HIV-associated cryptococcal meningitis

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Introduction

While the incidence of cryptococccal meningitis in the developed world has declined with widespread, early antiretroviral therapy (ART), cryptococcal disease remains a major opportunistic infection and leading cause of mortality in patients infected with HIV in much of the developing world. Most HIV-related cases are caused by *Cryptococcus neoformans* var. *grubii* (serotype A), while var. *neoformans* (serotype D) is responsible for a proportion, especially in Europe, and there are a small number of *Cryptococcus gatti* infections (formerly *C. neoformans* serotypes B and C) [1,2]. The last includes a small number of cases in HIV-infected individuals forming part of an unprecedented outbreak of *C. gattii* infections, predominantly in apparently immunocompetent patients, on Vancouver Island, Canada [3,4].

C. neoformans is distributed worldwide. An ubiquitous environmental saphrophyte, it is found in soil contaminated with pigeon droppings and has also been isolated from the heartwood of several tree species in South America [5] and India [6], and from the homes of African HIV-seropositive patients [7,8]. Exposure may be common [9], although the exact circumstances are usually unclear. Inhalation of small, thinly encapsulated yeasts, or basidiospores [10], may lead to an initial pulmonary infection, which, depending on host immune response and the number and virulence of the organisms, is cleared, contained within granulomata as a latent infection or disseminates. The minority in whom disease disseminates typically have defects in T cell function, through malignancy, immunosuppressive medication, autoimmune disease or sarcoidosis [11,12] or HIV infection, indicating the role of T cell-mediated immunity in host defence.

In HIV-seropositive patients, most episodes of cryptococcal meningitis probably represent reactivation of latent infection, which may have been acquired many years earlier. There is compelling evidence for latent infection in a rat model [13] and humans [14]. Dromer and colleagues [15] typed *C. neoformans* isolates from HIV-seropositive patients diagnosed with cryptococcosis in France, some of whom were from Africa but had lived in France for a median of over 9 years. There was a significant clustering of isolates from African compared with European patients, suggesting that the patients had acquired their isolates long before the development of clinical disease. A proportion of HIV-related cases, however, may result from dissemination of new or primary infection [16], as has been observed in the recent outbreak of *C. gattii* infection in British Columbia [17].

HIV-associated cryptococcal meningitis usually presents as a subacute meningo-encephalitis in profoundly immunosuppressed patients (CD4 cell counts < 100 cells/μl), with malaise, headache, fever and, later, visual disturbance and altered mental status. Signs, if present, may include meningism, papilloedema, cranial nerve palsies [particularly sixth nerve palsies reflective of raised pressure in cerebrospinal fluid (CSF)] and reduced conscious level. The diagnosis is usually straightforward. The high organism load in this setting means the sensitivity of India ink staining of CSF is high. Those who have a negative

result with India ink can be diagnosed by highly sensitive and specific cryptococcal antigen testing of CSF, or serum if CSF cannot be obtained [18]. Lumbar puncture often reveals markedly elevated opening pressures, an important complicating factor, with only modestly elevated or normal white cell counts (usually lymphocytes), elevated protein and low or normal glucose.

High organism burden at baseline (indicated by quantitative CSF culture or CSF antigen titre) and abnormal mental status are the most important predictors of death [19,20], while high opening pressures and a poor inflammatory response in the CSF have also been associated with poor outcome [19,21,22]. Autopsy series reveal the lack of a protective granulomatous response in HIV-seropositive patients with cryptococcal meningitis. There is extensive involvement of brain parenchyma in addition to meningitis and higher organism burdens (which are predominantly extracellular) compared with the infection in HIV-seronegative individuals [23–25].

The remainder of this review will focus on the current epidemiology and management of HIV-associated cryptococcal meningitis, with some emphasis on the developing world where the burden of disease is highest. In addition to antifungal therapy, the important complications of elevated CSF pressure and immune reconstitution inflammatory syndrome (IRIS) and future approaches to prevention and therapy are discussed.

Epidemiology

Increasing numbers of cases of cryptococcal meningitis were reported in young adults in the former Zaire throughout the 1960s, possibly representing the first signs of the evolving HIV epidemic [26,27]. The late 1970s and early 1980s saw a sharp increase in the numbers of cases both in Kinshasa [28,29] and in Zairian immigrants to Europe [30–32], many of whom had, in retrospect, features suggestive of AIDS [33].

As the HIV epidemic expanded in the 1980s, *C. neoformans* emerged as an important opportunistic infection in the United States, Europe and Australia, occurring in 5–10% of all AIDS patients [34–38]. Rates of infection declined through the 1990s, initially with the widespread and frequent use of azoles to treat candidiasis [38,39], and subsequently with the introduction of HAART [40,41]. The annual incidence in AIDS patients in Atlanta fell from 66 per 1000 in 1992 to 7 per 1000 in 2000 [41].

HIV-related cryptococcal meningitis is now a problem in the West in patients who present with late-stage HIV infection, typically those with limited access to healthcare [40,41]. However, it remains a major opportunistic infection in the developing world in areas of high HIV

seroprevalence [42,43]. *C. neoformans* is the leading cause of meningitis in central and southern Africa, accounting for 26.5% of cases in a series from Malawi [44], 31% in a series from the Central African Republic [45] and 45% from Zimbabwe [46]. In these areas, it is one of the main causes of mortality in cohorts of HIV-infected individuals, responsible for 13–44% of all deaths [47–49]. For comparison, 5–13% of deaths were attributed to tuberculosis in these studies. In Thailand, cryptococcosis accounts for up to 20% of AIDS-defining illnesses [43,50] and it is reported as a major opportunistic infection in India [51] and Brazil [52]. There are interesting geographical variations in incidence that presumably relate to differential rates of exposure. For example, cryptococcal disease appears to be more common in southern and east Africa than in west Africa [53], and in north and northeast Thailand compared with southern Thailand [43].

Even with current optimal treatment, the 10-week mortality of HIV-associated cryptococcal meningitis is high, ranging from 10 to 25% in developed countries, with no evidence of any decrease in recent years [54]. Of note, mortality is higher in less selected series [55] compared with clinical trials in which very sick patients are excluded [21]. In unselected series from resource-poor settings, acute mortality is up to 43% even with amphotericin B therapy [56]. In Zambia, median survival with low-dose fluconazole monotherapy was 19 days [57], barely better than that in the absence of antifungal therapy [57–59]. In South Africa, in a recent unselected prospective series [60], overall 10-week mortality was 37% despite initial treatment with amphotericin B for most patients and access to ART (Fig. 1).

However, this last study also confirmed that, once over the acute cryptococcal infection and established on ART, the long-term outlook is good, as in the developed country setting, with a levelling of the survival curve. Therefore, in the setting of expanding access to ART
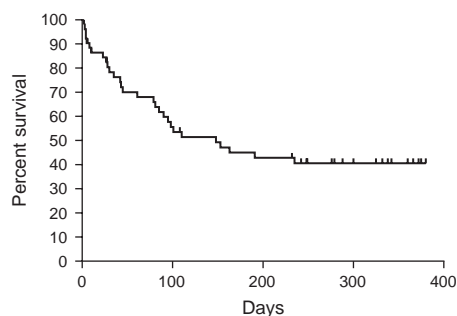

Fig. 1. Prospective observational study of survival of 54 patients with HIV-associated cryptococcal meningitis treated according local protocol (49 with amphotericin B 1 mg/kg daily, 5 with fluconazole) in Cape Town South Africa. Patients not already taking antiretroviral therapy at the time of presentation were started on therapy from 4 weeks after antifungal therapy. (From data in Bicanic *et al.* [60].)

across the developing world, the urgent challenge is to improve acute management and thereby increase the proportion of patients surviving the critical initial months.

Antifungal therapy

Current antifungal treatment guidelines (Table 1; [61,62]) are based in large part on the results of a large, randomized trial published a decade ago [21]. Initial therapy was with amphotericin B (0.7 mg/kg daily) with or without flucytosine (100 mg/kg daily) for 2 weeks, followed by an 8-week consolidation phase with either fluconazole (400 mg daily) or itraconazole (400 mg daily). The rationale was to gain control of infection with initial more rapidly active amphotericin B-based therapy but switch to well-tolerated azoles for consolidation treatment to minimize the dose-dependent toxicity of amphotericin B. The mortality was the lowest of any published trial, at 9.4% in the first 10 weeks. The addition of flucytosine was associated with a trend towards a higher proportion of patients with sterile CSF at 2 weeks and reduced relapse. Fluconazole was superior to itraconazole for consolidation treatment [21]. That the combination of amphotericin B plus flucytosine is more rapidly fungicidal than amphotericin B alone has been demonstrated in a subsequent study in Thailand using serial quantitative cultures to assess the rate of clearance of cryptococcal colony-forming units from the CSF or early fungicidal activity. The clearance of cryptococci from CSF was significantly faster with amphotericin B plus flucytosine than with amphotericin B alone, amphotericin B plus fluconazole (at 400 mg daily) or a combination of all three (Fig. 2 [20]).

Both these studies also demonstrated that, with appropriate monitoring, conventional amphotericin B is reasonably well tolerated, with drug discontinuations in 3% of patients in the first 2 weeks in the Mycoses Study Group trial [21]. Saline and fluid loading equivalent to 1 litre normal saline

daily should be given unless contraindicated, to minimize nephrotoxicity [63], and electrolytes replaced as required. Anaemia, secondary to suppression of erythropoietin transcription [64], is also a predictable side effect of amphotericin B [65–67]. This may be more clinically significant in populations with lower baseline haemoglobin levels, and where transfusion, when occasionally needed, is difficult.

Flucytosine, at the historically low daily dose of 100 mg/kg, was also well tolerated without real-time drug level monitoring in either trial. A substudy of the Thai trial comparing oral and intravenous flucytosine at the same daily dosage of 100 mg/kg has provided some insight into this observation. In contrast to earlier studies in other patient populations, oral bioavailability of flucytosine in these patients at a late stage of HIV infection was only around 50%, resulting in relatively low serum concentration, of an order not usually associated with toxicity. Nevertheless, despite the lower serum levels, patients on oral formulation had the same rate of clearance of infection as those on intravenous formulation [68], consistent with evidence for the dose-independent activity of flucytosine [69–71]. The data suggest that even 100 mg/kg daily, if given intravenously, may be in excess of that required for maximal additional fungicidal activity.

If renal impairment does develop, liposomal amphotericin B, at 3 mg/kg daily, provides a less nephrotoxic and equally effective alternative. A small study suggested liposomal amphotericin B, at 4 mg/kg daily, was more active than conventional amphotericin B [72], but a larger study found no difference in the proportion of patients with sterile CSF at 2 weeks in patients receiving daily liposomal amphotericn B at 3 or 6 mg/kg compared with conventional amphotericin B at 0.7 mg/kg daily [73].

Unfortunately, in many resource-poor settings, amphotericin B is not available or cannot be used safely because of lack of monitoring, and fluconazole, widely available, through a free access programme or in generic form, is the only treatment option. Outcomes with initial

Table 1. Antifungal treatment recommendations for HIV-associated cryptococcal meningitis[a].

	Preferred daily regimen [61,62]	Notes
First 2 weeks	Amphotericin B (AmB) 0.7–1 mg/kg *plus* flucytosine 100 mg/kg[b]	If flucytosine not tolerated or not available, consider AmB at 1 mg/kg daily
		If AmB not tolerated, consider liposomal AmB at 3mg/kg daily if available; if not, switch early to fluconazole at 800 mg daily
Next 8 weeks	Fluconazole 400 mg[c]	If no facility for i.v. therapy, consider fluconazole at ≥ 800 mg daily
Thereafter, until immune-reconstitution (see text)	Fluconazole 200 mg	

Am B, amphotericin B; i.v., intravenous.
[a]Adapted from Bicanic, 2005 (Bicanic T, Harrison TS. Cryptococcal meningitis. *Br Med Bull* 2005; **72**: 99–118).
[b]Adjust for renal impairment; oral or i.v. formulation; if i.v. formulation, consider 75 mg/kg daily [68].
[c]Adjust for renal impairment; increase dose by 50% if concomitant rifampicin.
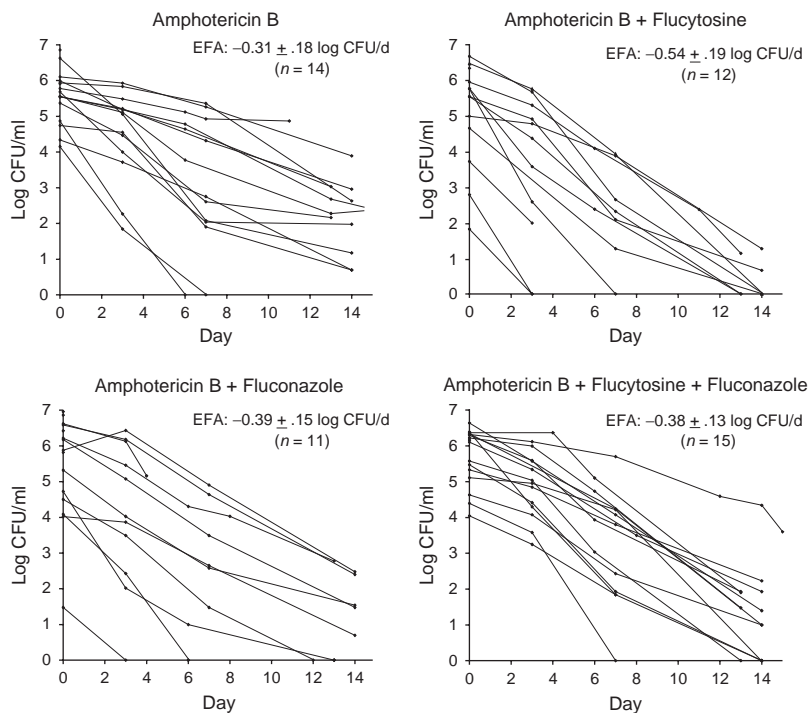
Fig. 2. Fall in *Cryptococcus neoformans* colony-forming units (CFU) in cerebrospinal fluid (CSF) over time by treatment group. The decrease in log CFU/ml CSF per day was calculated for each patient using the slope of the linear regression of log CFU against time. For each treatment group, early fungicidal activity (EFA) is shown as the mean ($\pm$SD) rate of fall in log CFU. EFA was significantly greater for amphotericin B (AmB) plus flucytosine compared with AmB alone ($P < 0.001$), AmB plus fluconazole ($P = 0.02$), or triple therapy with AmB, flucytosine and fluconazole ($P = 0.02$). (Adapted from Brouwer *et al.* [20], with permission).

fluconazole monotherapy at 200–400 mg daily have not been good, either in early US-based studies [19,74], including a small randomized study in which 400 mg daily was clinically inferior to amphotericin B plus flucytosine [74], or in more recent series from Africa [57,75,76]. Although the earlier randomized study comparing amphotericin B with fluconazole found no significant difference in clinical outcomes, time to sterilization was very long for fluconazole (median 64 days), and outcomes for both drugs were poor [19]. Furthermore, the dosages used for both drugs were lower than currently recommended, making interpretation difficult. The 10-week mortality of approximately 50% with initial fluconazole monotherapy reported by Schaars and colleagues [75] in South Africa represents a minimum estimate in this setting given the retrospective nature of the study with incomplete out-patient follow up. Recent work from Cape Town has demonstrated that 400 mg fluconazole daily is essentially fungistatic over the first 2 weeks of treatment [60]. The resulting prolonged period with a high viable organism load may predispose to the development of fluconazole resistance. Such resistance is a significant problem when initial therapy is with fluconazole [77]. A further concern is that prolonged active infection could also increase the risk of immune reconstitution reactions (see below) following introduction of ART, although data on this point are lacking.

Animal studies suggest a dose–response relationship with increasing fluconazole levels [78]. There is a linear plasma concentration–dose relationship with fluconazole at up to 2 g daily [79], and doses up to 1600 mg daily have appeared safe in small numbers of patients [79,80]. In addition there is a suggestion of a dose–response relationship in terms of the time to sterilization of CSF: with a median time to CSF sterilization of 64 days with 200–400 mg daily [19], a mean time of 41 days with 400 mg daily [74], and 21 [81] and 33 days [82] with 800 mg daily. On this basis, and given the unsatisfactory results of treatment at lower doses, a dose-escalation study of fluconazole therapy is currently underway in Uganda. In the meantime, in settings where amphotericin B cannot be used safely and fluconazole is the only option, the authors would suggest a starting dose of at least 800 mg daily (Table 1).

The combination of fluconazole plus flucytosine is additive or synergistic in murine models [83,84], although not in a study in rabbits [78]. A clinical study in Uganda suggested benefit with addition of flucytosine to fluconazole, although the dose of fluconazole was low (200 mg daily) [76]; and in a small series from the United States, the combination of flucytosine and fluconazole at 400 mg daily resulted in a relatively short median time to sterilization of CSF of 23 days, although side effects with the combination appeared frequent [85]. Further comparative trials to examine the fungicidal activity

and toxicity of this combination with higher doses of fluconazole are warranted in settings where intravenous amphotericin B-based therapy is not possible.

While not as effective, at conventional dosages, as amphotericin B for initial therapy, fluconazole is highly effective and safe as maintenance therapy [86−88]. Increasing evidence suggests discontinuation of this secondary prophylaxis is safe if there has been a significant and sustained immune reconstitution with ART (CD4 cell count > 200 cells/μl for > 6 months) [89−91].

Raised cerebrospinal fluid opening pressure

Significantly raised intracranial pressure is a major problem in cryptococcal meningitis, with over half of patients having pressures > 25 cmH$_2$O and nearly a third pressures > 35 cmH$_2$O in an analysis of the last Mycosis Study Group Trial [22]. Raised CSF pressure was associated with cognitive impairment, more cranial nerve lesions and increased short-term mortality. Prolonged raised CSF pressure usually manifests as severe headache, papilloedema, and progressive loss of vision, hearing impairment and decreased level of consciousness [92]. The mechanisms leading to increased pressure are debated [22,93,94]. Although a marked inflammatory response is not a feature of HIV-associated cryptococcal meningitis, it is possible cerebral oedema plays a role in some patients. Vascular endothelial growth factor, a mediator of vascular permeability, has been measured in the CSF of patients with cryptococcal disease [95,96], although no correlation between levels of this growth factor and CSF opening pressure has been demonstrated. Instead, the primary deficit is likely to be blockage of CSF reabsorption at the arachnoid villi because of the presence of organisms and shed polysaccharide [23−25]. This would be consistent with the association of raised pressure with higher CSF antigen titre and higher rates of India ink positivity [22], and it would explain why ventricular size usually remains normal, as there is no pressure gradient between the ventricles and the CSF over the convexities of the brain.

In terms of management, few controlled trials have been carried out, so recommendations are based on small series and expert opinion. Current US guidelines suggest daily lumbar punctures for all patients with elevated baseline opening pressures (> 25 cmH$_2$O), with the removal of sufficient CSF to reduce pressures by 50%, continued until pressure has been normal for several days [61]. The maximum volume of CSF that is safe to remove at a single lumbar puncture is unclear but 20−30 ml is probably reasonable [97]. If facilities allow, computed tomographic or magnetic resonance scanning of the head should be done prior to initial lumbar puncture or if suspected raised CSF pressure develops on therapy in order to exclude rare cases

of true hydrocephalus and space-occupying lesions. In the rare cases in which hydrocephalus develops, a ventriculoperitoneal shunt should be inserted [98]. When repeated lumbar punctures fail to control pressure and the patient's condition is deteriorating, CSF drainage can be achieved with a ventricular or, less invasive, lumbar drain [99,100]. It is unclear how long the defect in CSF reabsorption persists, but it may be that a significant proportion of patients will respond to relatively short-term drainage with a temporary system [99]. These allow the continual, controlled drainage of high volumes of fluid (approximately 200 ml daily) to a set pressure level, are relatively straightforward to insert, and have a low risk of complications with adequate monitoring and nursing and medical staff who are familiar with their use [101,102].

The use of mannitol, acetazolamide, and corticosteroids for raised CSF pressure is not supported by available evidence [61]. A randomized trial of acetazolamide was terminated early [103]; and high-dose steroids were associated with higher mortality in patients with elevated CSF pressures in a large, although uncontrolled, study [22].

Cryptococcal meningitis immune reconstitution syndrome

As has been described with other opportunistic infections [104−106], initiation of ART can lead to restoration of immune responses to viable or killed organisms or shed antigen; this, in turn, can lead to a paradoxical clinical deterioration; with the uncovering of previously subclinical cryptococcal disease or relapse of previously treated infection. Cryptococcal IRIS has been reported in 6−30% of patients with cryptococcal meningitis following commencement of ART [107,108] and may be fatal [108,109]. In the US study, the median time to onset of symptoms after starting ART was 30 days [107]; however, cases have been reported after many months [108]. Presentations include mediastinal lymphadenitis [110], abscesses [111,112] and cavitatory pneumonia [113], with the commonest being recurrence of meningitis [107−109]. Cases of IRIS have been characterized by a higher CSF white cell count and also higher opening pressures [107,114−116]. Risk factors for cryptococcal IRIS include higher cryptococcal antigen titres at baseline, fungaemia or widely disseminated disease [107,108] and ART within 1 month of antifungal therapy [107,108]. The diagnosis of cryptococcal IRIS is partly one of exclusion, the following factors supporting this diagnosis:

- temporal association between starting ART and clinical presentation
- evidence of immune restoration (rise in peripheral CD4 cell count)
- exclusion of alternative explanations (e.g., noncompliance or resistance to fluconazole, a second possible diagnosis)

- clinical features (i.e., new or increased lymphadenopathy), cytology (i.e., CSF white cell count) or histopathology consistent with an increased cell-mediated immune response
- negative cryptococcal cultures (restricted definition).

The role of IRIS is less in doubt when cryptococcal cultures are negative, but it is likely that immune reconstitution also contributes to the presentation and re-presentation of some patients who are still culture positive [77].

The occurrence of cryptococcal IRIS has implications for the timing of ART. The apparent increase in the risk of cryptococcal IRIS with earlier initiation of ART has to be balanced against the risk of other HIV-related complications if initiation of ART is delayed. Optimal timing of ART may be earlier in developing countries where rates of death prior to initiation of ART are high [60,117]. Until trials currently underway report, most investigators would start ART from 4 weeks into antifungal therapy, although it is possible earlier ART may be safe if a rapidly fungicidal regimen is used for initial antifungal therapy. Treatment of cryptococcal IRIS is another area requiring further investigation. If clinical progression occurs, despite appropriate antifungal therapy and aggressive management of any raised CSF pressure, short-term steroids, which have been used successfully in case reports [108,118,119], can be considered.

Prevention and screening

The need for primary antifungal prophylaxis is reducing as access to ART expands, the best prophylaxis being rapid immune reconstitution with ART. However in the absence of ART, or in those who fail to respond to treatment, a strong case exists for primary prophylaxis with fluconazole in those with CD4 cell counts < 100 cells/μl in areas with a high incidence of cryptococcal disease [120,121]. Such a policy was introduced in Thailand prior to widespread availability of ART and is under investigation in east Africa. In areas of high incidence, in view of the significant proportion of patients now presenting after starting ART [60], a case can also be made for screening with serum cryptococcal antigen [122] prior to ART in order to diagnose and treat subclinical infection before it is unmasked by immune reconstitution. However, studies are needed, and such a strategy is not justified and is not used in areas of lower incidence [18,123].

Future developments

Reasons for the ongoing high mortality of HIV-related cryptococcal disease include the inadequacy of current antifungal therapy, restricted access to some drugs in many areas [124], the problem of raised CSF pressure and the lack of data on optimal timing of ART. As discussed above, efforts are underway to address some of these questions.

Regarding access to antifungal drugs, fluconazole is widely available through a free access programme and in generic form. In contrast, although generic amphotericin B is also available, the cost is variable and may be significant in very resource-limited settings [124]. In addition, continuous supply has been an issue in some areas, including the United Kingdom, related perhaps to the reduced market for amphotericin B deoxycholate for treating other fungal infections in the developed world. Flucytosine is a simple and old molecule that nevertheless is not widely available either in Africa or Asia, where the burden of cryptococcal disease is so high. Only one manufacturer markets the drug to our knowledge. In countries where they are not marketed, flucytosine tablets can be obtained on a named patient basis from IDIS World Medicines (www.idispharma.com). If studies, currently underway, comparing high-dose fluconazole with flucytosine as a companion drug to give with amphotericin B show that flucytosine remains the second drug of choice, then advocacy is needed to expand access to flucytosine.

The rate of clearance of infection, or early fungicidal activity, from serial quantitative cultures of CSF provides a means by which the activity of new drugs or combinations for antifungal therapy can be accurately assessed in small numbers of patients; this would enable regimens for testing in phase III trials to be selected on a more rational basis [20]. Such clearance studies are underway to examine whether 1 mg/kg amphotericin B daily is associated with a significant increase in fungicidal activity compared with 0.7 mg/kg daily, and to compare flucytosine and high-dose fluconazole as a second drug to give with amphotericin B. Comparative studies are also needed with new azoles with activity against *C. neoformans*, such as voriconazole [125], although interactions with antituberculous and antiretroviral medication are a problem. Minimal inhibitory concentrations, animal model data and penetration into the central nervous system for voriconazole and posaconazole are shown in Table 2. Of note, echinocandins have limited anticryptococcal activity because they target 1–3-β-D-glucan linkages, which are not important in the cryptococcal cell wall [126].

Given the limitations of current antifungal drugs, and uncertainty over further drug development, there is continuing interest in adjunctive immunotherapy. A monoclonal antibody directed against the capsular polysaccharide of *C neoformans* has reached phase I human studies [127]; and a monoclonal antibody fragment, Mycograb, directed against candidal heat shock protein 90, and reported to be beneficial when given with amphotericin B in invasive candidiasis [128], also has in-vitro activity against *C. neoformans* [129]. Clinical trials in cryptococcal meningitis are planned.

Table 2. Data on activity of posaconazole and voriconazole in cryptoccocal infection.

	Posaconazole [134]	Voriconazole
In-vitro minimum concentration giving 90% inhibition (μg/ml)[a] [135–144]	0.25–0.5	0.12–0.25
Activity against fluconazole-resistant strains	Yes [135–140]	Yes [141–144]
Animal models	Equivalent efficacy to fluconazole in rabbit model [140]; active in murine model; at doses tested but appeared inferior to amphotericin B [145]	Good activity in murine model [146], in high doses equivalent efficacy to amphotericin B [147]
Penetration into CSF[b]	Poor; undetectable levels in CSF in animal models, despite therapeutic plasma levels and in-vivo efficacy [140]	Good; high levels demonstrated in CSF in animal models and immunosuppressed patients [148]
Penetration into brain tissue	No data	High levels demonstrated in guinea pig models and immunosuppressed patients [148]
Clinical data	Case series of activity in fungal CNS infections [149,150]; clinical success in 14 of 29 patients (48%)[c] with refractory cryptococcal meningitis [150]	Case series of activity in fungal CNS infections [151,152]; successful response in 7 of 18 (39%) patients[d] with refractory cryptococcal meningitis [125]

CSF, cerebrospinal fluid; CNS, central nervous system.
[a]For comparison, values for itraconazole and fluconazole are 0.5 and 8–16 μg/ml, respectively.
[b]Relevance unclear given the concentration of amphotericin B, a very active drug, is very low in CSF [153,154].
[c]Success required resolution of (seen in 4 patients) or improvement in (10 patients) attributable symptoms, signs and radiographic abnormalities; all successes also had negative CSF culture; of the other 15 patients, 6 classified as stable.
[d]Success required demonstration of reduction in antigen titre; of other 11 patients, 10 classified as stable; >90% patients alive at 90 days.

An alternative approach is use of interferon-γ (IFN-γ). There is direct in-vivo evidence that IFN-γ is important for clearance of cryptococcal infection in HIV-infected patients [130]. A placebo-controlled trial showed that adjuvant IFN-γ therapy was safe and well tolerated, with no detrimental effects on HIV viral load or CD4 cell counts. There was also a trend towards improved mycological outcomes, with 13% of placebo recipients achieving negative cultures at 2 weeks compared with 36% or 32% of those receiving IFN-γ [131]. The trend in favour of IFN-γ was already seen after 2 weeks of treatment, and studies have shown that endogenous IFN-γ in the CSF peaks at day 3 and is virtually undetectable by day 14 [130], suggesting that short courses of adjuvant IFN-γ, which would be more feasible to implement, may be effective.

Finally, studies continue to identify suitable cryptococcal antigens for vaccine development [132] and to address the formidable challenges inherent in the vaccination of immunodeficient hosts, such as those with HIV infection [133].

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