africa, and *C. neoformans* var. *neoformans* (lineage VNIV). Both *C. gattii* and *C. neoformans* are facultatively sexual with a bipolar mating system, and hybrids can form between species and between lineages. To date, no important differences in susceptibility to antifungal drugs has been found between cryptococcal species and lineages.

Box 2 | **Predisposing genetic and other conditions in non-HIV CM**

Syndromes and autoantibodies

- Idiopathic CD4+ lymphopenia[22,23]
- Pulmonary alveolar proteinosis with autoantibodies to GM-CSF[24–26]
- Autoantibodies to IFN-γ[27]

Monogenic disorders

- Primary immunodeficiency owing to *GATA2* mutations[28–30]
- Chronic granulomatous disease
- Hyperimmunoglobulin E recurrent infection syndrome (also known as Job syndrome)[31,32]
- X-Linked CD40L deficiency (also known as hyper-IgM syndrome)[33,34]

Polygenetic modifiers

- FCg receptor II polymorphism[35]

Comorbidities[18,19]

- Sarcoidosis, autoimmune disease, steroid treatment
- Hepatic disease
- Solid organ transplant conditioning

CM, cryptococcal mengitis; GM-CSF, granulocyte-macrophage colony stimulating factor.

of CM that often does not feature fever, which can delay diagnosis. Inflammatory sequelae, including hydrocephalus, can be present at diagnosis or occur during therapy, and are reported more frequently with *C. gattii* than with *C. neoformans*[40], perhaps because *C. gattii* infection induces secretion of higher levels of proinflammatory cytokines than does *C. neoformans* infection[41].

In an autopsy series, HIV-associated CM was characterized by the presence of numerous, predominantly extracellular organisms throughout the parenchyma and meninges, sometimes in grossly visible accumulations, with little inflammatory response[42]. In non-HIV individuals, there are fewer organisms, largely confined to the meninges and large perivascular Virchow–Robin spaces, and the infection is associated with a diverse inflammatory response ranging from granulomatous to a more disorganized macrophage infiltration[42,43]. Immunohistochemistry studies also demonstrate cryptococcal capsule polysaccharide throughout the brain, localized in macrophages and microglia, especially in HIV-associated cases[44].

Brain MRI can detect many of these pathological features, including dilated Virchow–Robin spaces, pseudocysts, cryptococcomas, and cortical and lacunar infarcts[45,46]. In HIV-negative cases, a larger proportion of patients have large space-occupying cryptococcomas with a marked surrounding inflammatory response, and hydrocephalus.

Outcomes and prognostic factors

Outcomes for patients with HIV-associated cryptococcal meningitis in Africa remain poor, with overall best estimates suggesting a 3-month mortality of 70%, driven by late presentation, and lack of access to drugs, manometers, and optimal monitoring. In prospective research studies, for patients treated with fluconazole, mortality at 10 weeks is 50–60%[47–49]. Although amphotericin B-based treatment is associated with better outcomes, in trial settings, mortality at 10 weeks is still 24–42%[16,50–52]. Mortality remains high even in resource-rich settings: studies from the USA and France suggest that 10-week mortality is 15–26%, and has not changed since the availability of ART[53–55]. In a US study, 90-day mortality in HIV-negative patients was 27% — higher than in HIV-positive patients[56]. The high mortality in HIV-negative patients is potentially caused by delays in diagnosis and dysfunctional immune responses[57]. In HIV-negative individuals, *C. gattii* tends to cause more numerous granulomatous lesions in both the lung and the brain, and these patients have more neurological sequelae[6,40,58]. In HIV-infected individuals, the presentation and outcomes of *C. gattii* and *C. neoformans* infections are not readily distinguishable[59].

A recent analysis of over 500 patients with HIV-related cryptococcal meningitis confirmed and extended our understanding of the factors associated with poor outcome[60]. The most important of these factors are altered mental status at presentation, and baseline fungal burden (assessed by colony forming unit count). Older age and low weight also confer a poor prognosis[60]. In addition, the rate of infection clearance, which depends on the effectiveness of antifungal therapy and host immune response, is independently associated with outcome[60,61], making infection clearance a clinically relevant endpoint for phase II studies of novel antifungal regimens (see below).

In HIV-negative individuals, altered mental status and fungal burden are important prognostic factors. In addition, markers of a poor inflammatory response, low CSF white cell count, and absence of headache, as well as underlying haematological malignancy or chronic renal and liver disease, have been linked with poor prognosis[18,58,62,63].

Immune responses in HIV-infected patients

Jarvis *et al.* have carried out detailed analyses of local and systemic immune responses in patients with HIV-associated cryptococcal meningitis, at baseline and over time on treatment. These analyses have involved cytokine and chemokine profiling of CSF, and determination of the pattern of intracellular cytokine production by CD4+ memory cells when peripheral blood mononuclear cells (PBMC) are stimulated *ex vivo* with cryptococcal mannoproteins[64,65]. This linkage of immune response data to clinical parameters and outcomes allows an investigation of responses associated with survival.

In the analyses conducted by Jarvis and colleagues, variation in CSF parameters at baseline could be largely explained by two principal components (PCs): PC1 was driven by increased levels of IL-6 and IFN-γ, and also IL-8, IL-10, IL-17, CCL5 (also known as RANTES) and tumour necrosis factor (TNF), and PC2 by levels of monocyte chemotactic protein 1 (MCP-1), inflammatory protein 1α (MIP-1α) and GM-CSF (discussed below). Consistent with prior work[66], the proinflammatory response represented by PC1 correlated with high peripheral CD4+ T cell and CSF white cell counts, markers of CSF macrophage activation, reduced fungal

Colony forming unit
A measure to quantify viable fungal cells on the basis of the cells' ability to grow to form visible colonies on an agar plate.

Rate of infection clearance
The rate of decrease in viable organisms in the cerebrospinal fluid (CSF) during treatment, derived from quantitative cultures of the CSF obtained from serial lumbar punctures done over the first 14 days of treatment. For a particular drug regimen, the early fungicidal activity is the mean rate of infection clearance for patients on that regimen.

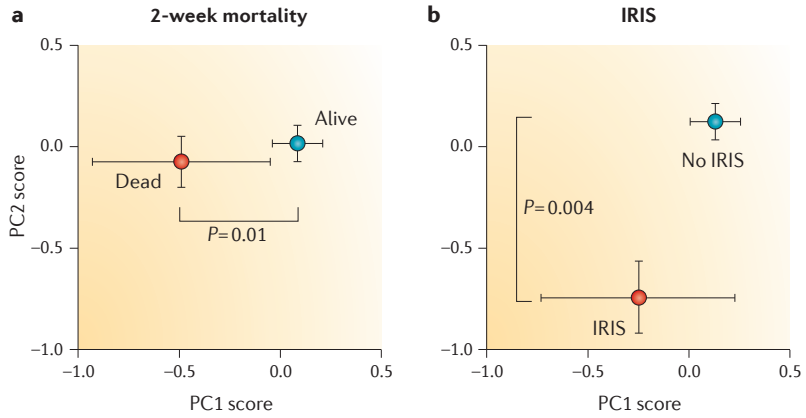


Figure 1 | **Associations between baseline cerebrospinal fluid immune response profiles and clinical outcome in HIV-associated CM.** Baseline cerebrospinal fluid (CSF) cytokine and chemokine concentrations were measured in 90 patients with HIV-associated cryptococcal meningitis (CM). Principal component analysis was used to identify co-correlated cytokine and chemokine measurements that accounted for the variance in the cytokine data set. A majority of this variance was accounted by two components, PC1 and PC2. PC1 was driven by increased levels of IL-6 and IFN-γ, and also IL-8, IL-10, CCL5 (also known as RANTES), tumour necrosis factor (TNF), and IL-17. PC2 was driven by high concentrations of chemokines, monocyte chemotactic protein 1 (MCP-1) and macrophage inflammatory protein 1α (MIP-1α), and granulocyte-macrophage colony stimulating factor (GM-CSF). **a** | PC1 score was associated with 2-week survival. **b** | In those who survived and were started on antiretroviral therapy, PC2 score was associated with the development of CM-associated immune reconstitution inflammatory syndrome (IRIS). The points on the graphs represent the mean values, and the error bars denote standard errors of the mean. Reproduced from Jarvis, J. N. *et al.* Cerebrospinal fluid cytokine profiles predict risk of early mortality and immune reconstitution inflammatory syndrome in HIV-associated cryptococcal meningitis. *PLoS Pathog.* **11**, e1004754 (2015); used under CC BY: http://creativecommons.org/licenses/by/2.0/legalcode.

burden, more rapid clearance of infection, and survival[65] (FIG. 1a). Analysis of systemic responses showed a similar pattern: patients who survived had a higher proportion of $CD4^+$ memory cells producing IFN-γ and TNF, and polyfunctional cells producing both of these cytokines, than patients who died, whose cryptococcal-specific $CD4^+$ memory cells were dominated by cells producing only MIP-1α[64] (FIG. 2). The results are consistent with recent independent work that related poor survival to decreased monocyte production of TNF and to reduced IFN-γ responses in whole blood when stimulated with LPS[67].

Diagnosis

Characteristic CSF parameters in CM include elevated white cell count, with lymphocyte predominance, elevated CSF protein, and low CSF glucose. In HIV-associated cryptococcal meningitis, the CSF white cell count is lower (median 15×10^6 cells/l (REF. 60)) and can often be normal. Diagnosis should not be an issue in HIV-associated cryptococcal meningitis, given the high fungal burden. Simple India Ink examination of the CSF has a 70–90% sensitivity, and cases tha t are negative on the India Ink test can be reliably diagnosed by detection of cryptococcal antigen (glucuronoxylomannan, the dominant capsular polysaccharide) and culture. However, until recently, antigen detection was based on latex agglutination tests, which, although sensitive and specific, were never widely available in high-burden, resource-limited settings.

In the context of limited resources, development of a lateral flow assay (LFA; manufactured by IMMY) has been a major advance. The test meets the ASSURED criteria[68] for point-of-care tests, has a 2-year shelf life at room temperature and requires no specimen preparation. In addition, it is in the order of 100-fold more sensitive to polysaccharide across the four cryptococcal serotypes than the older latex agglutination tests,[69,70]. Availability of this test makes the screen and pre-emptive therapy prevention strategy feasible (discussed below). It also enables earlier diagnosis (that is, diagnosis before lumbar puncture) through testing of serum, plasma, or whole-blood finger prick samples[71] in primary care settings, and through screening of medical inpatients in high-incidence areas. Of note, although urine samples contain antigen, they are prone to producing false positive results with the current LFA test[72,73]. More studies are needed to demonstrate whether earlier, LFA-based diagnosis translates to improved outcomes.

A second, semi-quantitative, lateral flow test is now in development by Biosynex and Institut Pasteur in Paris, France. The positive test result consists of either one (low antigen level) or two (high antigen level) bands; this gross approximation of fungal burden might prove useful in individualizing treatment, especially in the setting of screening.

Fungal burden can be low in some cases of HIV-associated unmasking CM that present after initiation of ART, as immune reconstitution can have a more dominant role than active infection (see below) in this context. In these patients, CSF cultures can be negative.

Similarly, in non-HIV CM, cultures and latex agglutination tests, especially for *C. gattii*, can be negative, and repeat large-volume CSF samples have sometimes been required in the past[74]. The improved sensitivity of the new LFA test for both *Cryptococcus* spp. is a major advantage[75,76], and means the diagnosis of CM should not be delayed, as long as the diagnosis is considered in all patients with a lymphocytic meningitis, even those who are apparently immunocompetent and/or afebrile.

Antifungal therapy

Current recommendations for first-line antifungal treatment have not changed notably for a decade[77–79] (TABLE 1), and are based on a three-step 'induction, consolidation, and maintenance' approach that was first used in the landmark 1997 Mycoses Study Group trial[80], and has been used in subsequent studies which showed that amphotericin B plus flucytosine led to the most rapid clearance of infection[81], and demonstrated a survival benefit with this combination over amphotericin alone[82]. In fact, the latter study found a reduction of almost 40% in the relative risk of death at 10 weeks with addition of flucytosine. Of note, the currently used dosage of flucytosine for 2 weeks in HIV-associated CM, with monitoring of full blood counts rather than drug serum levels, has been found to be well-tolerated[82–84]; in pharmacokinetic studies in patients, serum concentrations of

ASSURED criteria
Originally developed by the WHO Sexually Transmitted Diseases Diagnostics Initiative as a benchmark to determine whether new diagnostic tests addressed the needs of their disease control programmes in resource-limited settings: the ASSURED criteria include the test being affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end-users.

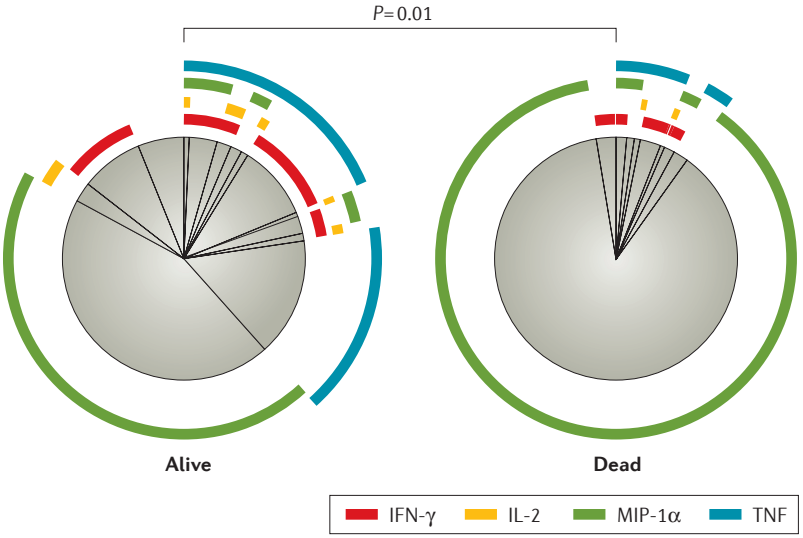


Figure 2 | **Systemic immune responses to cryptococcal antigen are associated with survival in HIV-associated cryptococcal meningitis.** Peripheral blood mononuclear cells were collected from patients with HIV-associated cryptococcal meningitis at first presentation to hospital, and stimulated *ex vivo* with cryptococcal mannoprotein antigen, known to contain important T-cell epitopes. The Figure depicts the functional phenotypes of the responses specific to cryptococcal antigen in patients who were alive 2 weeks after the infection and in patients who died. Flow cytometry results show the proportion of $CD4^+$ memory T cells that (as a specific response to cryptococcal antigen) produce IFN-γ, IL-2, macrophage inflammatory protein 1α (MIP-1α), tumour necrosis factor (TNF), and combinations of these cytokines at baseline. Patients who survived had a higher proportion of T cells that produced IFN-γ, TNF, and both, whereas in patients who died, the responses were dominated by T cells producing only MIP-1α. Adapted with permission from Oxford Journals. © 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 (2013).

flucytosine that are usually associated with bone marrow suppression were not found[85]. Care must be taken, however, to adjust the dose of flucytosine if renal impairment secondary to amphotericin develops.

Importantly, amphotericin B deoxycholate (D-AmB) is associated with renal impairment, hypokalaemia and hypomagnesaemia, and anaemia, especially during the second week of induction therapy[84]. Saline and fluid loading equivalent to giving 1 l of regular saline per day in addition to usual fluid requirements, has been shown to reduce renal impairment[86,87], and potassium and magnesium supplements under careful monitoring are recommended[77]. In a recent analysis, the average fall in haemoglobin during 14 days of treatment with D-AmB was 2.3 g/dl (REF. 84), which can pose a challenge in treatment centres where transfusion capacity is limited.

Liposomal amphotericin B (L-AmB) is equally as effective as D-AmB, and is better tolerated[88]. Optimal dosing and schedules for L-AmB are still not defined, and one or few large intermittent doses together with oral therapy could provide a safe and effective induction regimen, which would be more cost-effective and sustainable in resource-limited settings compared with current daily dosing. This approach is being tested in the ongoing Ambition-CM trial[89].

The rate of clearance of infection derived from quantitative cultures of CSF from serial lumbar punctures over the first 2 weeks of treatment provides a statistically powerful and clinically relevant endpoint to explore the activity of new dosages and drug combinations. Such early fungicidal activity (EFA) studies have influenced recommendations where resources are limited. Higher doses of fluconazole, up to 1,200 mg daily, are safe and more fungicidal than previously used lower doses[49], and the combination of high-dose fluconazole plus flucytosine has an EFA that approaches that measured for amphotericin B alone[90]. This oral combination could also prevent emergence of secondary fluconazole resistance. In addition, shorter, 7-day induction with D-AmB does not seem to lead to reduced EFA, perhaps because D-AmB has a prolonged half-life in brain tissues, and is much better tolerated[91–93]. On the basis of these phase II studies, high-dose fluconazole combined with flucytosine, and 1-week D-AmB-based induction are both being compared with the standard 2-week course of flucytosine plus D-AmB treatment regimen in African treatment centres in the phase III ACTA trial, which will report in 2017[94]. If these two regimens are as effective as 2 weeks of treatment with D-AmB, both would be much more readily and safely sustained in resource-limited settings.

EFA has also been used to explore the *in vivo* efficacy of established drugs that are known from preclinical studies to have anticryptococcal activity, for possible repurposing for CM. Addition of sertraline to amphotericin B plus fluconazole was associated with an increase in EFA in a Ugandan cohort, compared with historical controls treated without sertraline at the same centre[17], and is being tested now in phase III (results expected in 2018[95]). A number of other candidate agents are suitable for similar testing[96]. At least one new agent, Viamet 1129, is being developed specifically for CM. It is a novel oral azole-like compound that concentrates in brain tissue and shows impressive fungicidal activity in animal models. Phase I studies of Viamet 1129 have started, with phase II EFA studies under development.

In patients without HIV, the clinical response depends on control of aberrant immune responses as much as it depends on control of the initial infection. Initial therapy with amphotericin B and flucytosine is similar to that for HIV-related disease, but the induction phase is longer (4–6 weeks[6,78,97]) (TABLE 1) and lipid formulations have been favoured over the deoxycholate preparation because of reduced renal toxicity. Previously healthy patients with idiopathic $CD4^+$ lymphopenia can take longer to respond microbiologically but have less immune sequelae, whereas those with normal $CD4^+$ T cell counts can have immune sequelae on presentation or, in an important minority, such sequelae can develop despite microbiological control (discussed below).

Reduction of immunosuppression in solid organ transplant recipients is an intuitively logical approach after severe infections such as cryptococcosis, and is well tolerated. However, discontinuation of calcineurin agents and similar drugs has been associated with clinical deterioration and IRIS, although discontinuation of corticosteroids was not[98,39]. Calcineurin inhibitors have

an added benefit of being synergistic with antifungals against *Cryptococcus*, and have been associated with better outcomes in post-transplant cryptococcosis[99].

Raised CSF pressure

Half of HIV-infected patients with CM have a CSF opening pressure of >25 cmH_2O, with roughly a quarter of patients having a very high pressure of >35 cmH_2O (REFS 60,100). High pressure is associated with worse symptoms, including headache, nausea, diplopia secondary to sixth nerve palsies, and altered mental status.

Head scans show that hydrocephalus is rare in HIV-associated meningitis[45], and the pathophysiology is most likely attributed to blockage of CSF reabsorption by alive or dead organisms, and/or shed cryptococcal polysaccharide at the level of the arachnoid granulations and other sites of CSF reabsorption[101]. In a small postmortem patient series, arachnoid granulation tissue contained many fungal cells in comparison with the rest of the brain, and high numbers of organisms were associated with increased premorbid CSF pressure[101]. Interestingly, a high fungal burden appears necessary but not sufficient for the development of high pressure, suggesting that other pathogen or host factors must be involved[102].

Based on this understanding of mechanism, careful therapeutic lumbar punctures are recommended to control high CSF pressure. The safe maximum volume of CSF that can be drained at one lumbar puncture is unclear, but up to 30 ml are frequently removed in patients with high pressure, with checking of pressure after each 10 ml removed. Increasing evidence points convincingly to the efficacy of this procedure. In the original data from the 1997 Mycoses Study Group trial, high pressure was associated with increased acute mortality[100], whereas in a large cohort of patients in whom regular lumbar punctures were performed with additional therapeutic lumbar punctures if the pressure was high, there was no association of high baseline pressure with mortality. In fact, the opposite trend was seen[60]. In addition, adherence to guidelines regarding CSF pressure measurement and management has been associated with improved outcome[103], and, in a Ugandan study, therapeutic lumbar punctures were associated with reduced mortality, irrespective of baseline pressure[104].

Importantly from a clinical standpoint, it is well recognized that high CSF pressure, even if absent on presentation, can develop later, despite sterilization of CSF, typically in the second and third weeks of antifungal therapy[102]. Thus, it is vital that lumbar puncture is repeated and pressure is measured in patients who do not improve or in whom symptoms recur.

Repeated daily therapeutic lumbar punctures are sufficient to control raised pressure in the majority of patients. Occasionally, however, only a temporary lumbar drain, ideally managed on a neurosurgical facility, which allows an order of magnitude more CSF to be safely drained per day, is sufficient[105,106]. Our own and others' experience suggests that around 7 days of temporary drainage is usually required[106] (FIG. 3). Ventricular shunts are also effective, but can usually be avoided in HIV-associated CM.

By contrast, an important minority of HIV-negative individuals with CM have central obstruction of the choroid plexes within the foramina of the fourth ventricle that is accompanied by hydrocephalus, or a superior arachnoiditis and a communicating process of elevated pressures, both of which are associated with robust inflammatory responses[43]. Thus, in meningitis that is not associated with HIV, shunts are needed more often because of hydrocephalus or persistent obstruction, and can be used safely provided antifungal therapy has been started[107].

Cryptococcal IRIS

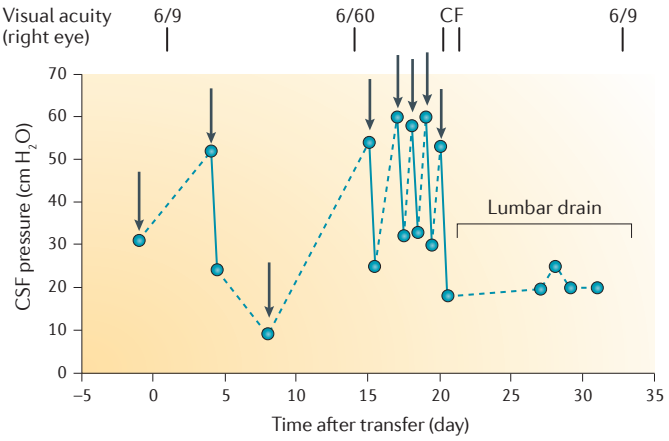
CM-associated IRIS is another common and life-threatening complication. In the context of HIV, there are two forms of IRIS: paradoxical IRIS in patients who

Table 1 | **Antifungal therapy in cryptococcal meningitis**

Stage	Pharmacological treatment regimen	Duration
Induction	L-AmB 3–6 mg/kg daily or D-AmB 0.7–1.0 mg/kg daily (L-AmB preferred in organ transplant patients and when >2-week induction is needed) in combination with flucytosine 100 mg/kg daily (75 mg/kg daily if intravenous formulation is used)	• HIV+ patients: 2 weeks • Transplant recipients:* ≥2 weeks • For all other patients, including patients with *Cryptococcus gatti* infection:* 4–6 weeks
Consolidation	• Fluconazole 400–800 mg daily‡ • In HIV+ patients, start ART at 4 weeks	8 weeks
Maintenance therapy	• Fluconazole 200 mg daily • In HIV+ patients, consider discontinuing maintenance after a minimum of 1 year if $CD4^+$ cell count is >100 cells/μL and HIV viral load is suppressed	≥1 year
Induction therapy in resource-limited settings	• If flucytosine is not available: • D-AmB 0.7–1 mg/kg daily intravenously in combination with fluconazole 800–1200 mg daily	2 weeks (1 week is better than no D-AmB)
	• If D-AmB is not available: • Fluconazole 1,200 mg daily§ in combination with flucytosine 100 mg/kg daily orally (if available)	2 weeks

ART, antiretroviral therapy; D-AmB, amphotericin B deoxycholate; L-AmB, liposomal amphotericin B. *see IDSA guidelines[78]. ‡800 mg daily preferred if second line induction regimens used. §Fluconazole increases nevirapine levels, and safety of high-dose fluconazole with nevirapine is unknown. Alternative antiretrovirals are preferred.

Paradoxical IRIS
Clinical deterioration in HIV-positive patients with cryptococcal meningitis who have responded to initial antifungal therapy, but then relapse after starting antiretroviral therapy owing to the resultant immune restoration and enhanced inflammatory immune response to residual cryptococcal antigens.



Time after transfer (day)	21	22	23	24	25	26	27	28	29	30	31	32	33
CSF drained (ml)	250	231	227	205	174	185	143	113	170	172	51		

Drain clamped overnight

Drain clamped, then removed

Figure 3 | **Raised cerebrospinal fluid pressure in a patient with HIV-associated cryptococcal meningitis.** Depicted here is time course of changes in cerebrospinal fluid (CSF) pressure, visual acuity, and volume of CSF drained through a temporary lumbar drain *in situ* over 11 days. Arrows indicate times of lumbar puncture. During the third week of antifungal therapy, and despite the fact that CSF cultures had become negative, the patient developed severely raised CSF pressure that was unresponsive to repeated daily lumbar punctures, but did respond to CSF drainage via a temporary lumbar drain. Symptoms of high CSF pressure recurred when the drain was clamped after 6 days, but did not recur when it was clamped and then removed after 10 days. CF, counting fingers (indicating severely reduced visual acuity). Adapted with permission from Elsevier Ltd © Macsween, K. F. *et al. J. Infect.* **51**, e221-e224 (2005).

respond to antifungal therapy for CM before starting ART and then have a relapse of CM symptoms after starting ART; and unmasking IRIS in individuals who present with CM for the first time after ART is started[108].

Paradoxical IRIS

Paradoxical CM-IRIS remains a diagnosis of exclusion, and the reported rates of paradoxical IRIS vary depending on how thoroughly alternative diagnoses can be investigated. Parodoxical IRIS probably occurs in 10–20% of patients who survive CM long enough to start ART, and the condition is most likely to develop at a median of 1–2 months after ART initiation[109–111].

Risk factors for CM-IRIS include a low pre-ART $CD4^+$ cell count that rises rapidly after ART initiation, a low initial CSF white cell count, low markers of inflammation and IFN-γ responses on initial presentation, and a high fungal burden at baseline and day 14 (REFS 112–114). In the principal component analysis of baseline CSF cytokine profiles by Jarvis *et al.* (described earlier), the second principal component — which was driven by high concentrations of chemokines, MCP-1 and MIP-1α, and GM-CSF — was associated with the subsequent development of IRIS[65] (FIG. 1b); consistent with prior findings by Chang *et al.*[115]. These chemokines might enhance T and myeloid cell trafficking into the CNS, resulting — once restoration of $CD4^+$ T_H1 subsets occurs following initiation of ART — in the excessive T_H1-type immune responses seen during CM-IRIS episodes[57,110,116,117] (FIG. 4).

As with other opportunistic infections, the question arises as to how to best time ART to avoid precipitating IRIS, while establishing HIV therapy as soon as possible. For cryptococcal disease, we know that ART initiation at 3–8 days after presentation[118,119] is too soon, and at 6 weeks is probably unnecessarily late[120]. In the Cryptococcal Optimal ART Timing (COAT) study[119], as early as day 14 after presentation, patients given early ART (median initiation at 8 days) were found to have higher CSF white cell counts and CSF markers of macrophage and/or microglial activation than patients not yet started on ART, suggesting the excess deaths in the early ART arm may have been immune mediated[121]. Thus, current guidelines suggest that ART is initiated at 4–6 weeks, although cohort evidence suggests that starting treatment during the fourth week is safe in the context of rapidly fungicidal induction therapy[60], which should itself help prevent IRIS.

Patients who re-present (that is, get worse and seek care) at the clinic after the start of ART should have an lumbar puncture to screen for ongoing active infection, and re-induction antifungal therapy (TABLE 1) should be considered while awaiting culture results. Moreover, lumbar puncture enables measurement and management of CSF pressure, which is essential given that high pressure is an important feature of paradoxical CM-IRIS. Alternative diagnoses should be actively pursued. While CM-IRIS is clearly life-threatening, it is possible to minimize CM-IRIS mortality with awareness and early recognition, and short courses of corticosteroids for patients who are deteriorating[112]. In some cases, steroid weaning leads to recurrent IRIS, and case reports have described the use of thalidomide and adalimumab in refractory CM-IRIS in both HIV and transplant patients[122,123].

Unmasking IRIS

Unmasking HIV-associated CM-IRIS cases are characterized by lower fungal burden compared with ART-naive cases, and some evidence, although not conclusive, points to increased inflammatory responses in unmasking CM-IRIS[124]. The balance between active infection and immune pathology can vary, depending on the interval between ART initiation and presentation. If this interval is just a few days, little difference is seen when compared with ART-naive patients, whereas patients presenting much later may be culture-negative, and are diagnosed on the basis of antigen testing only. Patients presenting with unmasking CM-IRIS are treated with the same antifungal regimens used for those who are ART-naive, although care is needed in view of interactions and overlapping adverse effects between antifungals and ART. Careful assessment is also needed to try to distinguish between unmasking cases, and the increasing number of patients who are ART-exposed but present with CM with persisting low $CD4^+$ cell counts attributed to ART non-adherence and/or resistance. In these latter cases, any switches or re-initiation of ART are best postponed until 4 weeks into antifungal therapy.

Unmasking IRIS
Individuals with HIV infection can present for the first time with cryptococcal meningitis after effective antiretroviral therapy (ART) has been initiated. These patients may have a mixture of active infection and immune-mediated pathology as a result of ART-mediated immune restoration.

Macrophage–T-cell dissociation
Despite appropriate T-cell signalling, macrophages fail to become classically activated and clear infection but rather remain in an alternatively activated state that is less effective at controlling infection and clearing antigen.

Immune responses in non-HIV patients

In the HIV-negative host, paradoxical immune reactions are an important cause of poor outcome in CM. In patients who have previously been immunosuppressed prior to bone marrow transplantation or given chemotherapy for haematological malignancies, reductions in immunosuppressive medications to boost the immune response frequently result in an IRIS-like reconstitution syndrome[39]. In previously healthy individuals in whom no immune reconstitution occurs, clinical deterioration commonly occurs during therapy, not from microbiological failure but from an aggravated immune response, known as post-infectious inflammatory response syndrome (PIIRS).

Primary differences in the non-HIV setting compared with the HIV setting include compartmentalization, with only intrathecal responses measurable, and in many non-HIV cases an alternatively activated M2-like skewing of CNS-tissue infiltrating macrophages, which has been found to indicate a nonprotective response in animal models of CM[125]. As shown in FIG. 4, PIIRS shares features with CM-IRIS, including activation of the dendritic cell-T-cell synapse and T-cell inflammatory responses evidenced by elevated cytokines IFN-γ and IL-6. Biomarkers of this inflammatory process are the CSF soluble T-cell activation marker sCD27, which is associated with release of the axonal damage protein, neurofilament light chain. However, the syndrome differs from CM-IRIS in that PIIRS lacks effective activation of macrophages, leading to macrophage–T-cell dissociation; as a result, these patients have persistent tissue antigen and inflammation, and can show clinical deterioration. As shown in FIG. 5, corticosteroids have been useful in these patients with inflammatory brain lesions, whether infected with *C. neoformans* or *C. gattii*, once microbiological clearance is documented by negative CSF cultures[43,126].

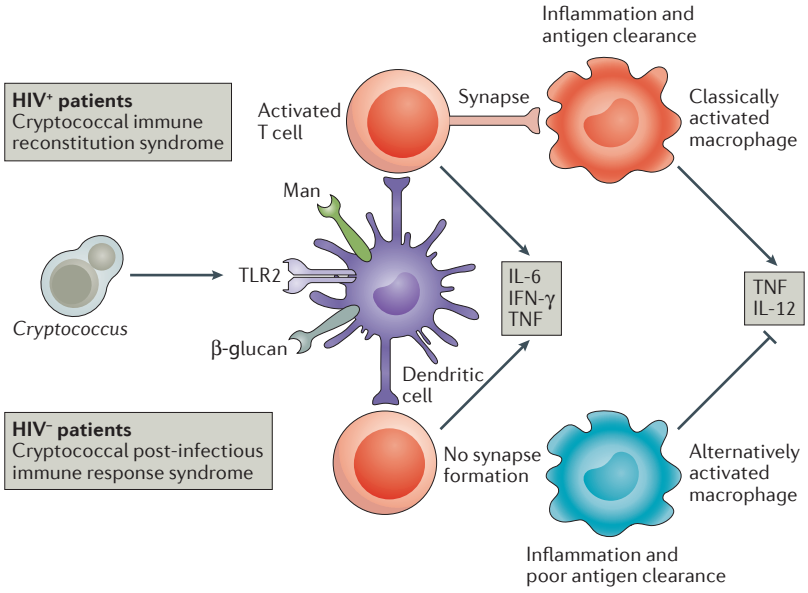


Figure 4 | **Host damage from infection-related inflammatory syndromes in HIV-positive and in HIV-negative cryptococcal meningitis.** Some HIV-positive cryptococcal meningitis (CM) patients who initially improve with antifungal therapy later re-present with CM-associated immune reconstitution inflammatory syndrome after initiation of effective antiretroviral therapy. Such patients present with clinical deterioration although CSF cultures do not show active infection, and an aggravated T-cell and proinflammatory macrophage response. HIV-negative, previously healthy patients with CM may deteriorate clinically despite effective antifungal therapy and negative CSF cultures, with a similar post-infectious immune response syndrome consisting of a similar T-cell response but a defective M2 macrophage polarization that is ineffective in clearing fungal antigen. Man, mannose; TLR2, Toll-like receptor 2.

Prospects for immunotherapies

As exemplified above, an increased understanding of the immunopathology in some non-HIV cases of CM, of immunological predispositions to CM in the heterogeneous group of HIV-negative patients with CM, and of protective immune responses in patients with HIV-associated CM should guide attempts to manipulate immune responses for patient benefit. Any such attempts will need to be tailored to the patient and their immune status at the time, moving the damage-response framework[127] to an optimal balance of effective fungal clearance without immune pathology.

Given that PIIRS in non-HIV CM can be persistent owing to macrophage clearance defects, and the adverse effects of prolonged corticosteroid use, active research is currently directed at identifying steroid-sparing immunosuppressants that are effective in neuroinflammation. In HIV-negative, previously healthy patients, identification of genetic or immunological defects could be useful to guide attempts to augment microbiological control and to identify family members at risk. In addition, diseases such as pulmonary alveolar proteinosis are important comorbidities to identify in non-HIV CM, because pulmonary pathology may be problematic after resolution of meningitis, and a number of therapies, such as aerosolized GM-CSF, have proven effective for pulmonary pathology[128,129].

In HIV-associated CM, clinical trial data are consistent with the immune data from patients, showing the importance of T_H1-type immunity in controlling infection. In a large randomized trial, adjunctive steroids at the time of CM diagnosis were associated with an increased risk of disability or death, increased adverse events, and a substantially reduced rate of fungal clearance[16]. By contrast, in two smaller trials, adjunctive IFN-γ appeared to be safe and augmented clearance without any indication of adverse effects on HIV viral control or IRIS[52,130]. Perhaps unsurprisingly, the greatest effect was seen in patients with poor baseline T_H1-type responses. Further studies on the risks and benefits of IFN-γ in HIV-associated CM are needed, and rapid assessment of immune status could enable targeting of IFN-γ to patients who are likely to benefit most. Even in HIV-associated CM, the patients' immune responses vary; augmentation of immune responses by IFN-γ should be avoided in patients with late unmasking IRIS, and in those with paradoxical CM-IRIS.

Screening and prevention

Cryptococcal infection can be diagnosed early through detection of cryptococcal polysaccharide antigen in the blood, so screening of patients at very high risk of cryptococcal infection, such as those with late-stage

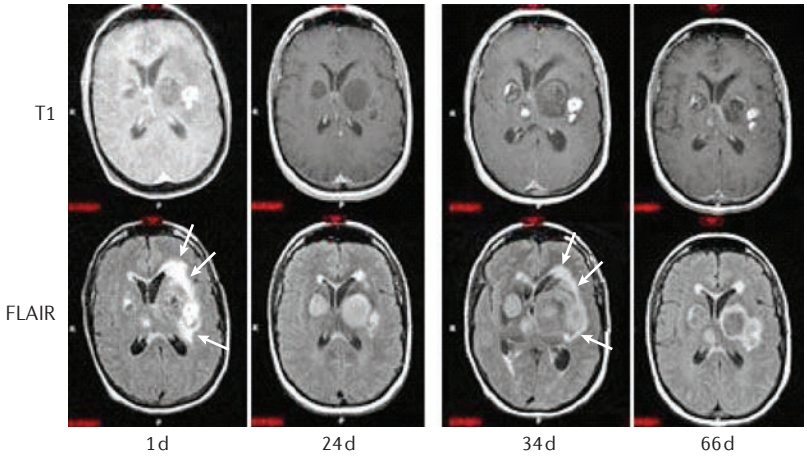


Figure 5 | **Corticosteroid treatment can reduce brain oedema in patients with HIV-negative cryptococcal meningitis.** MRI scans demonstrate reduced brain oedema (arrows) after treatment with corticosteroids in an HIV-negative patient with CM and PIIRS. T1 and FLAIR weighted MRI scans of a patient with *Cryptococcus gattii* infection and autoantibody against granulocyte-macrophage colony stimulating factor treated with amphotericin B from day 1 to day 66, with adjunctive prednisone (50 mg daily) between day 1 and day 24. Corticosteroid therapy was stopped on day 24, but then re-instituted at days 34–66 after clinical deterioration. Reproduced from Panackal, A. A. *et al.* Paradoxical immune responses in non-HIV cryptococcal meningitis. *PLoS Pathog.* **11**, e10047884 (2015); used under CC BY: http://creativecommons.org/licenses/by/2.0/legalcode.

HIV, could provide an opportunity for preventing the development of CM. In one study, the antigen was detectable in the blood at a median of 22 days before development of CNS symptoms[11]. In a retrospective cohort study of over 700 prospectively monitored patients in Cape Town, South Africa, blood samples were taken before initiation of ART[131]. None of the 661 patients who were cryptococcal antigen negative (93%) went on to develop CM in the first year of ART. By contrast, at least 7 of 25 patients (28%) who were antigen-positive with no prior history of CM developed CM during this time. This 100% negative predictive value of antigen-negativity supports the utility of antigen detection in a screen and pre-emptive therapy strategy, whereby patients at risk (with $CD4^+$ T cell counts <100 cells/μl) are tested for antigen and those who tested positive are given pre-emptive therapy with the widely available and safe oral fluconazole. Several modelling studies have suggested that such a strategy would be highly cost-effective, saving lives and money even at very low prevalences of antigenaemia, in light of the fact that the costs of admission and care of prevented cases would be avoided[132,133]. On this basis, screening was endorsed in WHO guidelines[77], and programmes initiated in South Africa[134] and elsewhere, and by Médecins Sans Frontières.

Some prospective data is now available that supports the screening-based prevention strategy: in a randomized controlled trial in Tanzania and Zambia (REMSTART), screening of patients presenting for ART with a $CD4^+$ T cell count <200 cells/μl, as described above, combined with simple lay-worker ART adherence support for the first month, reduced overall mortality in the first year of ART by 28%[135]. Nevertheless, questions remain regarding how best to implement screening and, in particular, over the best therapy for antigen-positive patients. Data from REMSTART, as well as studies in Uganda and Cape Town, all suggest that the mortality of antigen-positive patients remains much higher, at 25–30%, than antigen-negative patients, which is about 10–12%[73,135]. Moreover, when the researchers analysed data from patients who had consented to lumbar puncture, as many as ~40%, of serum antigen-positive patients, even if asymptomatic, were found to have CSF antigenaemia, which suggests meningitis[73]. Furthermore, high blood antigen titres can predict those with meningitis[73], and are associated with a poor outcome[136]. Although fluconazole may be sufficient therapy for many antigen-positive patients, patients with evidence of meningitis may need more-aggressive antifungal therapy. A planned follow-up trial for REMSTART will compare fluconazole with fluconazole plus flucytosine for antigen-positive patients. New semi-quantitative antigen tests could rapidly identify those who would benefit from more-intensive treatment.

Conclusions

Cryptococcal meningitis is a leading fungal cause of human disease and death worldwide. However, expanding access to current antifungal drugs[137] and optimizing their use in regimens that are sustainable in resource-limited settings, together with earlier diagnosis enabled by a new point-of-care immunodiagnostic test and therapeutic lumbar punctures to manage the common complication of raised CSF pressure, have the potential to markedly reduce the global disease burden. Drug discovery aimed specifically at *Cryptococcus* spp. is limited, but one promising new agent, Viamet-1129, is now entering clinical evaluation. Immunomodulatory adjunctive therapies, based on advances in our understanding of host immunity in different patient populations, hold promise, but need to be carefully tailored to the individual patient and their immune status at the time.

1. Durski, K. N. *et al.* Cost-effective diagnostic checklists for meningitis in resource-limited settings. *J. Acquir. Immune Defic. Syndr.* **63**, e101–e108 (2013).
2. Rajasingham, R. *et al.* Epidemiology of meningitis in an HIV-infected Ugandan cohort. *Am. J. Trop. Med. Hyg.* **92**, 274–279 (2015).
3. Jarvis, J. N. *et al.* Adult meningitis in a setting of high HIV and TB prevalence: findings from 4961 suspected cases. *BMC Infect. Dis.* **10**, 67 (2010).
4. Zhu, L. P. *et al.* Cryptococcal meningitis in non-HIV-infected patients in a Chinese tertiary care hospital, 1997–2007. *Med. Mycol.* **48**, 570–579 (2010).
5. Pyrgos, V. *et al.* Epidemiology of cryptococcal meningitis in the US: 1997–2009. *PLoS ONE* **8**, e56269 (2013).
6. Phillips, P. *et al.* Longitudinal clinical findings and outcome among patients with *Cryptococcus gattii* infection in British Columbia. *Clin. Infect. Dis.* **60**, 1368–1376 (2015).
7. May, R. C. *et al. Cryptococcus*: from environmental saprophyte to global pathogen. *Nat. Rev. Microbiol.* **14**, 106–117 (2016).
8. Alanio, A. *et al. Cryptococcus neoformans* host adaptation: toward biological evidence of dormancy. *mBio* **6**, e02580-14 (2015).
9. Fisher, M. C. *et al.* Emerging fungal threats to animal, plant and ecosystem health. *Nature* **484**, 186–194 (2012).
10. Park, B. J. *et al.* Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS* **23**, 525–530 (2009).
11. French, N. *et al.* Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. *AIDS* **16**, 1031–1038 (2002).
12. Boulware, D. R. *et al.* Update on the global burden of disease of HIV-associated cryptococcal meningitis. Oral Abstract presented at: 9th International Conference on Cryptococcus and Cryptococcosis; 2014 May; Amsterdam.
13. National Institute of Communicable Diseases. RapidGerms South Africa Annual Report 2014. http://www.nicd.ac.za/assets/files/GERMS-SA%20AR%202014.pdf [online] pages 8–11 (2014).
14. Wall, E. C. *et al.* Bacterial meningitis in Malawian adults, adolescents, and children during the era of

antiretroviral scale-up and *Haemophilus influenzae* type b vaccination, 2000–2012. *Clin. Infect. Dis.* **58**, e137–e145 (2014).
15. Tenforde, M. *et al.* HIV-associated cryptococcal meningitis in Botswana: national incidence and temporal trends following ART rollout. http://www.aids2016.org/Portals/0/File/AIDS2016_Abstracts_LOW.pdf?ver=2016-08-10-154247-087 [online] Abstract presented at: 21st International AIDS Conference; July 2016; Durban.
16. Beardsley, J. *et al.* Adjunctive dexamethasone in HIV-associated cryptococcal meningitis. *N. Engl. J. Med.* **374**, 542–554 (2016).
17. Rhein, J. *et al.* Efficacy of adjunctive sertraline for the treatment of HIV-associated cryptococcal meningitis: an open-label dose-ranging study. *Lancet Infect. Dis.* **16**, 809–818 (2016).
18. 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 (2001).
19. Bernard, C. *et al.* Cryptococcosis in sarcoidosis: cryptOsarc, a comparative study of 18 cases. *QJM* **106**, 523–539 (2013).
20. Jarvis, J. N. *et al.* Is HIV-associated tuberculosis a risk factor for the development of cryptococcal disease? *AIDS* **24**, 612–614 (2010).
21. Speed, B. & Dunt, D. Clinical and host differences between infections with the two varieties of *Cryptococcus neoformans*. *Clin. Infect. Dis.* **21**, 28–34 (1995).
22. 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 (2013).
23. Gorska, M. M. & Alam, R. A mutation in the human *Uncoordinated 119* gene impairs TCR signaling and is associated with CD4 lymphopenia. *Blood* **119**, 1399–1406 (2012).
24. Lee, Y. C., Chew, G. T. & Robinson, B. W. Pulmonary & meningeal cryptococcosis in pulmonary alveolar proteinosis. *Aust. N. Z. J. Med.* **29**, 843–844 (1999).
25. Rosen, L. B. *et al.* Anti-GM-CSF autoantibodies in patients with cryptococcal meningitis. *J. Immunol.* **190**, 3959–3966 (2013).
26. 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**, e00912-14 (2014).
27. Browne, S. K. *et al.* Adult-onset immunodeficiency in Thailand and Taiwan. *N. Engl. J. Med.* **367**, 725–734 (2012).
28. Vinh, D. C. *et al.* Autosomal dominant and sporadic monocytopenia with susceptibility to mycobacteria, fungi, papillomaviruses, and myelodysplasia. *Blood* **115**, 1519–1529 (2010).
29. Hsu, A. P. *et al.* Mutations in *GATA2* are associated with the autosomal dominant and sporadic monocytopenia and mycobacterial infection (MonoMAC) syndrome. *Blood* **118**, 2653–2655 (2011).
30. Spinner, M. A. *et al.* GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. *Blood* **123**, 809–821 (2014).
31. Jacobs, D. H. *et al.* Esophageal cryptococcosis in a patient with the hyperimmunoglobulin E-recurrent infection (Job's) syndrome. *Gastroenterology* **87**, 201–203 (1984).
32. Holland, S. M. *et al. STAT3* mutations in the hyper-IgE syndrome. *N. Engl. J. Med.* **357**, 1608–1619 (2007).
33. Winkelstein, J. A. *et al.* The X-linked hyper-IgM syndrome: clinical and immunologic features of 79 patients. *Medicine (Baltimore)* **82**, 373–384 (2003).
34. Iseki, M. *et al.* Hyper-IgM immunodeficiency with disseminated cryptococcosis. *Acta Paediatr.* **83**, 780–782 (1994).
35. Hu, X. P. *et al.* Association of Fcγ receptor IIB polymorphism with cryptococcal meningitis in HIV-uninfected Chinese patients. *PLoS ONE* **7**, e42439 (2012).
36. Coelho, C., Bocca, A. L. & Casadevall, A. The tools for virulence of Cryptococcus neoformans. *Adv. Appl. Microbiol.* **87**, 1–41 (2014).
This review discusses virulence factors specific to *Cryptococcus*.
37. Moodley, A. *et al.* Early clinical and subclinical visual evoked potential and Humphrey's visual field defects in cryptococcal meningitis. *PLoS ONE* **7**, e52895 (2012).
38. Jarvis, J. N. *et al.* Pulmonary cryptococcosis misdiagnosed as smear-negative pulmonary tuberculosis with fatal consequences. *Int. J. Infect. Dis.* **14** (Suppl. 3), e310–e312 (2010).
39. Sun, H. Y. *et al.* Predictors of immune reconstitution syndrome in organ transplant recipients with cryptococcosis: implications for the management of immunosuppression. *Clin. Infect. Dis.* **60**, 36–44 (2015).
40. Mitchell, D. H. *et al.* Cryptococcal disease of the CNS in immunocompetent hosts: influence of cryptococcal variety on clinical manifestations and outcome. *Clin. Infect. Dis.* **20**, 611–616 (1995).
41. Schoffelen, T. *et al. Cryptococcus gattii* induces a cytokine pattern that is distinct from other cryptococcal species. *PLoS ONE* **8**, e55579 (2013).
42. Lee, S. C., Dickson, D. W. & Casadevall, A. Pathology of cryptococcal meningoencephalitis: analysis of 27 patients with pathogenetic implications. *Hum. Pathol.* **27**, 839–847 (1996).
43. Panackal, A. A. *et al.* Paradoxical immune responses in non-HIV cryptococcal meningitis. *PLoS Pathog.* **11**, e1004884 (2015).
44. Lee, S. C., Casadevall, A. & Dickson, D. W. Immunohistochemical localization of capsular polysaccharide antigen in the central nervous system cells in cryptococcal meningoencephalitis. *Am. J. Pathol.* **148**, 1267–1274 (1996).
45. Loyse, A. *et al.* Neurological, visual, and MRI brain scan findings in 87 South African patients with HIV-associated cryptococcal meningoencephalitis. *J. Infect.* **70**, 668–675 (2015).
46. Charlier, C. *et al.* Cryptococcal neuroradiological lesions correlate with severity during cryptococcal meningoencephalitis in HIV-positive patients in the HAART era. *PLoS ONE* **3**, e1950 (2008).
47. Rothe, C. *et al.* A prospective longitudinal study of the clinical outcomes from cryptococcal meningitis following treatment induction with 800 mg oral fluconazole in Blantyre, Malawi. *PLoS ONE* **8**, e67311 (2013).
48. Gaskell, K. M. *et al.* A prospective study of mortality from cryptococcal meningitis following treatment induction with 1200 mg oral fluconazole in Blantyre, Malawi. *PLoS ONE* **9**, e110285 (2014).
49. Longley, N. *et al.* Dose response effect of high-dose fluconazole for HIV-associated cryptococcal meningitis in southwestern Uganda. *Clin. Infect. Dis.* **47**, 1556–1561 (2008).
50. Loyse, A. *et al.* Comparison of the early fungicidal activity of high-dose fluconazole, voriconazole, and flucytosine as second-line drugs given in combination with amphotericin B for the treatment of HIV-associated cryptococcal meningitis. *Clin. Infect. Dis.* **54**, 121–128 (2012).
51. Bicanic, T. *et al.* High-dose amphotericin B with flucytosine for the treatment of cryptococcal meningitis in HIV-infected patients: a randomized trial. *Clin. Infect. Dis.* **47**, 123–130 (2008).
52. Jarvis, J. N. *et al.* Adjunctive interferon-γ immunotherapy for the treatment of HIV-associated cryptococcal meningitis: a randomized controlled trial. *AIDS* **26**, 1105–1113 (2012).
53. Dromer, F. *et al.* Determinants of disease presentation and outcome during cryptococcosis: the CryptoA/D study. *PLoS Med.* **4**, e21 (2007).
54. Lortholary, O. *et al.* Long-term outcome of AIDS-associated cryptococcosis in the era of combination antiretroviral therapy. *AIDS* **20**, 2183–2191 (2006).
55. Robinson, P. A. *et al.* Early mycological treatment failure in AIDS-associated cryptococcal meningitis. *Clin. Infect. Dis.* **28**, 82–92 (1999).
56. Brizendine, K. D., Baddley, J. W. & Pappas, J. W. Predictors of mortality and differences in clinical features among patients with Cryptococcosis according to immune status. *PLoS ONE* **8**, e60431 (2013).
57. Panackal, A. A. *et al.* Fighting the monster: applying the host damage framework to human central nervous system infections. *mBio* **7**, e01906-15 (2016).
58. Chen, S. C. *et al.* Clinical manifestations of *Cryptococcus gattii* infection: determinants of neurological sequelae and death. *Clin. Infect. Dis.* **55**, 789–798 (2012).
59. Steele, K. T. *et al.* In-hospital mortality of HIV-infected cryptococcal meningitis patients with *C. gattii* and *C. neoformans* infection in Gaborone, Botswana. *Med. Mycol.* **48**, 1112–1115 (2010).
60. 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 (2014).
61. 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 (2009).
62. Diamond, R. D. & Bennett, J. E. Prognostic factors in cryptococcal meningitis: a study in 111 cases. *Ann. Intern. Med.* **80**, 176–181 (1974).
63. Dismukes, W. E. *et al.* Treatment of cryptococcal meningitis with combination amphotericin B and flucytosine for four as compared with six weeks. *N. Engl. J. Med.* **317**, 334–341 (1987).
64. 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 (2013).
65. Jarvis, J. N. *et al.* Cerebrospinal fluid cytokine profiles predict risk of early mortality and immune reconstitution inflammatory syndrome in HIV-associated cryptococcal meningitis. *PLoS Pathog.* **11**, e1004754 (2015).
66. Siddiqui, A. A. *et al.* IFN-γ at the site of infection determines rate of clearance of infection in cryptococcal meningitis. *J. Immunol.* **174**, 1746–1750 (2005).
67. Scriven, J. E. *et al.* A glucuronoxylomannan-associated immune signature, characterized by monocyte deactivation and an increased interleukin 10 level, is a predictor of death in cryptococcal meningitis. *J. Infect. Dis.* **213**, 1725–1734 (2016).
68. Peeling, R. W. *et al.* Rapid tests for sexually transmitted infections (STIs): the way forward. *Sex. Transm. Infect.* **82** (Suppl. 5). v1–v6 (2006).
69. Jarvis, J. N. *et al.* Evaluation of a novel point-of-care cryptococcal antigen test on serum, plasma, and urine from patients with HIV-associated cryptococcal meningitis. *Clin. Infect. Dis.* **53**, 1019–1023 (2011).
70. 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. *Clin. Vaccine Immunol.* **18**, 1292–1296 (2011).
71. Williams, D. A. *et al.* Evaluation of fingerstick cryptococcal antigen lateral flow assay in HIV-infected persons: a diagnostic accuracy study. *Clin. Infect. Dis.* **61**, 464–467 (2015).
72. Tenforde, M. W. *et al.* Poor specificity of urinary cryptococcal antigen testing: Reply to Drain *et al.* Prevalence of cryptococcal antigenuria at initial HIV diagnosis in KwaZulu-Natal. *HIV Med.* http://dx.doi.org/10.1111/hiv.12319 (2015).
73. Longley, N. *et al.* Cryptococcal antigen screening in patients initiating ART in South Africa: a prospective cohort study. *Clin. Infect. Dis.* **62**, 581–587 (2016).
74. Berlin, L. & Pincus, J. H. Cryptococcal meningitis. False-negative antigen test results and cultures in nonimmunosuppressed patients. *Arch. Neurol.* **46**, 1312–1316 (1989).
75. Jitmuang, A. *et al.* Performance of the cryptococcal antigen lateral flow assay in non-HIV-related cryptococcosis. *J. Clin. Microbiol.* **54**, 460–463 (2016).
76. Tintelnot, K. *et al.* Pitfalls in serological diagnosis of *Cryptococcus gattii* infections. *Med. Mycol.* **53**, 874–879 (2015).
77. World Health Organization. Rapid Advice: Diagnosis, Prevention and Management of Cryptococcal Disease in HIV-Infected Adults, Adolescents and Children. http://apps.who.int/iris/bitstream/10665/44786/1/9789241502979_eng.pdf (2011)
78. 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 (2010).
79. Govender, N. P. *et al.* Guideline for the prevention, diagnosis and management of cryptococcal meningitis among HIV-infected persons: 2013 update. *South Afr. J. HIV Med.* **14**, 76–86 (2013).
80. van der Horst, C. M. *et al.* Treatment of cryptococcal meningitis associated with the acquired immunodeficiency syndrome. *N. Engl. J. Med.* **337**, 15–21 (1997).
81. Brouwer, A. E. *et al.* Combination antifungal therapies for HIV-associated cryptococcal meningitis: a randomised trial. *Lancet* **363**, 1764–1767 (2004).
82. Day, J. N. *et al.* Combination antifungal therapy for cryptococcal meningitis. *N. Engl. J. Med.* **368**, 1291–1302 (2013).
83. Loyse, A. *et al.* Flucytosine and cryptococcosis: time to urgently address the worldwide accessibility of a 50-year-old antifungal. *J. Antimicrob. Chemother.* **68**, 2435–2444 (2013).

84. 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 (2015).
85. Brouwer, A. E. *et al.* Oral versus intravenous flucytosine in patients with human immunodeficiency virus-associated cryptococcal meningitis. *Antimicrob. Agents Chemother.* **51**, 1038–1042 (2007).
86. Girmenia, C. *et al.* Effects of hydration with salt repletion on renal toxicity of conventional amphotericin B empirical therapy: a prospective study in patients with hematological malignancies. *Support. Care Cancer* **13**, 987–992 (2005).
87. Thakur, C. P. *et al.* Improving outcome of treatment of kala-azar by supplementation of amphotericin B with physiologic saline and potassium chloride. *Am. J. Trop. Med. Hyg.* **83**, 1040–1043 (2010).
88. Hamill, R. J. *et al.* Comparison of 2 doses of liposomal amphotericin B and conventional amphotericin B deoxycholate for treatment of AIDS-associated acute cryptococcal meningitis: a randomized, double-blind clinical trial of efficacy and safety. *Clin. Infect. Dis.* **51**, 225–232 (2010).
89. Molefi, M. *et al.* AMBITION-*cm*: intermittent high dose AmBisome on a high dose fluconazole backbone for cryptococcal meningitis induction therapy in sub-Saharan Africa: study protocol for a randomized controlled trial. *Trials* **16**, 276 (2015).
90. Nussbaum, J. C. *et al.* Combination flucytosine and high-dose fluconazole compared with fluconazole monotherapy for the treatment of cryptococcal meningitis: a randomized trial in Malawi. *Clin. Infect. Dis.* **50**, 338–344 (2009).
91. Jackson, A. *et al.* A phase II randomised controlled trial adding oral flucytosine to high dose fluconazole, with short-course amphotericin B, for cryptococcal meningitis in Malawi. *AIDS* **26**, 1363–1370 (2012).
92. Muzoora, C. K. *et al.* Short course amphotericin B with high dose fluconazole for HIV-associated cryptococcal meningitis. *J. Infect.* **64**, 76–81 (2011).
93. Livermore, J. *et al.* Efficacy of an abbreviated induction regimen of amphotericin B deoxycholate for cryptococcal meningoencephalitis: 3 days of therapy is equivalent to 14 days. *mBio* **5**, e00725-13 (2013).
94. ISRCTN registry. ISRCTN.com [online], http://www.isrctn.com/ISRCTN45035509 (2015).
95. US National Library of Medicine. ClinicalTrials.gov, https://clinicaltrials.gov/ct2/show/NCT01802385 (2016).
96. Butts, A. *et al.* Estrogen receptor antagonists are anti-cryptococcal agents that directly bind EF hand proteins and synergize with fluconazole in vivo. *mBio* **5**, e00765-13 (2014).
97. Chen, S. C. *et al.* Antifungal therapy and management of complications of cryptococcosis due to *Cryptococcus gattii*. *Clin. Infect. Dis.* **57**, 543–551 (2013).
98. Singh, N. How I treat cryptococcosis in organ transplant recipients. *Transplantation* **93**, 17–21 (2012).
99. Kontoyiannis, D. P. *et al.* Calcineurin inhibitor agents interact synergistically with antifungal agents *in vitro* against *Cryptococcus neoformans* isolates: correlation with outcome in solid organ transplant recipients with cryptococcosis. *Antimicrob. Agents Chemother.* **52**, 735–738 (2008).
100. Graybill, J. R. *et al.* Diagnosis and management of increased intracranial pressure in patients with AIDS and cryptococcal meningitis. *Clin. Infect. Dis.* **30**, 47–54 (2000).
101. Loyse, A. *et al.* Histopathology of the arachnoid granulations and brain in HIV-associated cryptococcal meningitis: correlation with cerebrospinal fluid pressure. *AIDS* **24**, 405–410 (2010).
102. 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 (2009).
103. Shoham, S. *et al. Cryptococcus neoformans* meningitis at 2 hospitals in Washington, D. C.: adherence of health care providers to published practice guidelines for the management of cryptococcal disease. *Clin. Infect. Dis.* **40**, 477–479 (2005).
104. Rolfes, M. A. *et al.* The effect of therapeutic lumbar punctures on acute mortality from cryptococcal meningitis. *Clin. Infect. Dis.* **59**, 1607–1614 (2014).
105. Macsween, K. F. *et al.* Lumbar drainage for control of raised cerebrospinal fluid pressure in cryptococcal meningitis: case report and review. *J. Infect.* **51**, e221–e224 (2005).
106. Manosuthi, W. *et al.* Temporary external lumbar drainage for reducing elevated intracranial pressure in HIV-infected patients with cryptococcal meningitis. *Int. J. STD AIDS* **19**, 268–271 (2008).
107. Park, M. K., Hospenthal, D. R. & Bennett, J. E. Treatment of hydrocephalus secondary to cryptococcal meningitis by use of shunting. *Clin. Infect. Dis.* **28**, 629–633 (1999).
108. Haddow, L. J. *et al.* Cryptococcal immune reconstitution inflammatory syndrome in HIV-1-infected individuals: proposed clinical case definitions. *Lancet Infect. Dis.* **10**, 791–802 (2010).
109. Bicanic, T. *et al.* Immune reconstitution inflammatory syndrome in HIV-associated cryptococcal meningitis: a prospective study. *J. Acquir. Immune Defic. Syndr.* **51**, 130–134 (2009).
110. 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 (2010).
111. Muller, M. *et al.* Immune reconstitution inflammatory syndrome in patients starting antiretroviral therapy for HIV infection: a systematic review and meta-analysis. *Lancet Infect. Dis.* **10**, 251–261 (2010).
112. Longley, N., Harrison, T. S. & Jarvis, J. N. Cryptococcal immune reconstitution inflammatory syndrome. *Curr. Opin. Infect. Dis.* **26**, 26–34 (2013).
113. 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 (2010).
114. Chang, C. C. *et al.* Cryptococcosis-IRIS is associated with lower *cryptococcus*-specific IFN-γ responses before antiretroviral therapy but not higher T-cell responses during therapy. *J. Infect. Dis.* **208**, 898–906 (2013).
115. Chang, C. C. *et al.* Chemokine levels and chemokine receptor expression in the blood and the cerebrospinal fluid of HIV-infected patients with cryptococcal meningitis and cryptococcosis-associated immune reconstitution inflammatory syndrome. *J. Infect. Dis.* **208**, 1604–1612 (2013).
116. Worsley, C. M. *et al.* Multi-analyte profiling of ten cytokines in South African HIV-infected patients with immune reconstitution inflammatory syndrome (IRIS). *AIDS Res. Ther.* **7**, 36 (2010).
117. 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 (2015).
118. 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 (2010).
119. Boulware, D. R. *et al.* Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis. *N. Engl. J. Med.* **370**, 2487–2498 (2014).
120. 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 (2009).
121. 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 (2015).
122. Scemla, A. *et al.* Dramatic improvement of severe cryptococcosis-induced immune reconstitution syndrome with adalimumab in a renal transplant recipient. *Am. J. Transplant.* **15**, 560–564 (2015).
123. Brunel, A. S. *et al.* Thalidomide for steroid-dependent immune reconstitution inflammatory syndromes during AIDS. *AIDS* **26**, 2110–2112 (2012).
124. Jarvis, J. N., Meintjes, G. & Harrison, T. S. Outcomes of cryptococcal meningitis in antiretroviral naive and experienced patients in South Africa. *J. Infect.* **60**, 496–498 (2010).
125. Hardison, S. E. *et al.* Pulmonary infection with an interferon-gamma-producing *Cryptococcus neoformans* strain results in classical macrophage activation and protection. *Am. J. Pathol.* **176**, 774–785 (2010).
126. Phillips, P. *et al.* Dexamethasone in *Cryptococcus gattii* central nervous system infection. *Clin. Infect. Dis.* **49**, 591–595 (2009).
127. Casadevall, A. & Pirofski, L. A. The damage-response framework of microbial pathogenesis. *Nat. Rev. Microbiol.* **1**, 17–24 (2003).
128. Tazawa, R. *et al.* Inhaled granulocyte/macrophage-colony stimulating factor as therapy for pulmonary alveolar proteinosis. *Am. J. Respir. Crit. Care Med.* **181**, 1345–1354 (2010).
129. Tazawa, R. *et al.* Duration of benefit in patients with autoimmune pulmonary alveolar proteinosis after inhaled granulocyte-macrophage colony-stimulating factor therapy. *Chest* **145**, 729–737 (2014).
130. Pappas, P. G. *et al.* Recombinant interferon-γ1b as adjunctive therapy for AIDS-related acute cryptococcal meningitis. *J. Infect. Dis.* **189**, 2185–2191 (2004).
131. 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 (2009).
132. 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 (2013).
133. 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 (2010).
134. Govender, N. P. *et al.* Phased implementation of screening for cryptococcal disease in South Africa. *S. Afr. Med. J.* **102**, 914–917 (2012).
135. 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 (2015).
136. Morawski, B. M. *et al.* Pre-ART cryptococcal antigen titer associated with preemptive fluconazole failure. http://www.croiconference.org/sessions/pre-art-cryptococcal-antigen-titer-associated-preemptive-fluconazole-failure [online] Abstract presented at: CROI; February 2016; Boston.
137. Loyse, A. *et al.* Cryptococcal meningitis: improving access to essential antifungal medicines in resource-poor countries. *Lancet Infect. Dis.* **13**, 629–637 (2013).
138. Kuris, A. M., Lafferty, K. D. & Sokolow, S. H. Sapronosis: a distinctive type of infectious agent. *Trends Parasitol.* **30**, 386–393 (2014).
139. Casadevall, A. Evolution of intracellular pathogens. *Annu. Rev. Microbiol.* **62**, 19–33 (2008).
140. McDonald, T., Wiesner, D. L. & Nielsen, K. *Cryptococcus*. *Curr. Biol.* **22**, R554–R555 (2012).
141. Casadevall, A., Steenbergen, J. N. & Nosanchuk, J. D. 'Ready made' virulence and 'dual use' virulence factors in pathogenic environmental fungi — the *Cryptococcus neoformans* paradigm. *Curr. Opin. Microbiol.* **6**, 332–337 (2003).
142. Zaragoza, O. *et al.* The capsule of the fungal pathogen *Cryptococcus neoformans*. *Adv. Appl. Microbiol.* **68**, 133–216 (2009).
143. Xu, J. Fundamentals of fungal molecular population genetic analyses. *Curr. Issues Mol. Biol.* **8**, 75–89 (2006).
144. Hagen, F. *et al.* Recognition of seven species in the *Cryptococcus gattii*/*Cryptococcus neoformans* species complex. *Fungal Genet. Biol.* **78**, 16–48 (2015).
145. Farrer, R. A. *et al.* Genome evolution and innovation across the four major lineages of *Cryptococcus gattii*. *mBio* **6**, e00868-15 (2015).
146. Engelthaler, D. M. *et al. Cryptococcus gattii* in North American Pacific Northwest: whole-population genome analysis provides insights into species evolution and dispersal. *mBio* **5**, e01464-14 (2014).

Author contributions

P.R.W., J.N.J., A.A.P., M.C.F. and T.S.H. wrote the article. All authors participated in researching data for article, provided substantial contribution to discussion of content, and reviewed and edited the manuscript before submission.

Competing interests statement

P.W. has a CRADA (cooperative research and development agreement) with Matinas BioPharma regarding an oral amphotericin formulation. J.J. has received an Investigator Award (to institution) from Gilead Sciences. T.H. has received an Investigator Award (to institution) from Gilead Sciences and has received a donation of cryptococcosis test kits for research purposes from Immuno-Mycologics, received honoraria from Pfizer, and is on the advisory board for Viamet.

FURTHER INFORMATION

A semi-quantitative, lateral flow test in development by Biosynex: www.biosynex.com/fr/test-cryptops/ [French]
IMMY (manufacturer of diagnostic tools for fungal diseases): www.immy.com
Viamet 1129: http://www.viamet.com/pipeline/vt-1129.php