Combination Therapy for Mucormycosis: Why, What, and How?

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The high mortality rate of mucormycosis with currently available monotherapy, particularly in hematology patients, has stimulated interest in studying novel combinations of antifungal agents to determine whether superior outcomes might be achieved. Combination lipid polyene-echinocandin therapy is the most promising of such regimens based on safety profile, the availability of parenteral formulations of echinocandins, their synergy in murine models of mucormycosis, and observational clinical data that are concordant. Other options include combination lipid polyene plus deferasirox or posaconazole therapy. Definitive, randomized, placebo-controlled phase III clinical trials are needed to determine whether combination therapy with any of these options is superior to monotherapy. Until such studies are conducted, clinicians will continue to be placed in the unacceptable position of not knowing if and when to administer combination therapy. Such a state of confusion may lead to undertreatment if combination therapy is indeed superior but is not used and, conversely, may lead to unacceptable toxicity and cost to patients if combination therapy is not superior but is used. It is critical that sponsors step forward with funding to conduct these clinical trials to determine whether outcomes from these devastating infections can be improved.

IMPORTANCE OF STUDIES OF COMBINATION THERAPY FOR MUCORMYCOSIS

The mortality rate of mucormycosis treated with available antifungal monotherapy remains unacceptable [1–7]. In recent years, promising combination-therapy strategies have been described in preclinical models and in retrospective and open label case series [8]. Concordance between preclinical efficacy and observational clinical studies offers hope that such strategies may improve survival in patients with mucormycosis, yet small, retrospective, observational studies cannot serve as the basis for introducing combination therapy as the standard of care. It is critical to conduct well-controlled, large-scale, randomized, double-blinded, placebo-controlled trials to definitively compare the efficacy of combination therapy with that of monotherapy for this disease. Failure to conduct such studies will leave clinicians in permanent therapeutic limbo, unsure of which strategies are most effective and least toxic for this deadly infection. If outcomes of monotherapy cannot be improved on, it is incumbent on the scientific community to prove this, so that more expensive, potentially toxic combination regimens are not de facto adopted as the standard of care.

CHOOSING WHICH COMBINATION-THERAPY STUDIES TO CONDUCT

A reasonable underlying principle for prioritization of therapeutic strategies to advance to large-scale, expensive,
phase III clinical trials is a requirement that the strategies be shown to (1) markedly improve survival in relevant animal models of mucormycosis, (2) have available retrospective or observational clinical data that are concordant with the preclinical models both for efficacy and safety, and (3) involve therapeutic agents that are already approved by relevant governmental regulatory agencies for use in humans. Based on these criteria, the following 3 potential strategies are selected for discussion: (1) combination lipid polyene-echinocandin clinical studies, (2) combination lipid polyene-deferasirox clinical studies, and (3) combination lipid polyene-posaconazole (or isavuconazole) clinical studies (Table 1).

A variety of other adjunct strategies are also now available, including novel cytokine therapies, and other biological strategies, such as white blood cell transfusions and hyperbaric oxygen [8]. For example, liposomal amphotericin B (LAmB) combined with granulocyte-macrophage colony-stimulating factor recently was shown to be efficacious in a murine model of invasive mucormycosis [9]. However, these approaches are not yet as developed as novel antifungal agent combinations, and they require additional proof of concept in animal models before being prioritized for large-scale clinical investigations.

**Lipid Polyene as Backbone Therapy**

Any potential combination-therapy study under consideration for testing in patients with mucormycosis should use a polyene as backbone therapy. An equally important consideration for a combination-therapy study will be which polyene is optimal for use as backbone therapy. Amphotericin B deoxycholate (AmB) remains the only antifungal agent licensed by the US Food and Drug Administration for primary therapy of mucormycosis. However, 2 lipid formulations of amphotericin, amphotericin B lipid complex (ABLC) and LAmB, are licensed for the treatment of invasive mold infections that are refractory to or occur in patients intolerant of AmB.

For numerous reasons, AmB should not be used as the backbone therapy for a combination-therapy study. First, the drug is clearly more toxic than lipid formulations [10–16]. Second, in mouse models of mucormycosis and in retrospective clinical studies, high-dose LAmB therapy was more effective than AmB [6, 17]. Third, given the marked decline in acceptability of use of AmB to many physicians due to its toxicity, it is likely that a study requiring use of AmB would not meet enrollment targets in the US or Europe. Fourth, neither AmB nor lipid amphotericin B derivatives have ever been, or will ever be, tested against placebo in a monotherapy randomized trial of mucormycosis; the indication of AmB for primary treatment of mucormycosis is based strictly on the fact that for many decades, AmB was the only antifungal agent available for such agents. Hence, a lipid polyene is the preferred backbone agent in a placebo-controlled, combination superiority clinical trial, and relevant government regulatory agencies should comfortably accept lipid polyene plus placebo as the comparator regimen in a superiority combination-therapy clinical trial.

Either LAmB or ABLC are reasonable choices for backbone therapy; few data are available to guide selection of one drug over the other, although limited data suggest that LAmB may be less nephrotoxic and may result in superior eradication of fungus from the central nervous system (CNS) and higher cure rates with CNS mucormycosis [18–20]. Selection of which dose of lipid polyene for a combination-therapy study is also difficult based on available data [8]. Based on pharmacokinetic data, a reasonable dose for LAmB would be 5 mg/kg per day, with possible escalation to 10 mg/kg/d in patients with CNS infection [8]. Of note, a pilot study of initial high-dose (10 mg/kg/d) LAmB for mucormycosis has been recently completed in France.

### Table 1. Options for Combination-Therapy Studies for Mucormycosis

<table>
<thead>
<tr>
<th>Combination Therapy With Lipid Polyene</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echinocandins</td>
<td>Very safe; intravenous formulations available; strong preclinical data; concordant observational clinical study</td>
<td>Will industrial sponsor pay for study?</td>
</tr>
<tr>
<td>Deferasirox</td>
<td>Strong preclinical data; concordant observational clinical data (highly limited)</td>
<td>Phase II DEFEAT Mucor study failed to show benefit, and there was excess mortality in patients who received deferasirox therapy, particularly in those with active hematological malignancy; no intravenous formulation; will industrial sponsor pay for study?</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>Safe; in vitro activity against some Mucorales</td>
<td>Preclinical data poor; no clinical data; pharmacokinetic-pharmacodynamic concerns; no intravenous formulation; will industrial sponsor pay for study?</td>
</tr>
<tr>
<td>Isavuconazole</td>
<td>In vitro activity against Mucorales; intravenous formulation in late-stage development</td>
<td>No combination-therapy data in mice and no clinical data yet available; will industrial sponsor pay for study?</td>
</tr>
<tr>
<td>Echinocandins plus deferasirox</td>
<td>Maximal aggressiveness</td>
<td>Study would be large to enable comparison with both dual-therapy options; safety concerns and no intravenous formulation for deferasirox</td>
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**Abbreviation:** DEFEAT Mucor, Deferasirox–AmBisome Therapy for Mucormycosis.
with 40 patients included (AmbiZygo study, NCT00467883). Dose escalation beyond 10 mg/kg/d does not result in enhanced serum levels of LAmB [21]. ABLC can also be dosed at 5 mg/kg/d, and no data support dose escalation beyond 5 mg/kg/d of ABLC.

**Combination Polyene-Echinocandin Clinical Trials**

Although the echinocandins do not have in vitro activity against the Mucorales in standard susceptibility tests, *Rhizopus oryzae* does express the target enzyme for echinocandins [22]. Furthermore, during disseminated mucormycosis in mice with diabetic ketoacidosis, combination caspofungin plus ABLC therapy markedly and synergistically improved survival compared with monotherapy or placebo [23]. Combination therapy with LAmB plus either micafungin or anidulafungin also markedly improved survival in neutropenic and DKA mice with disseminated mucormycosis [24].

The notable synergy seen in murine models is concordant with the results of a small, retrospective clinical study in which combination ABLC or LAmB plus caspofungin therapy was associated with significantly improved survival for patients with rhino-orbitocerebral mucormycosis compared with polyene monotherapy [19]. By multivariate analysis, only combination therapy was significantly associated with superior outcomes (odds ratio, 10.9 for success vs monotherapy; $P = .02$). The study was limited by its small size (a total of 41 patients), retrospective nature, and the fact that almost all patients enrolled were diabetic, few had malignancy and none were hematopoietic stem cell transplant recipients. Nevertheless, the established safety profile of echinocandins, and the concordance of the robust murine efficacy data with the available clinical data, provide strong rationale for a phase III, randomized, double-blinded, placebo-controlled study to determine whether lipid polyene plus echinocandins are indeed superior to lipid polyene plus placebo therapy (Table 1).

**Combination Polyene-Deferasirox Clinical Trials**

Deferasirox is an orally available iron chelator, approved in the United States and Europe for the treatment of iron overload in transfusion-dependent anemias [25], which is fungicidal for clinical isolates of Mucorales in vitro [26, 27]. In DKA mice with disseminated mucormycosis, deferasirox was as effective as LAmB therapy, and combination deferasirox-LAmB therapy synergistically improved survival [26]. Based on these animal data, deferasirox was successfully used as salvage therapy in a patient with advanced rhinocerebral mucormycosis who had progressive brain-stem disease despite LAmB therapy [28]. In a recent open-label experience, 8 patients received deferasirox as combination therapy, 5 for primary therapy and 3 for salvage therapy. Seven of the 8 patients survived, of whom 5 were considered cured and were not receiving any antifungal therapy after months to years of follow-up; 2 patients continued to receive antifungal therapy at last observation. The patient who died had demonstrated clinical improvement while receiving deferasirox therapy, but the disease progressed after cessation of the drug. The only toxic effects observed occurred in 2 patients who developed mild to moderate rashes that reversed on cessation of deferasirox therapy. There was no evidence of any laboratory or other toxic effects, including nephrotoxicity, which was seen in up to one-third of patients with iron overload given long-term deferasirox treatment in phase III clinical trials [29].

Unfortunately, a double-blinded, randomized, placebo-controlled, phase II safety/exploratory efficacy study of adjunctive deferasirox therapy (20 mg/kg/d for 14 days) for mucormycosis (the Deferasirox-AmBisome Therapy for Mucormycosis [DEFEAT Mucor] study, NCT00419770) has failed to demonstrate a benefit of combination therapy [30]. Rather, there was excess mortality in patients treated with adjunctive deferasirox. Unfortunately, there were more leukemic and neutropenic patients in the deferasirox arm, which could have contributed to the excess mortality. No specific toxicity was identified to account for the increased mortality in patients receiving deferasirox, and the deaths were due to progression of underlying disease and/or progression of infection. Nevertheless, no subpopulation was found in which deferasirox adjunctive therapy seemed to demonstrate benefit. Only a large study, focused on a diabetic or steroid-treated population, and excluding patients with active malignancy (particularly hematological), can clarify the potential for deferasirox to add benefit to lipid polyene therapy for mucormycosis.

In contrast to echinocandins, the safety profile of deferasirox in patients with mucormycosis is not well established. Furthermore, the drug caused mild, reversible declines in glomerular function in up to one third of patients in its pivotal studies in patients with iron overload [31, 32]. There have been rare, postmarketing reports of severe acute renal failure resulting in hemodialysis or death in iron-overloaded patients taking deferasirox [33]. There is also no parenteral formulation of deferasirox, and Souther et al [34] recently reported the failure of salvage deferasirox in a patient who had undergone partial colectomy to resect mucormycosis. Combined with the failure of the small, phase II DEFEAT Mucor study to demonstrate benefit of adjunctive deferasirox, the above factors warrant caution in protocol development for a future phase III trial of this drug for the treatment of mucormycosis, at least in patients with hematological malignancies (Table 1).

**Posaconazole or Isavuconazole Combination-Therapy Study**

The reported 90% minimum inhibitory concentrations for posaconazole with Mucorales range from 1 to $\geq 4$ μg/mL [35–39]. In febrile neutropenic patients or those with invasive fungal infections, posaconazole dosed at 400 mg orally twice daily
therapy [52]. In terms of clinical experience, one adolescent with and reduced the tissue fungal burden of mice infected with mucormycosis, demonstrating that triple combination therapy enhanced survival in late stage treatment. Furthermore, a recent study in mice posaconazole plus deferasirox, which would represent maximal aggressiveness in treatment. No clinical studies have evaluated combination posaconazole-polyene therapy for mucormycosis. These facts, combined with the current absence of a parenteral formulation, place the potential for a posaconazole combination-therapy study at lower priority (Table 1).

Isavuconazole is another expanded spectrum azole with in vitro activity against the Mucorales, which has a parenteral formulation in late stage development [49–51]. Combination-therapy studies with isavuconazole warrant consideration if and when the drug is approved for treatment of other invasive fungal infections.

**Triple Combination Therapy**

The rationale for both echinocandins and deferasirox as second agents in combination therapy against mucormycosis raises the possibility of a triple-therapy study, with a polyene plus echinocandins plus deferasirox, which would represent maximal aggressiveness in treatment. Furthermore, a recent study in mice demonstrated that triple combination therapy enhanced survival and reduced the tissue fungal burden of mice infected with mucormycosis compared with placebo, monotherapy, or dual drug therapy [52]. In terms of clinical experience, one adolescent with osteosarcoma and disseminated *Lichtheimia coryniformis* infection received successful triple combination therapy with high doses of LAmB, posaconazole, and caspofungin [33]. These collective experiences support the concept of a triple-therapy study.

Nevertheless, a triple-therapy study would have to be very large, given the need to compare the triple-therapy regimen with both dual-therapy regimens and monotherapy. Novel strategies, such as adaptive Bayesian study designs could help mitigate sample size for such a study, but it is not clear that sufficient preliminary data exist to build a Bayesian model robust enough to design such an adaptive study. Further exploration of this possibility is warranted. In the meantime, it is likely that dual-therapy studies will have to be conducted first. If dual therapy is found to be superior to monotherapy, a triple-therapy study could subsequently be conducted.

**HOW TO CONDUCT COMBINATION-THERAPY STUDIES**

Unfortunately, by far the greatest barriers to conducting a phase III, randomized, double-blinded, placebo-controlled, combination-therapy study for mucormycosis are financial and logistical, owing to the relative rarity of the disease. Such a study is likely to require enrollment of 200–300 patients and cost in excess of $10–$20 million. For a market as small as mucormycosis, a primary challenge is raising the funds to pay for such a study from either private or public sources.

Mucormycosis is a disease of increasing frequency [54]. It continues to have a higher mortality rate than most other infections. It frequently affects young patients, resulting in a devastating impact on families, as is illustrated dramatically by the case of Henry Schueler. It causes devastating morbidity even in survivors. Hence, there is a great need for new therapies for these infections. It is hoped that sponsors will recognize the critical need for combination-therapy studies for this infection, and the public good that could result from such studies. All the authors of this article, both American and European, have recently demonstrated their commitment to recruiting such patients in reference centers and are ready to be involved with both the design and conduct of future trials.

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