

Target product profile and discovery and development path for novel cryptococcal disease treatments  
TPP for novel cryptococcal disease treatments

David B. Meya, Manu De Rycker, Ian H. Gilbert, William Hope, Justine Jelagat Odionyi, Michael Keegan, Angela Loyse, Pablo Moral-Lopez, Peter R. Williamson, Lionel K. Tan, sabela Ribeiro, Timothy J. Miles



PII: S0163-4453(25)00243-9

DOI: <https://doi.org/10.1016/j.jinf.2025.106643>

Reference: YJINF106643

To appear in: *Journal of Infection*

Received date: 30 May 2025

Revised date: 21 October 2025

Accepted date: 25 October 2025

Please cite this article as: David B. Meya, Manu De Rycker, Ian H. Gilbert, William Hope, Justine Jelagat Odionyi, Michael Keegan, Angela Loyse, Pablo Moral-Lopez, Peter R. Williamson, Lionel K. Tan, sabela Ribeiro and Timothy J. Miles, Target product profile and discovery and development path for novel cryptococcal disease treatmentsTPP for novel cryptococcal disease treatments, *Journal of Infection*, (2025) doi:<https://doi.org/10.1016/j.jinf.2025.106643>

This is a PDF of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability. This version will undergo additional copyediting, typesetting and review before it is published in its final form. As such, this version is no longer the Accepted Manuscript, but it is not yet the definitive Version of Record; we are providing this early version to give early visibility of the article. Please note that Elsevier's sharing policy for the Published Journal Article applies to this version, see: <https://www.elsevier.com/about/policies-and-standards/sharing#4-published-journal-article>. Please also note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

# Target product profile and discovery and development path for novel cryptococcal disease treatments

**Running title:** TPP for novel cryptococcal disease treatments

David B. Meya <sup>a</sup>, Manu De Rycker <sup>b</sup>, Ian H. Gilbert <sup>b</sup>, William Hope <sup>c</sup>, Justine Jelagat Odionyi <sup>d</sup>, Michael Keegan <sup>e</sup>, Angela Loyse <sup>f</sup>, Pablo Moral-Lopez <sup>g</sup>, Peter R. Williamson <sup>h</sup>, Lionel K. Tan <sup>e,†</sup>, Isabela Ribeiro <sup>d,†</sup>, Timothy J. Miles <sup>g,\*,†</sup>

<sup>a</sup> *Department of Internal Medicine, College of Health Sciences, Makerere University, Kampala, Uganda*

<sup>b</sup> *University of Dundee, Dundee, UK*

<sup>c</sup> *University of Liverpool, Antimicrobial Pharmacodynamics and Therapeutics, Liverpool, UK*

<sup>d</sup> *Drugs for Neglected Diseases Initiative (DNDi), Geneva, Switzerland*

<sup>e</sup> *ViiV Healthcare Ltd., London, UK*

<sup>f</sup> *City St George's University of London, London, UK*

<sup>g</sup> *GSK, Tres Cantos, Spain*

<sup>h</sup> *Laboratory of Clinical Immunology and Microbiology (LCIM), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, Maryland, USA*

\* Corresponding author at: GSK, Severo Ochoa 2, PTM, Tres Cantos, Madrid ES 28760, Spain. Tel: 00 34 91 807 03 00.

*E-mail addresses:* david.meya@gmail.com (D. B. Meya), M.DeRycker@dundee.ac.uk (M. De Rycker), I.H.Gilbert@dundee.ac.uk (I. H. Gilbert), william.hope@liverpool.ac.uk (W. Hope), jodionyi@dndi.org (J. J. Odionyi), michael.r.keegan@viivhealthcare.com (M. Keegan), aloyse@sgul.ac.uk (A. Loyse), pablo.x.moral@gsk.com (P. Moral-Lopez),

peter.williamson2@nih.gov (P. R. Williamson), lionel.x.tan@viivhealthcare.com (L. K. Tan), iribeiro@dndi.org (I. Ribeiro), tim.j.miles@gsk.com (T. J. Miles).

<sup>†</sup> These authors contributed equally.

#### Author details

First name	Last name	ORCID
David	Meya	0000-0002-8854-0321
Manu	De Rycker	0000-0002-3171-3519
Ian	Gilbert	0000-0002-5238-1314
William	Hope	0000-0001-6187-878X
Justine Jelagat	Odionyi	0000-0002-8578-1832
Michael	Keegan	0000-0002-6924-7242
Angela	Loyse	0000-0001-7853-1344
Pablo	Moral-Lopez	0009-0004-4140-1887
Peter	Williamson	/
Lionel K	Tan	0000-0002-3822-497X
Isabela	Ribeiro	0000-0001-7402-4628
Timothy	Miles	0000-0001-7407-7404

#### Summary

*Cryptococcus neoformans* and *Cryptococcus gattii* are World Health Organization critical and medium priority pathogens, respectively. These mainly impact people with human immunodeficiency virus residing in low- and middle-income countries, but other patient groups and settings are also affected. The high global morbidity and mortality and the limitations of current treatments provided an impetus for the development of a target product profile (TPP) for new anti-cryptococcal agents. Key attributes of the TPP include improved

safety, superior (or at least comparable) activity to current treatments against all syndromes across the full disease spectrum (cryptococcal meningitis, cryptococcal pneumonia, etc.), relevance for *C. neoformans* and *C. gattii*, suitability for all age groups, oral and intravenous formulations, an acceptable treatment regimen, minimal/manageable drug-drug interactions, thermostability, and a barrier to resistance at least as high as current options. The aim of this TPP, along with the suggested discovery and development paths, is to assist all stakeholders in the development of novel cryptococcal disease treatments.

*Keywords:*

Cryptococcal meningitis

*Cryptococcus neoformans*

Drug development

Human immunodeficiency virus

Low- and middle-income countries

## Introduction

*Cryptococcus* spp. are encapsulated yeasts that are typically found in decaying organic matter and soil, particularly that containing bird and animal droppings.<sup>1</sup> Cryptococcal infection typically results from the inhalation of fungal spores or desiccated small yeasts.<sup>2</sup> During initial pulmonary infection, clinical manifestations range from asymptomatic to pneumonia to acute respiratory distress syndrome.<sup>3</sup> *Cryptococcus* spp. can survive and replicate within macrophages, with subsequent haematogenous dissemination throughout the host.

*Cryptococcus* spp. are neurotropic and invasion of the central nervous system (CNS) results in cryptococcal meningitis (CM) and encephalitis. The term “cryptococcal meningoencephalitis” is an all-encompassing term that reflects the fact that multiple CNS sub-compartments are involved.<sup>4</sup> Clinical manifestations of CM are variable and include fever, headache, lethargy, seizures, altered mental status, memory loss, raised intracranial pressure, and in severe cases, coma.<sup>5,6</sup> CM has been associated with an all-cause mortality rate of approximately 25–55% at 10 weeks in various studies,<sup>7-9</sup> depending on the treatment regimen and clinical context. However, prognosis (morbidity and mortality) among apparently immunocompetent people without classical risk factors tends to be poorer due to delayed diagnosis and frequent development of post-infectious inflammatory response syndromes.<sup>5,10,11</sup>

*Cryptococcus neoformans* is responsible for the vast majority of cryptococcal disease globally.<sup>3</sup> It currently tops the World Health Organization (WHO) fungal priority pathogens list based on mortality, incidence, global distribution, 10-year trends, inpatient care, complications and sequelae, antifungal resistance, preventability, access to diagnostic tests, and evidence-based treatments.<sup>12</sup> *Cryptococcus gattii* is less common, but has been associated with cryptococcal disease in apparently immunocompetent individuals<sup>13</sup> as well as

autoantibodies to the cytokine granulocyte-macrophage colony stimulating factor in 76% of immunocompetent patients.<sup>14</sup> *C. gattii* is medium priority in the WHO fungal priority pathogens list.<sup>12</sup>

Herein, we discuss the global burden of cryptococcal disease, the limitations of currently available treatments, the development of a target product profile (TPP) for much-needed novel cryptococcal disease treatments, the minimal and preferred attributes of these novel treatments, and potential drug discovery and development pathways.

## Global burden

Cryptococcal disease most often occurs in people with impaired cellular immunity, especially in people with human immunodeficiency virus (HIV) who have advanced HIV disease (defined as cluster of differentiation 4 (CD4) count  $< 200$  cells/mm<sup>3</sup>)<sup>15</sup> or present with acquired immunodeficiency syndrome (AIDS).<sup>3,5,16</sup> Others at increased risk of cryptococcal disease include solid organ transplant recipients and people with cancer, diabetes mellitus, autoimmune diseases, and those on long-term corticosteroids.<sup>5,16,17</sup> However, some people with cryptococcal disease have no identifiable predisposing conditions.<sup>16,17</sup>

Before the HIV epidemic in the 1980s, cryptococcal disease was rare, but cases then increased rapidly.<sup>6</sup> Following the introduction of antiretroviral therapy (ART) in the mid-1990s, there was a decline in cryptococcal disease in high-income countries.<sup>16</sup> However, in low- and middle-income countries (LMICs), cryptococcal disease continues to be a considerable problem despite ART roll-out due to ART non-adherence and failure.<sup>15</sup>

A review<sup>18</sup> of three studies that estimated the global burden of CM among people with HIV in 2007,<sup>19</sup> 2014,<sup>20</sup> and 2020<sup>15</sup> highlighted a downward trend in estimated CM cases and cryptococcal deaths, likely due to improvements and expanded access to ART.<sup>15,18-20</sup>

However, CM was still estimated to account for 19% of AIDS-related deaths in 2020, with more than 80% of CM cases and deaths occurring in Africa and Asia & the Pacific.<sup>15,18</sup> In addition to the estimated 152,000 CM cases among people with HIV in 2020, there were also an estimated 41,000 CM cases among people without HIV (approximately half of which were among those with no known underlying disease), for a total global estimate of over 190,000 cases that year.<sup>21</sup>

### **Current CM treatment options and their limitations**

The WHO currently recommends three phases for the treatment of CM in people with HIV: induction, consolidation, and maintenance.<sup>22</sup> Based on the AMBIsome Therapy Induction Optimisation (AMBITION) trial,<sup>7</sup> the preferred induction regimen is one dose of liposomal amphotericin B (L-AmB) followed by 14 days of flucytosine and fluconazole (Table 1).<sup>22</sup> Alternative induction regimens include amphotericin B deoxycholate (D-AmB) + flucytosine + fluconazole OR fluconazole + (flucytosine or L-AmB or D-AmB), depending on availability (Table 1).<sup>22</sup> The WHO also recommends pre-emptive fluconazole for screened individuals who test positive for cryptococcal antigen (i.e. asymptomatic antigenaemia), and fluconazole prophylaxis for people with HIV with CD4 count < 100 cells/mm<sup>3</sup> if screening is not available.<sup>22</sup>

D-AmB, which was discovered in 1955,<sup>23</sup> is used to treat various invasive fungal infections.<sup>24</sup> As D-AmB is associated with acute and chronic toxicity (e.g. nephrotoxicity, anaemia, and hypokalaemia), this needs to be prevented, monitored, and managed.<sup>22</sup> This need can be reduced, though not removed, with the use of a formulation in which the amphotericin molecule is incorporated within the lipid bilayer of liposomes (L-AmB).<sup>25</sup> L-AmB is preferred as it has an improved therapeutic index (i.e. better safety) and similar efficacy.<sup>7,22,25</sup> Other lipid complex formulations are available, but are not currently recommended by the WHO due to a lack of clinical evidence and because the lipid

formulations are not interchangeable.<sup>22</sup> Flucytosine is a pyrimidine analogue that was identified in the 1950s and fluconazole is a triazole that was patented in the 1980s.<sup>23</sup> Before administering flucytosine, electrolytes, haematologic status, and renal status should be determined, and flucytosine should be used with extreme caution in patients with impaired renal function (including dose adjustments and monitoring).<sup>26</sup> Fluconazole has rarely been associated with serious hepatic toxicity, anaphylaxis, exfoliative skin disorders, and QT interval prolongation.<sup>27</sup>

One disadvantage of most of the current treatment regimens is the requirement for intravenous administration of AmB, which can be challenging in resource-limited and rural settings. Fluconazole + flucytosine for 14 days is the only oral regimen, but 10-week mortality with this combination was higher in the Advancing Cryptococcal Meningitis Treatment for Africa (ACTA) trial than for D-AmB + flucytosine for 7 days (35% vs 24%).<sup>8</sup> Additionally, flucytosine availability may be limited in LMICs, and WHO guidelines do not recommend the use of high-dose fluconazole monotherapy due to limited supportive evidence.<sup>22</sup>

Another disadvantage is the need for patient monitoring. For D-AmB (or standard-dose L-AmB plus fluconazole), potassium and creatinine (every other day) and haemoglobin (every week) monitoring is recommended for the duration of therapy, as is pre-hydration and electrolyte replacement before D-AmB infusions,<sup>22</sup> which can be challenging in some settings. Patients on a single high dose of L-AmB should have their potassium and creatinine levels monitored on days 1 and 3 and haemoglobin tested on day 1 (and day 7 if they are still in hospital).<sup>22</sup> Patients on flucytosine are recommended to have their full blood counts monitored.<sup>22</sup> Therefore, there remains a need for safer and more tolerable treatments.

After induction, consolidation is with fluconazole for 8 weeks followed by maintenance with lower-dose fluconazole until immune reconstitution (defined as CD4 count



> 200 cells/mm<sup>3</sup>) and viral load suppression on ART (Table 1).<sup>22,28</sup> Other triazoles (i.e. itraconazole, voriconazole, posaconazole, isavuconazole) are not recommended in the WHO guidelines<sup>22</sup> due to their different bioavailability, variable cerebrospinal fluid (CSF) penetration, cost, drug interactions, and lack of robust trials.<sup>3</sup> Routine use of adjunctive systemic corticosteroids are not recommended in the WHO guidelines,<sup>22</sup> based on the CryptoDex trial.<sup>29</sup>

ART-naïve people with HIV and CM can develop cryptococcal immune reconstitution inflammatory syndrome (IRIS) after initiating ART.<sup>30-32</sup> While immune recovery is essential for managing CM, some trials have shown superior outcomes with delayed versus early initiation of ART,<sup>30-32</sup> although a recent trial does not.<sup>33</sup> Currently, WHO guidelines recommend that ART should be delayed until 4–6 weeks after initiation of anti-cryptococcal therapy.<sup>22</sup>

The WHO guidelines only cover the treatment of CM in people with HIV, while other guidelines from around the world cover HIV-negative populations with CM, including transplant recipients and patients with cancer.<sup>28,34-38</sup> Of note, some of these do include itraconazole, voriconazole, posaconazole, and isavuconazole, but generally only if fluconazole is unavailable or contraindicated.<sup>28,36,38</sup>

## **Development of the TPP**

While recommended treatment regimens for cryptococcal disease have evolved over the last decade, we continue to use drugs that were developed in the last century. Considering that 10-week mortality in the AMBITION trial was almost 25% among people with HIV and CM,<sup>7</sup> development of new drugs that could further reduce mortality and improve efficacy, feasibility, access, safety, and drug-drug interactions is a priority. We therefore developed a TPP to establish key features and performance specifications of new cryptococcal disease

treatments. A TPP is a prerequisite to ensure that new products are developed to meet pre-specified health-related goals.<sup>39</sup>

Our TPP was the result of an initial workshop involving industry and academic drug developers, clinicians, and non-governmental organisations, with further refinement following review by other key stakeholders.

### **Initial drafting phase**

The initial drafting phase included a review of the advantages and challenges of current treatment regimens, including efficacy, adverse event profiles, and routes of administration. We also reviewed the epidemiology of cryptococcal disease to understand patient populations, geographical locations, and the spectrum of disease. After consideration of whether different TPPs would be required for different populations (e.g. HIV, non-HIV immunocompromised, and apparently immunocompetent), it was suggested that one TPP could be used for all populations. However, we note that these may have different drug-drug interaction and comorbidity considerations. Initially, LKT (an infectious disease physician), MK (an HIV clinical scientist), PM-L (a drug discovery scientist), and TJM (an infectious disease drug discovery leader) drafted and refined the TPP.

### **Initial consultative phase**

The draft TPP was reviewed by experts from ViiV Healthcare, the University of Dundee, and the Drugs for Neglected Diseases initiative (DNDi). This included expertise on drug discovery and development, clinical management of HIV and opportunistic infections, and general infectious diseases in resource-limited settings. The draft TPP was adjusted to include “minimal” and “preferred” TPPs and the preference for one TPP for HIV and non-HIV populations was confirmed.

### **Expert consultative phase and finalisation of the TPP**

A virtual workshop was held (AL, DBM, IR, LT, MDR, MK, PM-L, TJM, WH) to review all aspects of the TPP. The panel included experts in antifungal pharmacodynamics (PD) and therapeutics, infectious diseases drug development, clinical, laboratory research, and management of cryptococcal disease. The objectives were to review the draft TPP and understand the strengths and areas for further development. Each area of the TPP was discussed and debated during the workshop, with off-line follow-up to review the outputs of the workshop and further develop and refine the TPP.

In October 2023, the TPP was presented to the End AIDS Action Group ([www.endaidsaction.group](http://www.endaidsaction.group)), which includes a broad range of participants including world-leading specialists in antifungal development, clinical research, and management of cryptococcal disease. Their feedback was incorporated into the TPP. The group was subsequently expanded to include PRW, who has expertise in managing non-HIV immunocompromised patients.

After consideration of the input from all the experts and groups consulted, the TPP was further refined. However, we acknowledge that the proposed TPP may require revision in the future as new scientific evidence emerges and following user engagement activities.

### **Proposed minimal and preferred TPPs**

The proposed minimal and preferred TPPs are detailed below and summarised in Table 2, while Fig. 1 provides an overview in an easy-to-understand format that can be shared with end users.

### **Indications for use**

Any new treatment for cryptococcal disease, whether monotherapy or a combination of drugs, should be suitable for treating all disease severities, which can range from

asymptomatic cryptococcal antigenaemia (i.e. cryptococcal antigen-positive [CrAg+]) to subclinical CM and overt/clinical CM.<sup>40</sup>

Any new treatment regimen should ideally be active against all cryptococcal species (particularly *C. neoformans*, but also *C. gattii*) and would be suitable for use as prophylaxis in individuals receiving a range of immunosuppressive modalities or regimens. As is common practice in anti-infective drug development, the inclusion of clinical strains of *C. neoformans* that are representative of different geographical areas early in the drug discovery process would be critical to ensure a robust therapeutic value of any novel antifungal. If any new cryptococcal drug was also active against other fungal species, this could be an additional benefit and increase the size of the population that would potentially use the drug.

### **Target patient populations**

A new drug or combination of drugs should be suitable for the treatment of immunocompromised and apparently immunocompetent adults (preferably also children). While there are some differences in the clinical presentation between immunocompromised and apparently immunocompetent individuals, there should be minimal difference in activity of the drugs depending on immune status. Ideally, new treatments would be suitable for use during pregnancy and lactation, which are not usually investigated early during drug development.

### **Clinical efficacy**

Any new treatment regimen would ideally be clinically and statistically superior to the gold standard of care, but, as this may be difficult to demonstrate, at a minimum it should have non-inferior efficacy and be superior in terms of other clinically relevant endpoints (e.g. tolerability and adverse event profile). A commonly used endpoint is 10-week mortality,<sup>7,8</sup> while early fungicidal activity (i.e. rate of fungal clearance) is a potential surrogate endpoint

that can reduce trial sample size, although this is still being validated.<sup>41,42</sup> Currently, we recommend all-cause mortality as the primary endpoint, as it incorporates outcomes from drug efficacy as well as drug toxicity and drug interactions, but this could change if other endpoints are validated and approved by regulatory authorities.

Cryptococcal species are intrinsically resistant to the echinocandin class of antifungals and can also develop resistance to azoles and other antifungal drugs (as discussed below). It would be preferable if resistance was not a problem for any new cryptococcal disease treatment regimen, particularly considering the “One Health” approach focused on the interplay between human, animal, and environmental health and the use of antifungals within this ecosystem. It would, therefore, be useful to understand the resistance mechanisms and liabilities of any new medications.

### **Safety/tolerability**

Any new cryptococcal disease medication should have an improved safety and tolerability profile compared to the gold standard of care. Ideally, minimal clinical monitoring/supportive care would be required, as current therapy requires renal, electrolyte, hepatic, neutrophil, and haemoglobin monitoring, which is burdensome to both user and healthcare providers, particularly in resource-limited settings. As a preference, any new drugs should have good tolerability in children, who should not be precluded from novel treatments despite accounting for a small proportion of the disease burden.<sup>43</sup> Ideally, any new drug would be non-teratogenic and be safe during breastfeeding to maximise its applicability.

### **Formulation/presentation**

Any new drug should ideally be easier to prepare and administer than current options. Given the high cost of intravenous administration (including the requirement for skilled workforce and monitoring) of drugs in hospital settings, oral agents are generally preferable

for LMICs, with the option for nasogastric tube administration in those with CM and low levels of consciousness. Consideration should be given for alternative oral options for adults who cannot swallow tablets and children, e.g. oral or dispersible tablets or syrups, although these would need to be palatable. Other dosage forms should preferably be available for very severe disease (i.e. intravenous, intramuscular, or subcutaneous), although this may prove challenging for drug development, as different physicochemical properties are preferred for different dosage forms.

### **Dose regimen**

The current WHO-recommended treatment regimen for people with HIV is long (2-week induction, 8-week consolidation, and maintenance until immune reconstitution),<sup>22</sup> as are regimens for other populations.<sup>28,34-38</sup> Any new drugs should shorten this burdensome regimen. If possible, new treatments would have sufficiently long half-life, potency, and fungicidal activity so that a single agent could be administered once or twice daily for 2–4 weeks. Failing this, a twice-daily combination therapy with a 1-week induction, 4–6-week consolidation, and maintenance until immune reconstitution could be considered.

### **Drug-drug interactions**

Ideally, there would be few (or at least manageable) drug-drug interactions with common medications in appropriate populations, including ART for people with HIV, transplant-conditioning agents (e.g. sirolimus), antimalarials, antituberculosis agents, cotrimoxazole, and antifungals, considering the common comorbidities (e.g. HIV, cancer, diabetes). Additionally, due to the neurological localisation of CM, use of concomitant anticonvulsants may be necessary and hence a lack of interaction with all anticonvulsant therapies would be preferred. Lastly, although corticosteroids are not routinely recommended in the WHO guidelines for the treatment of CM in people with HIV,<sup>22</sup> these may be necessary

in other populations<sup>35,44</sup> and patients on corticosteroids are at increased risk of CM,<sup>5,17</sup> so any new treatments should not interact with these. Although minimal drug-drug interactions would make any new treatments suitable for a wider range of patients, we acknowledge that some interactions may preclude certain populations.

### **Product stability and storage**

The product should be heat stable, with a 3-year shelf life in hot/humid climates. A shelf-life of 3 years in hot/humid conditions is considered the minimum acceptable to allow distribution and administration of the product to the target patient population. If possible, it would have no requirement for refrigeration, considering the likely use in settings with limited resources to support cold chain maintenance.

### **Product registration path**

Although any new cryptococcal drugs will primarily be used in LMICs, by first intent, the data package from any clinical trials should support approval by a WHO Listed Authority.<sup>45</sup> These can then be used to help with registration in countries with the highest burden of disease and WHO pre-qualification to enable access to the drug in these countries.

### **Patient and payor value proposition**

Overall, any new products should be novel, effective, safe, and suitable for the full spectrum of cryptococcal disease. It should not require hospitalisation for administration or monitoring and should not (or minimally) require monitoring for adverse events. Also, it should have no (or limited) contraindications, no (or minimal/manageable) interactions, and be suitable for combination use. Preferably, it should also have a reduced risk of inducing IRIS, have improved compliance (due to reduced toxicity, shorter duration, less frequent dosing, etc.), be CNS-penetrant (although this depends on blood–brain barrier [BBB] permeability as discussed below), and enable increased access to treatment. Although

cryptococcal IRIS is due to an interplay of host immunological factors and pathogen factors, there is evidence to suggest that one of the factors associated with the risk of IRIS is fungal load pre-ART initiation.<sup>46-48</sup> We thus hypothesise that if a cryptococcal sterilising cure could be successfully developed, or at least a treatment that significantly reduces the fungal burden rapidly, this has the potential to decrease the risk of cryptococcal IRIS, although this would need to be proven in clinical studies.

Cost is also an important factor, given that most cases occur in Africa.<sup>15</sup> Preferably, it would be affordable and supported by robust cost-effectiveness analysis taking into consideration the cost of manufacture of the product (cost of goods) and also any cost savings to the health service due to improved efficacy and safety.

### **Discovery and development path**

Antifungal development is challenging because fungal pathogens have various biological processes in common with humans.<sup>49</sup> Activity against fungal pathogens is therefore likely to result in toxicity to humans.<sup>49</sup> Drug discovery efforts for CM are limited, with the furthest developed series being new formulations or repurposed from other antifungal drug discovery efforts. These include an oral lipid nanocrystal formulation of AmB (MAT2203),<sup>50</sup> azoles (quileseconazole [VT-1129],<sup>51</sup> oteseconazole [VT-1161],<sup>52</sup> VT-1598,<sup>53</sup> and opelconazole [PC945]<sup>54</sup>), glycosylphosphatidylinositol anchor biosynthesis inhibitors (fosmanogepix [APX001]<sup>55</sup> and APX2039<sup>55,56</sup>), a diamidine (ATI-2307 [T-2307]),<sup>57</sup> an acetyl coenzyme A ligase inhibitor (AR-12 [OSU-03012]),<sup>58</sup> and a next-generation polyene drug (SF001).<sup>59</sup> Limited earlier-stage efforts are also ongoing.<sup>60-63</sup> Further details can be found in a recently published review of antifungals that are in development.<sup>64</sup> *Cryptococcus* spp. are unique pathogens, evolutionarily divergent from most other human fungal pathogens such as *Aspergillus* spp. and *Candida* spp. In addition to the current



repurposing efforts from other antifungal programmes, there is a need to develop fit-for-purpose *Cryptococcus*-specific drug discovery approaches.

### ***In vitro* assays**

Successful drug discovery relies on appropriate cell-based models that are predictive of clinical efficacy.<sup>65</sup> In infectious diseases, this frequently requires testing compounds in multiple cell-based models that reflect the complexity of the pathogen and its interactions with the host.<sup>66</sup> Despite being unicellular, *C. neoformans* displays remarkable heterogeneity in human infections,<sup>3</sup> with various degrees of encapsulation, melanisation, and Titan cell formation as well as infection of macrophages and microglia. Assessing the value of preclinical compound screening assays that cover this heterogeneity is a key aspect of developing the critical path for the discovery and development of new cryptococcosis drugs, with the relevant assays potentially differing by indication (e.g. CM vs systemic disease). Capsule size, for example, could be a key factor in drug discovery as the capsule may limit compound permeability, which is particularly relevant as cryptococci isolated from the CSF of people with HIV show a range of capsule sizes, with capsule size associated with virulence.<sup>67</sup> Backtranslation experiments, testing reference treatments, and new lead compounds in a range of preclinical cell-based models will be essential in this respect. Further triaging of hits should consider chemical tractability, potential to cross the BBB, and mode of action (avoiding compounds with the same mode of action as clinically used drugs).

### ***In vivo* models**

Laboratory animal models of CM are well characterised and highly predictive of therapeutic outcomes.<sup>56,68,69</sup> These experimental tools have been pivotal in understanding the PD of antifungal agents including the emergence of resistance (e.g. to fluconazole),<sup>70</sup> defining the optimal duration of induction therapies (e.g. D-AmB, L-AmB),<sup>69,71</sup> defining the optimal combinations of antifungal agents,<sup>53</sup> and defining the PD of new antifungal agents

(e.g. APX2039 and ATI-2307).<sup>56,72</sup> There are two model systems (murine and rabbits) that have strengths and limitations and yield complementary information.<sup>69</sup> Data from both are often required to achieve a detailed understanding of dose-effect-response relationships. Both models offer the opportunity to describe the temporal change in fungal burden from unrestrained growth or drug-induced killing. Increased use of organoids has recently been proposed to minimise the use of animal models in drug development, including that stated in the Food and Drug Administration Modernization Act 2.0 in the U.S. However, more work will be needed to optimise these model systems for effective drug development generally and antifungal drug development specifically.<sup>73</sup>

### *Murine model*

Mice are inherently susceptible to cryptococcal infection and do not require systemic immunosuppression to enable infection to be established. Intravenous inoculation results in disseminated infection with highly reproducible encephalitis. The endpoint for the estimation of the PD of antifungal agents is fungal burden in the brain.<sup>69</sup> The mouse is too small to enable drug partitioning and PD in sub-compartments of the CNS. Rather, the cerebrum is considered a single and fully homogenous compartment.

Advantages of murine studies are that they are relatively straightforward to conduct, result in a highly reproducible encephalitis, and are highly predictive of clinical outcomes for different antifungal agents and classes.<sup>74</sup> One key disadvantage is the inability to examine the CSF<sup>69</sup> (which is the most relevant compartment for clinical disease and clinical studies). Uncertainty about effect site pharmacokinetics (PK) (i.e. concentrations in the cerebrum) means that PK–PD relationships are generally quantified in terms of plasma concentrations. This requires an assumption that tracking of drug from plasma to the site of infection is the same in mice as in humans. The use of PK to predict tissue concentrations can also be

problematic, especially for lipid formulations that may have very different patterns of tissue partitioning.

### *Rabbit model*

The rabbit model of CM requires intracisternal inoculation of *Cryptococcus*, which results in a reproducible meningoencephalitis.<sup>72</sup> The model enables serial quantification of fungal burden in the CSF, which mimics clinical therapeutics. The rate of decline of fungal burden can be used for regimen identification and estimation of PD relationships.

Advantages of rabbit studies are that they closely mimic clinical disease and therapeutics, and effect site drug partitioning studies are relatively straightforward. Disadvantages are that they are resource intensive and significant technical expertise is required for intracisternal inoculation and serial CSF sampling, including advanced expertise in anaesthesia.

### **Compound properties**

Defining the appropriate compound properties for a new CM drug is important to guide drug discovery efforts but is not straightforward. Compounds need to penetrate the capsule and cell wall, a challenge exacerbated by their increased size and density in Titan cells.<sup>75</sup> The physicochemical properties required to penetrate the *Cryptococcus* capsule and cell wall remain poorly understood. In addition, the complex pathophysiology of CM poses a challenge for establishing the required compound distribution properties. *Cryptococcus* is typically present in the meninges but can also be found in the brain parenchyma, usually in cryptococcomas.<sup>76</sup> Increased BBB permeability in patients with CM<sup>77</sup> allows non-brain penetrant compounds to reach the yeast cells and BBB permeability is thus not a prerequisite for efficacy. However, whether the BBB is always compromised, particularly in patients with subclinical CM, remains to be understood. As compounds progress, detailed CNS partitioning studies in both uninfected laboratory animal models and disease models are advised and

should be considered in conjunction with pharmacodynamic data when deciding to advance compounds. From a safety perspective, it must be considered that for non-BBB penetrant compounds, standard preclinical toxicology and Phase 1 clinical trials may not identify CNS toxicities that could manifest in CM patients with a compromised BBB. Of note, early assessment of potential toxicity liabilities is paramount. Amphotericin B, developed in the 1950s, when regulatory requirements were less stringent, displays excellent efficacy, but its safety profile limits widespread use.<sup>24</sup> In current drug discovery practice, safety is integrated right from the start, and new treatments are only developed if they combine efficacy with a suitable therapeutic index. Finally, compounds should ideally be orally available and stable in tropical zones.

### **Clinical trials**

The European Medicines Agency published guidelines on the clinical development of antifungals for treating invasive fungal disease in 2010.<sup>78</sup> This includes guidance on the collection of preclinical data, including PK/PD and resistance considerations. Also, study design considerations for clinical trials, including disease categories, end users (including children), treatment types, biomarkers, and outcomes (including safety).<sup>78</sup> Adaptive designs<sup>79</sup> may be useful for assessing monotherapies, while combinations could be compared to one part of the combination or an approved monotherapy.<sup>78</sup> Ideally, the comparator regimen for HIV-associated CM should consist of the preferred induction regimen recommended by WHO guidelines and the WHO-listed regulatory authorities at the time of the trial. For example, the WHO 2022 guidelines (see Table 1)<sup>22</sup> are based on the results of the AMBITION study.<sup>7</sup> This allows any medicine developed to be compared to the best available regimen.

Of note, there is a marked paucity of data on the use of antifungal CM regimens in non-HIV patients, especially in apparently immunocompetent individuals. The optimal

duration of therapy at all stages (induction, consolidation, and maintenance) in this group is unclear and adequate evidence is necessary. Studies have been hampered by the lower number of cases<sup>21</sup> and differences in treatment centres from those caring for people with HIV, leading to difficulty in conducting appropriately powered studies. Potential solutions could be to create patient post-approval registries or ongoing cohorts of patients such as the *Cryptococcus* Infection Network in non-HIV Cohort (CINCH),<sup>80</sup> layering on adaptive designs that might explore multiple treatments, doses, durations, or combinations with options to ‘drop losers’ or ‘select winners’ early.<sup>79</sup> Another challenge related to non-HIV patients is the frequency of post-infectious inflammatory sequelae that may require adjunctive immune modulation to optimise clinical efficacy.<sup>44,81</sup> Treatment guidelines based on expert consensus have been published and the recommended regimens could serve as possible control regimens in studies.<sup>28,34-37</sup>

One way to decrease the required sample size of randomised controlled trials in CM could be to use early fungicidal activity as a surrogate endpoint for all-cause mortality.<sup>42</sup> Such an approach may also be useful to select candidate interventions in phase 2 studies.<sup>42</sup> In a study that investigated predictors of clinical outcome in people hospitalised with CM in a hospital in Brazil, higher CSF yeast count was found to predict in-hospital mortality and severity.<sup>82</sup> However, such tests are invasive and have an associated cost, and given the acute nature of CM, all-cause mortality remains a suitable endpoint.<sup>42</sup>

## Resistance

While resistance to AmB is limited,<sup>24</sup> *in vitro* resistance to flucytosine monotherapy emerges rapidly,<sup>83</sup> and resistance to fluconazole is increasing.<sup>84,85</sup> Human-to-human transmission of *Cryptococcus* is uncommon,<sup>18</sup> but has been reported in transplant recipients.<sup>86</sup> Most resistance is, therefore, either pre-existing in the natural population or emerges during patient treatment. Two resistance mechanisms are common in fungi, namely heritable and

transient.<sup>87</sup> Heritable resistance refers to stable point mutations or gene duplications, whereas transient resistance or heteroresistance is usually due to unstable aneuploidy. Of note, heteroresistance may be transient, but not all transient resistance is heteroresistance.

Resistance to flucytosine could result from mutations in the enzymes involved in flucytosine uptake and metabolism, or upregulation of pyrimidine synthesis.<sup>83</sup> For fluconazole, both mutations in its target gene (*ERG11*) and heteroresistance have been observed.<sup>84,88</sup> Heteroresistance is intrinsic in *Cryptococcus* (i.e. is found prior to treatment) and is driven by transient aneuploidy in a sub-population of cells.<sup>70,89</sup> The generation of heteroresistance in *Cryptococcus* is facilitated by their unique Titan cells, which produce large numbers of aneuploid progeny.<sup>87</sup> To overcome the resistance challenge, combination treatments can be used, as well as the development of drugs with novel modes of action.<sup>90</sup>

## Conclusions

*C. neoformans* was highlighted as critical in the 2022 WHO fungal priority pathogens list<sup>12</sup> due to its public health importance and unmet research and development needs. As current drugs have important limitations, a new, affordable, oral therapy that enables safe and effective treatment in the community for cryptococcal disease could lead to increased access and the avoidance of costs associated with hospitalisation for parenteral dosing and the need for adverse event monitoring. Ideally, a new oral therapy would be adaptable for intravenous/intramuscular/subcutaneous use in the most severe cases and be suitable for treating immunocompromised and apparently immunocompetent individuals. A novel mode of action also opens up the possibility for its use as part of combination treatment to shorten treatment durations and minimise the potential for resistance. If possible, a new therapy should have superior safety and efficacy, a less cumbersome treatment regimen, and have no – or at least manageable – drug interactions.

Drug discovery efforts for CM are limited, with most being repurposed from other drugs. Given the unique nature of *Cryptococcus*, there is a need to develop targeted approaches.

This TPP provides a foundation for the development of a novel cryptococcal disease treatment to allow academic drug developers, pharmaceutical companies, and key stakeholders to understand the required characteristics. Given the advancement in the drug development arena, we believe we can be optimistic for the community to develop new drugs that can approach our idealistic TPP. As new scientific evidence is generated, together with input from patient engagement on the TPP for CM, this TPP may require further review and revision.

## **Funding**

This work was supported by GSK, which funded the development of this publication, was involved in all stages of the process, and took charge of all costs associated with the development and publication of this manuscript.

## **Contributors**

GSK, the University of Dundee and the University of Exeter are working together on drug discovery for cryptococcal disease in a Wellcome funded programme. To inform the drug discovery programme, GSK Global Health, ViiV Healthcare Limited, the DNDi, and the University of Dundee have worked together with several key opinion leaders to develop this target product profile for cryptococcal disease.

**MK, PM-L, PRW, LKT, and TJM:** drafted and refined the initial target product profile (TPP). **DBM, MDR, WH, MK, AL** (including through the involvement of the End AIDS Action Group), **PM-L, LKT, IR, and TJM:** further refined the TPP. **DBM, MDR, IHG, WH, MK, LKT, IR, and TJM:** wrote the first draft of the manuscript. All authors

helped to refine the manuscript, approved the final version, and were responsible for the decision to submit it for publication.

### **Declaration of Competing Interest**

**DBM** received grants from the National Institutes of Health and from the UK Medical Research Council, outside of the submitted work. He also received payment for consulting from GSK, support from the National Institutes of Health for attending meetings, participated on Data Safety Monitoring Boards or Advisory Boards for the 5FC HIV-Crypto Study, the FIEBRE study, and the New Strat TB study. He also has a leadership role in MARA NGO and chairs the Advanced HIV/TB Technical Working Group, Ministry of Health in Uganda.

**MDR** and **IHG** received Wellcome grant funding for their attendance at the International Conference on Cryptococcus & Cryptococcosis in Uganda (January 2023), and a grant from the Tres Cantos Open Lab Foundation to develop cell-based assays for *Cryptococcus* drug discovery.

**WH** received grants from Bugworks and GARDP, personal fees from GSK, Mundipharma, and Pulmocide, and grants and personal fees from F2G, Pfizer, and Phico Therapeutics, outside of the submitted work. He also declares having participated in Advisory Boards for F2G and Basilea; and being an NIHR Speciality Co-Lead for Infection.

**MK** is employed by ViiV Healthcare and holds financial equities in GSK.

**AL** received grants from NIHR (Imprint project), NIH (Effect trial), and EDCTP (DREAMM project, 5FC HIV Crypto) and consulting fees from the Unitaid-CHAI AHD programme to her institution (City St George's University of London), outside of the submitted work. She is a member of the ENCORE study data safety monitoring board and leads the End AIDS Action group that encompasses the work of the Cryptococcal Meningitis Action Group (CryptoMAG) (unfunded roles). She also received CrAg LFA tests from



Biosynex for the DREAMM project, outside of the submitted work. No payment was received for this work.

**PM-L** is employed by GSK.

**JJO** is the lead for DNDi, which is a sub-recipient under Aurum Institute for the IMPAACT4HIV advanced HIV disease grant, and part of her salary is paid through this grant. JJO is also the project lead for a European & Developing Countries Clinical Trial Partnership (EDCTP) funded grant at DNDi, and her salary is partially paid through this grant. **LKT** is employed by ViiV Healthcare and holds financial equities in GSK.

**PRW** reports Division of Intermural Research (DIR) grant supports at NIH for the present manuscript: National Institutes of Health, NIAID AI001123; and National Institutes of Health, NIAID AI001124. He also reports two patents: Williamson, PR, Panepinto, JC, Qiu J. “Small molecule imaging of fungi by positron emission tomography scanning” U.S. Patent Number 9968693, Issued May 15, 2018, DIR/NIAID/NIH; and Hammoud DL, et al. “Radiolabeled Sugars for imaging of fungal infections” Application: E-163-2019-0-PCT-02. Outside of the submitted work, he also declares being a non-voting member of the CLSI.

**TJM** is employed by GSK, has a non-paid adjunct professor role at the University of Dundee, and holds financial equities in GSK.

**IR** is employed by the Drugs for Neglected Diseases initiative, which is a not-for-profit drug development organisation. She received payments of her salary as Viral Disease Cluster, overseeing exploratory and drug development activities in cryptococcal meningitis.

The authors declare no other financial and non-financial relationships and activities.

## Acknowledgements

The authors thank Jose M Gatell (ViiV) for his contribution to the TPP, and the End AIDS Action Group ([www.endaidsaction.group](http://www.endaidsaction.group)) for the feedback provided on the TPP

during the End AIDS Action Group call that took place in October 2023. The authors also thank Enovalife Medical Communication Service Center for editorial assistance and manuscript coordination; and Jenny Lloyd (Compass Healthcare Communications Ltd.) for medical writing support, on behalf of GSK.

Journal Pre-proof

## References

1. Pappas PG. *Cryptococcal infections in non-HIV-infected patients*. *Trans Am Clin Climatol Assoc* 2013;**124**:61-79.
2. Wang Y, Pawar S, Dutta O, Wang K, Rivera A, Xue C. *Macrophage mediated immunomodulation during cryptococcus pulmonary infection*. *Front Cell Infect Microbiol* 2022;**12**:859049. <https://doi.org/10.3389/fcimb.2022.859049>
3. Maziarz EK, Perfect JR. *Cryptococcosis*. *Infect Dis Clin North Am* 2016;**30**(1):179-206. <https://doi.org/10.1016/j.idc.2015.10.006>
4. Sabiiti W, May RC. *Mechanisms of infection by the human fungal pathogen Cryptococcus neoformans*. *Future Microbiol* 2012;**7**(11):1297-313. <https://doi.org/10.2217/fmb.12.102>
5. Yoon HA, Felsen U, Wang T, Pirofski LA. *Cryptococcus neoformans infection in human immunodeficiency virus (HIV)-infected and HIV-uninfected patients at an inner-city tertiary care hospital in the Bronx*. *Med Mycol* 2020;**58**(4):434-43. <https://doi.org/10.1093/mmy/myz082>
6. Perfect JR, Casadevall A. *Cryptococcosis*. *Infect Dis Clin North Am* 2002;**16**(4):837-74, v-vi. [https://doi.org/10.1016/s0891-5520\(02\)00036-3](https://doi.org/10.1016/s0891-5520(02)00036-3)
7. Jarvis JN, Lawrence DS, Meya DB, Kagimu E, Kasibante J, Mpoza E, et al. *Single-dose liposomal amphotericin B treatment for cryptococcal meningitis*. *N Engl J Med* 2022;**386**(12):1109-20. <https://doi.org/10.1056/NEJMoa2111904>
8. Molloy SF, Kanyama C, Heyderman RS, Loyse A, Kouanfack C, Chanda D, et al. *Antifungal combinations for treatment of cryptococcal meningitis in Africa*. *N Engl J Med* 2018;**378**(11):1004-17. <https://doi.org/10.1056/NEJMoa1710922>
9. Mfinanga S, Kanyama C, Kouanfack C, Nyirenda S, Kivuyo SL, Boyer-Chammard T, et al. *Reduction in mortality from HIV-related CNS infections in routine care in Africa (DREAMM): a before-and-after, implementation study*. *Lancet HIV* 2023;**10**(10):e663-e73. [https://doi.org/10.1016/S2352-3018\(23\)00182-0](https://doi.org/10.1016/S2352-3018(23)00182-0)
10. Ecevit IZ, Clancy CJ, Schmalfuss IM, Nguyen MH. *The poor prognosis of central nervous system cryptococcosis among nonimmunosuppressed patients: a call for better disease recognition and evaluation of adjuncts to antifungal therapy*. *Clin Infect Dis* 2006;**42**(10):1443-7. <https://doi.org/10.1086/503570>
11. Zhou LH, Zhao HZ, Wang X, Wang RY, Jiang YK, Huang LP, et al. *Immune reconstitution inflammatory syndrome in non-HIV cryptococcal meningitis: cross-talk between pathogen and host*. *Mycoses* 2021;**64**(11):1402-11. <https://doi.org/10.1111/myc.13361>
12. World Health Organization. WHO fungal priority pathogens list to guide research, development and public health action; 2022. (<https://www.who.int/publications/i/item/9789240060241>). [Accessed March 4, 2025].
13. Chen SC-A, Meyer W, Sorrell TC. *Cryptococcus gattii infections*. *Clin Microbiol Rev* 2014;**27**(4):980-1024. <https://doi.org/10.1128/CMR.00126-13>
14. Yang DH, England MR, Salvator H, Anjum S, Park YD, Marr KA, et al. *Cryptococcus gattii species complex as an opportunistic pathogen: underlying medical conditions associated with the infection*. *mBio* 2021;**12**(5):e0270821. <https://doi.org/10.1128/mBio.02708-21>
15. Rajasingham R, Govender NP, Jordan A, Loyse A, Shroufi A, Denning DW, et al. *The global burden of HIV-associated cryptococcal infection in adults in 2020: a modelling analysis*. *Lancet Infect Dis* 2022;**22**(12):1748-55. [https://doi.org/10.1016/S1473-3099\(22\)00499-6](https://doi.org/10.1016/S1473-3099(22)00499-6)

16. Mirza SA, Phelan M, Rimland D, Graviss E, Hamill R, Brandt ME, et al. *The changing epidemiology of cryptococcosis: an update from population-based active surveillance in 2 large metropolitan areas, 1992-2000*. *Clin Infect Dis* 2003;**36**(6):789-94. <https://doi.org/10.1086/368091>
17. Pappas PG, Perfect JR, Cloud GA, Larsen RA, Pankey GA, Lancaster DJ, et al. *Cryptococcosis in human immunodeficiency virus-negative patients in the era of effective azole therapy*. *Clin Infect Dis* 2001;**33**(5):690-9. <https://doi.org/10.1086/322597>
18. Zhao Y, Ye L, Zhao F, Zhang L, Lu Z, Chu T, et al. *Cryptococcus neoformans, a global threat to human health*. *Infect Dis Poverty* 2023;**12**(1):20. <https://doi.org/10.1186/s40249-023-01073-4>
19. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. *Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS*. *AIDS* 2009;**23**(4):525-30. <https://doi.org/10.1097/QAD.0b013e328322ffac>
20. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, et al. *Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis*. *Lancet Infect Dis* 2017;**17**(8):873-81. [https://doi.org/10.1016/S1473-3099\(17\)30243-8](https://doi.org/10.1016/S1473-3099(17)30243-8)
21. Denning DW. *Global incidence and mortality of severe fungal disease*. *Lancet Infect Dis* 2024;**24**(7):e428-e38. [https://doi.org/10.1016/S1473-3099\(23\)00692-8](https://doi.org/10.1016/S1473-3099(23)00692-8)
22. World Health Organization. Guidelines for diagnosing, preventing and managing cryptococcal disease among adults, adolescents and children living with HIV; 2022. (<https://www.who.int/publications/i/item/9789240052178>). [Accessed March 4, 2025].
23. Ngan NTT, Flower B, Day JN. *Treatment of cryptococcal meningitis: how have we got here and where are we going?* *Drugs* 2022;**82**(12):1237-49. <https://doi.org/10.1007/s40265-022-01757-5>
24. Cavassin FB, Baú-Carneiro JL, Vilas-Boas RR, Queiroz-Telles F. *Sixty years of amphotericin B: an overview of the main antifungal agent used to treat invasive fungal infections*. *Infect Dis Ther* 2021;**10**(1):115-47. <https://doi.org/10.1007/s40121-020-00382-7>
25. Gulati M, Bajad S, Singh S, Ferdous AJ, Singh M. *Development of liposomal amphotericin B formulation*. *J Microencapsul* 1998;**15**(2):137-51. <https://doi.org/10.3109/02652049809006844>
26. Bausch Health Companies Inc. ANCOBON (flucytosine) capsules; 2022. ([https://www.accessdata.fda.gov/drugsatfda\\_docs/label/2022/017001Orig1s034lbl.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/label/2022/017001Orig1s034lbl.pdf)). [Accessed April 2, 2025].
27. Pfizer. Diflucan (fluconazole tablets) (fluconazole for oral suspension); 2024. ([https://www.accessdata.fda.gov/drugsatfda\\_docs/label/2024/019949s072lbl.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/019949s072lbl.pdf)). [Accessed April 2, 2025].
28. Perfect JR, Dismukes WE, Dromer F, Goldman DL, Graybill JR, Hamill RJ, et al. *Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America*. *Clin Infect Dis* 2010;**50**(3):291-322. <https://doi.org/10.1086/649858>
29. Beardsley J, Wolbers M, Kibengo FM, Ggayi ABM, Kamali A, Cuc NTK, et al. *Adjunctive dexamethasone in HIV-associated cryptococcal meningitis*. *N Engl J Med* 2016;**374**(6):542-54. <https://doi.org/10.1056/NEJMoa1509024>

30. Boulware DR, Mehta DB, Muzoora C, Rolfes MA, Huppler Hullsiek K, Musubire A, et al. *Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis*. *N Engl J Med* 2014;**370**(26):2487-98. <https://doi.org/10.1056/NEJMoa1312884>
31. Bisson GP, Molefi M, Bellamy S, Thakur R, Steenhoff A, Tamuhla N, et al. *Early versus delayed antiretroviral therapy and cerebrospinal fluid fungal clearance in adults with HIV and cryptococcal meningitis*. *Clin Infect Dis* 2013;**56**(8):1165-73. <https://doi.org/10.1093/cid/cit019>
32. Makadzange AT, Ndhlovu CE, Takarinda K, Reid M, Kurangwa M, Gona P, et al. *Early versus delayed initiation of antiretroviral therapy for concurrent HIV infection and cryptococcal meningitis in sub-saharan Africa*. *Clin Infect Dis* 2010;**50**(11):1532-8. <https://doi.org/10.1086/652652>
33. Ingle SM, Miro JM, May MT, Cain LE, Schwimmer C, Zangerle R, et al. *Early antiretroviral therapy not associated with higher cryptococcal meningitis mortality in people with human immunodeficiency virus in high-income countries: an international collaborative cohort study*. *Clin Infect Dis* 2023;**77**(1):64-73. <https://doi.org/10.1093/cid/ciad122>
34. Baddley JW, Forrest GN, AST Infectious Diseases Community of Practice. *Cryptococcosis in solid organ transplantation - guidelines from the American Society of Transplantation Infectious Diseases Community of Practice*. *Clin Transplant* 2019;**33**(9):e13543. <https://doi.org/10.1111/ctr.13543>
35. Ssebambulidde K, Anjum SH, Hargarten JC, Chittiboina P, Shoham S, Seyedmousavi S, et al. *Treatment recommendations for non-HIV associated cryptococcal meningoencephalitis including management of post-infectious inflammatory response syndrome*. *Front Neurol* 2022;**13**:994396. <https://doi.org/10.3389/fneur.2022.994396>
36. Izumikawa K, Kakeya H, Sakai F, Shibuya K, Sugita T, Takazono T, et al. *Executive summary of JSMM clinical practice guidelines for diagnosis and treatment of cryptococcosis 2019*. *Med Mycol J* 2020;**61**(4):61-89. <https://doi.org/10.3314/mmj.20.001>
37. Chang CC, Hall V, Cooper C, Grigoriadis G, Beardsley J, Sorrell TC, et al. *Consensus guidelines for the diagnosis and management of cryptococcosis and rare yeast infections in the haematology/oncology setting, 2021*. *Intern Med J* 2021;**51** Suppl 7:118-42. <https://doi.org/10.1111/imj.15590>
38. Chang CC, Harrison TS, Bicanic TA, Chayakulkeeree M, Sorrell TC, Warris A, et al. *Global guideline for the diagnosis and management of cryptococcosis: an initiative of the ECMM and ISHAM in cooperation with the ASM*. *Lancet Infect Dis* 2024;**24**(8):e495-512. [https://doi.org/10.1016/S1473-3099\(23\)00731-4](https://doi.org/10.1016/S1473-3099(23)00731-4)
39. Cocco P, Ayaz-Shah A, Messenger MP, West RM, Shinkins B. *Target product profiles for medical tests: a systematic review of current methods*. *BMC Med* 2020;**18**(1):119. <https://doi.org/10.1186/s12916-020-01582-1>
40. Wake RM, Molloy SF, Jarvis JN, Harrison TS, Govender NP. *Cryptococcal antigenemia in advanced human immunodeficiency virus disease: pathophysiology, epidemiology, and clinical implications*. *Clin Infect Dis* 2023;**76**(4):764-70. <https://doi.org/10.1093/cid/ciac675>
41. Pullen MF, Hullsiek KH, Rhein J, Musubire AK, Tugume L, Nuwagira E, et al. *Cerebrospinal fluid early fungicidal activity as a surrogate endpoint for cryptococcal meningitis survival in clinical trials*. *Clin Infect Dis* 2020;**71**(7):e45-9. <https://doi.org/10.1093/cid/ciaa016>
42. Montezuma-Rusca JM, Powers JH. *Outcome assessments in clinical trials of cryptococcal meningitis: considerations on use of early fungicidal activity as a*



- potential surrogate endpoint for all-cause mortality. *Curr Treat Options Infect Dis* 2014;**6**(3):326-36. <https://doi.org/10.1007/s40506-014-0026-0>
43. Tenforde MW, Mokomane M, Leeme T, Patel RKK, Lekwape N, Ramodimoosi C, et al. *Advanced human immunodeficiency virus disease in Botswana following successful antiretroviral therapy rollout: incidence of and temporal trends in cryptococcal meningitis. Clin Infect Dis* 2017;**65**(5):779-86. <https://doi.org/10.1093/cid/cix430>
  44. Anjum S, Dean O, Kosa P, Magone MT, King KA, Fitzgibbon E, et al. *Outcomes in previously healthy cryptococcal meningoencephalitis patients treated with pulse taper corticosteroids for post-infectious inflammatory syndrome. Clin Infect Dis* 2021;**73**(9):e2789-98. <https://doi.org/10.1093/cid/ciaa1901>
  45. World Health Organization. A Framework for evaluating and publicly designating regulatory authorities as WHO Listed Authorities (WLA); (<https://www.who.int/initiatives/who-listed-authority-reg-authorities>). [Accessed March 4, 2025].
  46. Chang CC, Dorasamy AA, Gosnell BI, Elliott JH, Spelman T, Omarjee S, et al. *Clinical and mycological predictors of cryptococcosis-associated immune reconstitution inflammatory syndrome. AIDS* 2013;**27**(13):2089-99. <https://doi.org/10.1097/QAD.0b013e3283614a8d>
  47. Wiesner DL, Boulware DR. *Cryptococcus-related immune reconstitution inflammatory syndrome(IRIS): pathogenesis and its clinical implications. Curr Fungal Infect Rep* 2011;**5**(4):252-61. <https://doi.org/10.1007/s12281-011-0064-8>
  48. Shi ZW, Chen Y, Ogoke KM, Strickland AB, Shi M. *Cryptococcal immune reconstitution inflammatory syndrome: from clinical studies to animal experiments. Microorganisms* 2022;**10**(12):2419. <https://doi.org/10.3390/microorganisms10122419>
  49. Roemer T, Krysan DJ. *Antifungal drug development: challenges, unmet clinical needs, and new approaches. Cold Spring Harb Perspect Med* 2014;**4**(5):a019703. <https://doi.org/10.1101/cshperspect.a019703>
  50. Boulware DR, Atukunda M, Kagimu E, Musubire AK, Akampurira A, Tugume L, et al. *Oral lipid nanocrystal amphotericin B for cryptococcal meningitis: a randomized clinical trial. Clin Infect Dis* 2023;**77**(12):1659-67. <https://doi.org/10.1093/cid/ciad440>
  51. Lockhart SR, Fothergill AW, Iqbal N, Bolden CB, Grossman NT, Garvey EP, et al. *The investigational fungal Cyp51 inhibitor VT-1129 demonstrates potent in vitro activity against Cryptococcus neoformans and Cryptococcus gattii. Antimicrob Agents Chemother* 2016;**60**(4):2528-31. <https://doi.org/10.1128/AAC.02770-15>
  52. Wang L, Zhang M, Guo J, Guo W, Zhong N, Shen H, et al. *In vitro activities of the tetrazole VT-1161 compared with itraconazole and fluconazole against Cryptococcus and non-albicans Candida species. Mycologia* 2021;**113**(5):918-25. <https://doi.org/10.1080/00275514.2021.1913949>
  53. Garvey EP, Sharp AD, Warn PA, Yates CM, Schotzinger RJ. *The novel fungal CYP51 inhibitor VT-1598 is efficacious alone and in combination with liposomal amphotericin B in a murine model of cryptococcal meningitis. J Antimicrob Chemother* 2018;**73**(10):2815-22. <https://doi.org/10.1093/jac/dky242>
  54. Murray A, Cass L, Ito K, Pagani N, Armstrong-James D, Dalal P, et al. *PC945, a novel inhaled antifungal agent, for the treatment of respiratory fungal infections. J Fungi (Basel)* 2020;**6**(4):373. <https://doi.org/10.3390/jof6040373>
  55. Shaw KJ, Schell WA, Covell J, Duboc G, Giamberardino C, Kapoor M, et al. *In vitro and in vivo evaluation of APX001A/APX001 and other Gwt1 inhibitors against*

- cryptococcus*. *Antimicrob Agents Chemother* 2018;**62**(8):e00523-18. <https://doi.org/10.1128/AAC.00523-18>
56. Giamberardino CD, Schell WA, Tenor JL, Toffaletti DL, Palmucci JR, Marius C, et al. *Efficacy of APX2039 in a rabbit model of cryptococcal meningitis*. *mBio* 2022;**13**(6):e0234722. <https://doi.org/10.1128/mbio.02347-22>
  57. Wiederhold NP. *Review of T-2307, an investigational agent that causes collapse of fungal mitochondrial membrane potential*. *J Fungi (Basel)* 2021;**7**(2):130. <https://doi.org/10.3390/jof7020130>
  58. Koselny K, Green J, DiDone L, Halterman JP, Fothergill AW, Wiederhold NP, et al. *The celecoxib derivative AR-12 has broad-spectrum antifungal activity in vitro and improves the activity of fluconazole in a murine model of cryptococcosis*. *Antimicrob Agents Chemother* 2016;**60**(12):7115-27. <https://doi.org/10.1128/AAC.01061-16>
  59. Vahedi-Shahandashti R, Lass-Flörl C. *In vitro activity of SF001: a next-generation polyene versus amphotericin B*. *Antimicrob Agents Chemother* 2025;**69**(6):e00322-25. <https://doi.org/10.1128/aac.00322-25>
  60. Li L, Wu H, Zhu S, Ji Z, Chi X, Xie F, et al. *Discovery of novel 7-hydroxy-5-oxo-4,5-dihydrothieno[3,2-b]pyridine-6-carboxamide derivatives with potent and selective antifungal activity against Cryptococcus species*. *J Med Chem* 2022;**65**(16):11257-69. <https://doi.org/10.1021/acs.jmedchem.2c00794>
  61. Kryštůfek R, Šácha P, Starková J, Brynda J, Hradilek M, Tloušťová E, et al. *Re-emerging aspartic protease targets: examining Cryptococcus neoformans major aspartyl peptidase 1 as a target for antifungal drug discovery*. *J Med Chem* 2021;**64**(10):6706-19. <https://doi.org/10.1021/acs.jmedchem.0c02177>
  62. Donlin MJ, Lane TR, Riabova O, Lepioshkin A, Xu E, Lin J, et al. *Discovery of 5-nitro-6-thiocyanatopyrimidines as inhibitors of Cryptococcus neoformans and Cryptococcus gattii*. *ACS Med Chem Lett* 2021;**12**(5):774-81. <https://doi.org/10.1021/acsmedchemlett.1c00038>
  63. Iyer KR, Li SC, Revie NM, Lou JW, Duncan D, Fallah S, et al. *Identification of triazenyl indoles as inhibitors of fungal fatty acid biosynthesis with broad-spectrum activity*. *Cell Chem Biol* 2023;**30**(7):795-810.e8. <https://doi.org/10.1016/j.chembiol.2023.06.005>
  64. Puumala E, Fallah S, Robbins N, Cowen LE. *Advancements and challenges in antifungal therapeutic development*. *Clin Microbiol Rev* 2024;**37**(1):e0014223. <https://doi.org/10.1128/cmr.00142-23>
  65. Scannell JW, Bosley J, Hickman JA, Dawson GR, Truebel H, Ferreira GS, et al. *Predictive validity in drug discovery: what it is, why it matters and how to improve it*. *Nat Rev Drug Discov* 2022;**21**(12):915-31. <https://doi.org/10.1038/s41573-022-00552-x>
  66. De Rycker M, Baragaña B, Duce SL, Gilbert IH. *Challenges and recent progress in drug discovery for tropical diseases*. *Nature* 2018;**559**(7715):498-506. <https://doi.org/10.1038/s41586-018-0327-4>
  67. Robertson EJ, Najjuka G, Rolfes MA, Akampurira A, Jain N, Anantharanjit J, et al. *Cryptococcus neoformans ex vivo capsule size is associated with intracranial pressure and host immune response in HIV-associated cryptococcal meningitis*. *J Infect Dis* 2014;**209**(1):74-82. <https://doi.org/10.1093/infdis/jit435>
  68. Kovanda LL, Giamberardino C, McEntee L, Toffaletti DL, Franke KS, Bartuska A, et al. *Pharmacodynamics of isavuconazole in a rabbit model of cryptococcal meningoencephalitis*. *Antimicrob Agents Chemother* 2019;**63**(9):e00546-19. <https://doi.org/10.1128/AAC.00546-19>

69. Lestner J, McEntee L, Johnson A, Livermore J, Whalley S, Schwartz J, et al. *Experimental models of short courses of liposomal amphotericin B for induction therapy for cryptococcal meningitis. Antimicrob Agents Chemother* 2017;**61**(6):e00090-17. <https://doi.org/10.1128/AAC.00090-17>
70. Sionov E, Chang YC, Garraffo HM, Kwon-Chung KJ. *Heteroresistance to fluconazole in Cryptococcus neoformans is intrinsic and associated with virulence. Antimicrob Agents Chemother* 2009;**53**(7):2804-15. <https://doi.org/10.1128/AAC.00295-09>
71. Livermore J, Howard SJ, Sharp AD, Goodwin J, Gregson L, Felton T, 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* 2014;**5**(1):e00725-13. <https://doi.org/10.1128/mBio.00725-13>
72. Giamberardino CD, Tenor JL, Toffaletti DL, Palmucci JR, Schell W, Boua J-VK, et al. *Pharmacodynamics of ATI-2307 in a rabbit model of cryptococcal meningoencephalitis. Antimicrob Agents Chemother* 2023;**67**(10):e0081823. <https://doi.org/10.1128/aac.00818-23>
73. Pang PD, Ahmed SM, Nishiga M, Stockbridge NL, Wu JC. *Tackling the challenges of new approach methods for predicting drug effects from model systems. Nat Rev Drug Discov* 2024;**23**(8):565-6. <https://doi.org/10.1038/d41573-024-00081-9>
74. Roosen L, Maes D, Musetta L, Himmelreich U. *Preclinical models for cryptococcosis of the CNS and their characterization using in vivo imaging techniques. J Fungi (Basel)* 2024;**10**(2):146. <https://doi.org/10.3390/jof10020146>
75. Zaragoza O, García-Rodas R, Nosanchuk JD, Cuenca-Estrella M, Rodríguez-Tudela JL, Casadevall A. *Fungal cell gigantism during mammalian infection. PLoS Pathog* 2010;**6**(6):e1000945. <https://doi.org/10.1371/journal.ppat.1000945>
76. Chastain DB, Rao A, Yaseyyedi A, Henao-Martínez AF, Borges T, Franco-Paredes C. *Cerebral cryptococcomas: a systematic scoping review of available evidence to facilitate diagnosis and treatment. Pathogens* 2022;**11**(2):205. <https://doi.org/10.3390/pathogens11020205>
77. Charlier C, Chrétien F, Baudrimont M, Mordelet E, Lortholary O, Dromer F. *Capsule structure changes associated with Cryptococcus neoformans crossing of the blood-brain barrier. Am J Pathol* 2005;**166**(2):421-32. [https://doi.org/10.1016/S0002-9440\(10\)62265-1](https://doi.org/10.1016/S0002-9440(10)62265-1)
78. European Medicines Agency. Guideline on the clinical evaluation of antifungal agents for the treatment and prophylaxis of invasive fungal disease; 2010. ([https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-clinical-evaluation-antifungal-agents-treatment-and-prophylaxis-invasive-fungal-disease\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-clinical-evaluation-antifungal-agents-treatment-and-prophylaxis-invasive-fungal-disease_en.pdf)). [Accessed March 4, 2025].
79. Pallmann P, Bedding AW, Choodari-Oskoei B, Dimairo M, Flight L, Hampson LV, et al. *Adaptive designs in clinical trials: why use them, and how to run and report them. BMC Med* 2018;**16**(1):29. <https://doi.org/10.1186/s12916-018-1017-7>
80. Marr KA, Sun Y, Spec A, Lu N, Panackal A, Bennett J, et al. *A multicenter, longitudinal cohort study of cryptococcosis in human immunodeficiency virus-negative people in the United States. Clin Infect Dis* 2020;**70**(2):252-61. <https://doi.org/10.1093/cid/ciz193>
81. Pirofski LA, Casadevall A. *Immune-mediated damage completes the parabola: Cryptococcus neoformans pathogenesis can reflect the outcome of a weak or strong immune response. mBio* 2017;**8**(6):e02063-17. <https://doi.org/10.1128/mBio.02063-17>
82. de Oliveira L, Melhem MSC, Buccheri R, Chagas OJ, Vidal JE, Diaz-Quijano FA. *Early clinical and microbiological predictors of outcome in hospitalized patients with*



- cryptococcal meningitis. BMC Infect Dis* 2022;**22**(1):138.  
<https://doi.org/10.1186/s12879-022-07118-7>
83. Loyse A, Dromer F, Day J, Lortholary O, Harrison TS. *Flucytosine and cryptococcosis: time to urgently address the worldwide accessibility of a 50-year-old antifungal. J Antimicrob Chemother* 2013;**68**(11):2435-44.  
<https://doi.org/10.1093/jac/dkt221>
  84. Bermas A, Geddes-McAlister J. *Combating the evolution of antifungal resistance in Cryptococcus neoformans. Mol Microbiol* 2020;**114**(5):721-34.  
<https://doi.org/10.1111/mmi.14565>
  85. Bongomin F, Oladele RO, Gago S, Moore CB, Richardson MD. *A systematic review of fluconazole resistance in clinical isolates of Cryptococcus species. Mycoses* 2018;**61**(5):290-7. <https://doi.org/10.1111/myc.12747>
  86. Singh N, Huprikar S, Burdette SD, Morris MI, Blair JE, Wheat LJ, et al. *Donor-derived fungal infections in organ transplant recipients: guidelines of the American Society of Transplantation, infectious diseases community of practice. Am J Transplant* 2012;**12**(9):2414-28. <https://doi.org/10.1111/j.1600-6143.2012.04100.x>
  87. Zafar H, Altamirano S, Ballou ER, Nielsen K. *A titanic drug resistance threat in Cryptococcus neoformans. Curr Opin Microbiol* 2019;**52**:158-64.  
<https://doi.org/10.1016/j.mib.2019.11.001>
  88. Stone NR, Rhodes J, Fisher MC, Mfinanga S, Kivuyo S, Rugemalila J, et al. *Dynamic ploidy changes drive fluconazole resistance in human cryptococcal meningitis. J Clin Invest* 2019;**129**(3):999-1014. <https://doi.org/10.1172/JCI124516>
  89. Sionov E, Lee H, Chang YC, Kwon-Chung KJ. *Cryptococcus neoformans overcomes stress of azole drugs by formation of disomy in specific multiple chromosomes. PLoS Pathog* 2010;**6**(4):e1000848. <https://doi.org/10.1371/journal.ppat.1000848>
  90. Iyer KR, Revie NM, Fu C, Robbins N, Cowen LE. *Treatment strategies for cryptococcal infection: challenges, advances and future outlook. Nat Rev Microbiol* 2021;**19**(7):454-66. <https://doi.org/10.1038/s41579-021-00511-0>

## Figure legend

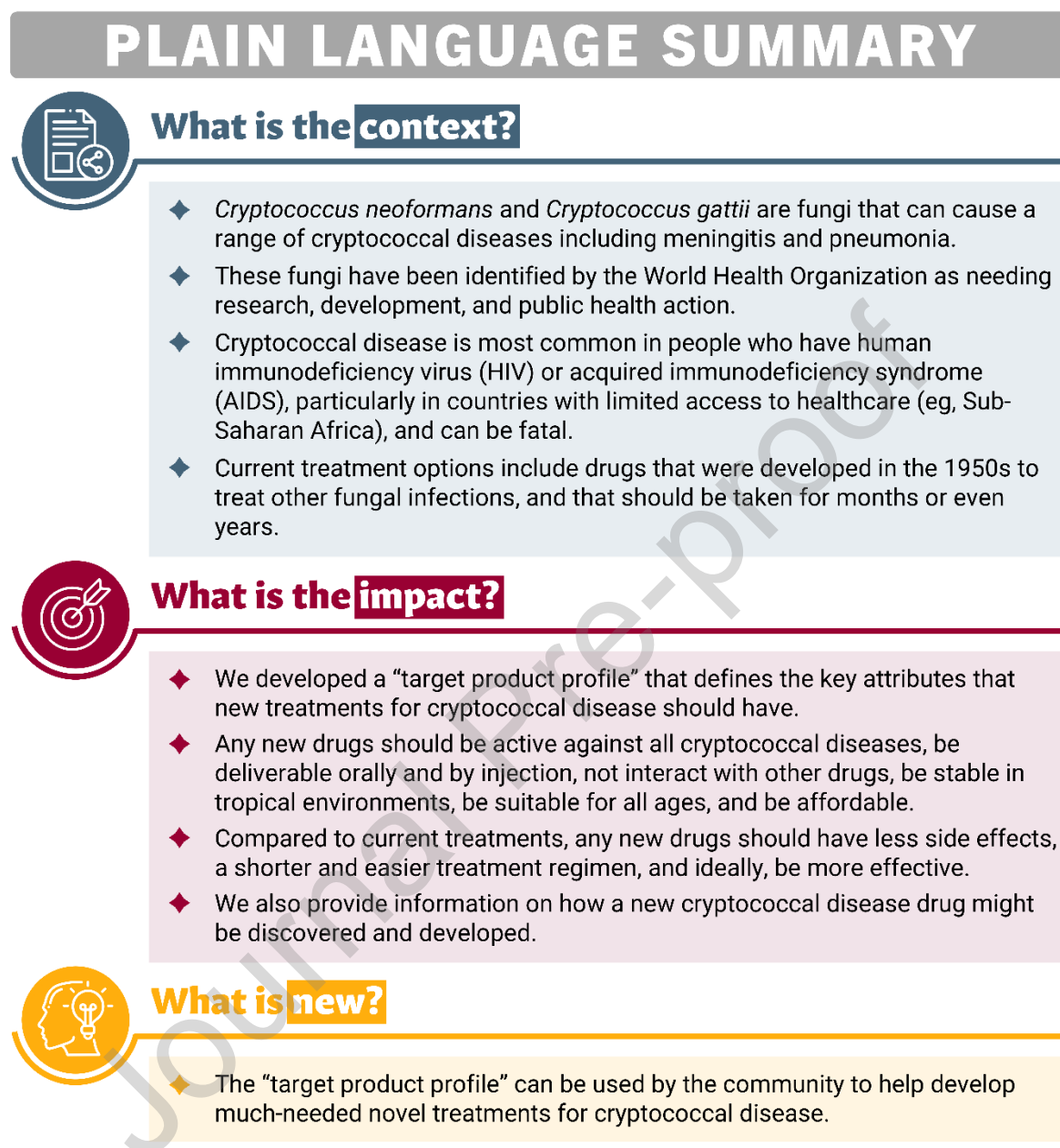


Fig. 1: Plain Language Summary

Table 1

2022 WHO guidelines for the treatment of CM in people with HIV.<sup>22</sup>

Preferred induction phase regimen

<ul style="list-style-type: none"> <li>• One high dose of L-AmB (10 mg/kg) and</li> <li>• 14 days of flucytosine (100 mg/kg/day<sup>a</sup>) and fluconazole (1200 mg/day for adults<sup>b</sup>)</li> </ul>
Alternative induction phase regimens
<p>If L-AmB is not available:</p> <ul style="list-style-type: none"> <li>• 7 days of D-AmB (1 mg/kg/day) and flucytosine (100 mg/kg/day<sup>a</sup>) then</li> <li>• 7 days of fluconazole (1200 mg/day for adults<sup>b</sup>)</li> </ul> <p>If L-AmB and D-AmB are not available:</p> <ul style="list-style-type: none"> <li>• 14 days of flucytosine (100 mg/kg/day<sup>a</sup>) and fluconazole (1200 mg/day for adults<sup>b</sup>)</li> </ul> <p>If flucytosine is not available:</p> <ul style="list-style-type: none"> <li>• 14 days of L-AmB (3–4 mg/kg/day) and fluconazole (1200 mg/day for adults<sup>b</sup>)</li> </ul> <p>If L-AmB and flucytosine are not available:</p> <ul style="list-style-type: none"> <li>• 14 days of D-AmB (1 mg/kg/day) and fluconazole (1200 mg/day for adults<sup>b</sup>)</li> </ul>
Consolidation phase
<ul style="list-style-type: none"> <li>• Fluconazole (800 mg/day for adults<sup>c</sup>) for 8 weeks following induction</li> </ul>
Maintenance phase
<ul style="list-style-type: none"> <li>• Fluconazole (200 mg/day for adults<sup>d</sup>) until immune reconstitution (CD4 &gt; 200 cells/mm<sup>3</sup>) and suppression of viral loads on ART</li> </ul>

Abbreviations: ART: antiretroviral therapy, CD4: cluster of differentiation 4, CM: cryptococcal meningitis, D-AmB: amphotericin B deoxycholate, HIV: human immunodeficiency virus, L-AmB: liposomal amphotericin B, WHO: World Health Organization.

<sup>a</sup>Divided into four doses per day.

<sup>b</sup>12 mg/kg/day for children and adolescents up to a maximum of 800 mg/day.

<sup>c</sup>6–12 mg/kg/day for children and adolescents up to a maximum of 800 mg/day.

<sup>d</sup>6 mg/kg/day for adolescents and children.

**Table 2**

TPP for cryptococcal disease including CM.

Attribute	Minimal TPP	Preferred TPP
Indication(s) for use	<ul style="list-style-type: none"> <li>• Tx of symptomatic and subclinical CM</li> <li>• Tx of symptomatic systemic cryptococcal disease (i.e. pulmonary,</li> </ul>	<ul style="list-style-type: none"> <li>• Tx of symptomatic and subclinical CM</li> <li>• Tx of symptomatic systemic cryptococcal disease (i.e. pulmonary, skin, lymph node, and bone)</li> </ul>

	skin, lymph node, and bone) <ul style="list-style-type: none"> <li>• Tx of asymptomatic people who are CrAg+</li> <li>• Activity against all species</li> </ul>	<ul style="list-style-type: none"> <li>• Tx of asymptomatic people who are CrAg+</li> <li>• Activity against all species</li> <li>• Prophylaxis against cryptococcal disease</li> </ul>
Target patient populations	<ul style="list-style-type: none"> <li>• Apparently immunocompetent and immunocompromised adults and adolescents with symptomatic CM</li> <li>• Apparently immunocompetent and immunocompromised adults and adolescents with symptomatic systemic cryptococcal disease (i.e. pulmonary, skin, lymph node, and bone)</li> <li>• Apparently immunocompetent and immunocompromised CrAg+ adults and children</li> </ul>	<ul style="list-style-type: none"> <li>• Apparently immunocompetent and immunocompromised adults and children with CM</li> <li>• Apparently immunocompetent and immunocompromised adults and children with symptomatic systemic cryptococcal disease (i.e. pulmonary, skin, lymph node, and bone)</li> <li>• Apparently immunocompetent and immunocompromised CrAg+ adults and children</li> <li>• Asymptomatic CrAg+ adults and children</li> <li>• Including use during pregnancy and lactation</li> </ul>
Clinical efficacy	<ul style="list-style-type: none"> <li>• Non-inferior to SoC<sup>a</sup> with activity against all causative species</li> </ul>	<ul style="list-style-type: none"> <li>• Superior to SoC<sup>a</sup> with activity against all causative species</li> <li>• High barrier to resistance</li> </ul>
Safety/tolerability	<ul style="list-style-type: none"> <li>• Improved safety and tolerability profile compared to current SoC<sup>b</sup></li> </ul>	<ul style="list-style-type: none"> <li>• Improved safety and tolerability profile compared to current SoC<sup>b</sup></li> <li>• Minimal or no requirement for monitoring (current therapy requires renal, hepatic, and anaemia monitoring)</li> <li>• Good tolerability in children</li> <li>• Non-teratogenic and improved safety during breastfeeding</li> </ul>
Formulation/presentation	<ul style="list-style-type: none"> <li>• Oral (+ nasogastric) and intravenous injection</li> </ul>	<ul style="list-style-type: none"> <li>• Easier administration</li> <li>• Oral (+ nasogastric) and intravenous, intramuscular, and subcutaneous injections</li> <li>• Good palatability and swallowability in adults with swallowing difficulties and children</li> </ul>

Dose regimen	<ul style="list-style-type: none"> <li>• Twice daily</li> <li>• Part of combination therapy</li> <li>• Induction: 1 week; consolidation: 4–6 weeks; (maintenance: until immune reconstitution<sup>c</sup>)</li> </ul>	<ul style="list-style-type: none"> <li>• Once or twice daily</li> <li>• Single drug</li> <li>• Total duration: 2–4 weeks (sterilising – no requirement for induction / consolidation / maintenance)</li> </ul>
Drug–drug interactions	<ul style="list-style-type: none"> <li>• Minimal/manageable drug interactions with concomitant medications (i.e. ART, transplant-conditioning agents, antimalarials, antituberculosis, cotrimoxazole, antifungals, anticonvulsants, corticosteroids, etc.)</li> </ul>	<ul style="list-style-type: none"> <li>• No drug interactions with concomitant medications (i.e. ART, antimalarials, antituberculosis, cotrimoxazole, antifungals, anticonvulsants, corticosteroids, etc.)</li> </ul>
Product stability and storage	<ul style="list-style-type: none"> <li>• Heat stable, 3-year shelf-life in hot tropic/humid climate (simulated with 30°C and 65% relative humidity)</li> </ul>	<ul style="list-style-type: none"> <li>• Heat stable, 3-year shelf-life in hot tropic/humid climate (simulated with 30°C and 65% relative humidity)</li> <li>• Stability of formulated suspension/intravenous formulations for multiple days without requirement for refrigeration</li> </ul>
Product registration path	<ul style="list-style-type: none"> <li>• WHO-listed authority approval followed by WHO pre-qualification</li> </ul>	<ul style="list-style-type: none"> <li>• WHO-listed authority approval followed by WHO pre-qualification</li> </ul>
Patient and payor value proposition	<ul style="list-style-type: none"> <li>• Novel, effective, and safe</li> <li>• Suitable for full spectrum of affected populations<sup>d</sup></li> <li>• No requirement for hospitalisation specifically to administer or monitor treatment</li> <li>• Reduction in required monitoring for adverse events</li> <li>• Contraindication: pregnancy/lactation</li> <li>• Minimal/manageable drug interactions</li> <li>• Potential for use as part of combination regimen</li> </ul>	<ul style="list-style-type: none"> <li>• Novel, effective, and safe</li> <li>• Suitable for full spectrum of affected populations<sup>d</sup></li> <li>• No requirement for hospitalisation specifically to administer or monitor treatment</li> <li>• No adverse events requiring close monitoring</li> <li>• No contraindications/interactions (suitable for use in combination)</li> <li>• Reduction in incidence of IRIS</li> <li>• Improved compliance due to shorter duration of therapy</li> <li>• CNS penetrant<sup>e</sup></li> <li>• Increased access to treatment</li> </ul>

	<ul style="list-style-type: none"> <li>• CNS penetrant<sup>e</sup></li> <li>• Increased access to treatment</li> <li>• Affordable target cost informed by cost-effectiveness analysis</li> </ul>	<ul style="list-style-type: none"> <li>• Affordable target cost informed by cost-effectiveness analysis</li> </ul>
--	--	--

Abbreviations: ART: antiretroviral therapy, BBB: blood–brain barrier, CM: cryptococcal meningitis, CNS: central nervous system, CrAg+: cryptococcal antigen positive, CYP2C9: Cytochrome P450 family 2 subfamily C member 9, CYP3A4: Cytochrome P450 family 3 subfamily A member 4, D-AmB: amphotericin B deoxycholate, HIV: human immunodeficiency virus, IRIS: immune reconstitution inflammatory syndrome, L-AmB: liposomal amphotericin B, QT: length of time between the start of the Q-wave and the end of the T-wave, SoC: standard of care, TPP: target product profile, Tx: treatment, WHO: World Health Organization.

<sup>a</sup>L-AmB + flucytosine + fluconazole followed by fluconazole for people with HIV (see Table 1) or other gold standard treatment in other populations.

<sup>b</sup>D-AmB/L-AmB – anaphylaxis, gastrointestinal adverse events, rigors, fever, hypertension or hypotension, hypoxia, nephrotoxicity; flucytosine – gastrointestinal adverse events, hepatic adverse events, bone-marrow suppression, teratogenicity; fluconazole – gastrointestinal adverse events, hepatic adverse events, rash, QT prolongation, CYP2C9 and CYP3A4 inhibition; teratogenicity.

<sup>c</sup>Only for people with HIV.

<sup>d</sup>From CrAg+, asymptomatic individuals to obtunded patients requiring hospitalisation, including patients with renal impairment, anaemia, and/or neutropenia, and safe in pregnancy or whilst breastfeeding.

<sup>e</sup>BBB integrity is uncertain in patients with subclinical CM, but in patients with CM, increased permeability could allow non-CNS permeable compounds.

## Declaration of Interest Statement

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The author is an Editorial Board Member/Editor-in-Chief/Associate Editor/Guest Editor for this journal and was not involved in the editorial review or the decision to publish this article.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

**DBM** received grants from the National Institutes of Health and from the UK Medical Research Council, outside of the submitted work. He also received payment for consulting from GSK, support from the National Institutes of Health for attending meetings, participated on Data Safety Monitoring Boards or Advisory Boards for the 5FC HIV-Crypto Study, the FIEBRE study, and the New Strat TB study. He also has a leadership role in MARA NGO and chairs the Advanced HIV/TB Technical Working Group, Ministry of Health in Uganda.

**MDR** and **IHG** received Wellcome grant funding for their attendance at the International Conference on *Cryptococcus* & *Cryptococcosis* in Uganda (January 2023), and a grant from the Tres Cantos Open Lab Foundation to develop cell-based assays for *Cryptococcus* drug discovery.

**WH** received grants from Bugworks and GARDP, personal fees from GSK, Mundipharma, and Pulmocide, and grants and personal fees from F2G, Pfizer, and Phico Therapeutics, outside of the submitted work. He also declares having participated in Advisory Boards for F2G and Basilea; and being an NIHR Speciality Co-Lead for Infection.

**MK** is employed by ViiV Healthcare and holds financial equities in GSK.

**AL** received grants from NIHR (Imprint project), NIH (Effect trial), and EDCTP (DREAMM project, 5FC HIV Crypto) and consulting fees from the Unitaid-CHAI AHD programme to her institution (City St George's University of London), outside of the submitted work. She is a member of the ENCORE study data safety monitoring board and leads the End AIDS Action group that encompasses the work of the Cryptococcal Meningitis Action Group (CryptoMAG) (unfunded roles). She also received CrAg LFA tests from Biosynex for the DREAMM project, outside of the submitted work. No payment was received for this work.

**PM-L** is employed by GSK.

**JJO** is the lead for DNDi, which is a sub-recipient under Aurum Institute for the IMPAACT4HIV advanced HIV disease grant, and part of her salary is paid through this grant. JJO is also the project lead for a European & Developing Countries Clinical Trial Partnership (EDCTP) funded grant at DNDi, and her salary is partially paid through this grant. **LKT** is employed by ViiV Healthcare and holds financial equities in GSK.

**PRW** reports Division of Intermural Research (DIR) grant supports at NIH for the present manuscript: National Institutes of Health, NIAID AI001123; and National Institutes of Health, NIAID AI001124. He also reports two patents: Williamson, PR, Panepinto, JC, Qiu J.



“Small molecule imaging of fungi by positron emission tomography scanning” U.S. Patent Number 9968693, Issued May 15, 2018, DIR/NIAID/NIH; and Hammoud DL, et al.

“Radiolabeled Sugars for imaging of fungal infections” Application: E-163-2019-0-PCT-02.

Outside of the submitted work, he also declares being a non-voting member of the CLSI.

**TJM** is employed by GSK, has a non-paid adjunct professor role at the University of Dundee, and holds financial equities in GSK.

**IR** is employed by the Drugs for Neglected Diseases initiative, which is a not-for-profit drug development organisation. She received payments of her salary as Viral Disease Cluster, overseeing exploratory and drug development activities in cryptococcal meningitis.

The authors declare no other financial and non-financial relationships and activities.

## Highlights

- *Cryptococcus neoformans* is a World Health Organization priority pathogen.
- A target product profile (TPP) for new anti-cryptococcal agents is described.
- Key attributes: tolerability, activity across disease subtypes, regimen simplicity.
- Other attributes: widespread suitability, manageable drug-drug interactions.
- This TPP aims to assist the development of novel cryptococcal disease treatments.