

Defining Responses to Therapy and Study Outcomes in Clinical Trials of Invasive Fungal Infections: Mycoses Study Group (MSG) and European Organization for Research and Treatment of Cancer (EORTC) Consensus Criteria

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Abstract

Invasive fungal infections (IFI) have become a major cause of morbidity and mortality in highly immunocompromised patients. Authoritative consensus criteria to diagnose IFIs have recently been established and have been useful in establishing eligibility criteria for antifungal trials. There is an important need for generating consensus definitions of outcomes of IFIs that will form a standard for evaluating treatment success and failure in clinical trials. Therefore, an expert international panel consisting of the Mycoses Study Group (MSG) and European Organization for Research and Treatment of Cancer (EORTC) and other experts was convened to propose guidelines for assessing treatment responses in clinical trials of IFI and defining study outcomes. Major fungal infections that are discussed include invasive disease due to *Candida* species, *Aspergillus* species, other moulds, *Cryptococcus neoformans*, histoplasmosis and coccidioidomycosis. Potential pitfalls in assessing outcome, such as conflicting clinical radiological, and/or mycological data, and gaps in knowledge are discussed.

Introduction

Invasive fungal infections (IFIs) have become a major cause of morbidity and mortality in highly immunocompromised patients. The European Organization for Research and Treatment of Cancer (EORTC) and the Mycoses Study Group (MSG) published international consensus guidelines on definitions of IFIs to ensure standardization between trials [1]. These guidelines or modified versions have been widely used as eligibility criteria in clinical trials and are currently undergoing revision.

Just as controversies exist in the definition of IFIs, there is also considerable debate as to how a successful response to therapy should be defined. The design of the trial and study endpoints must be focused on demonstrating the benefit and safety of a new therapy in the specific setting that it is being evaluated. Fulfilling pre-specified study endpoints is a pre-requisite for drug approval.

Bennett and colleagues discussed the important challenges in the design of clinical antifungal trials [2-4]. Our objective is to generate consensus definitions of outcomes of IFIs that will form a standard for evaluating treatment success and failure in future clinical trials. Aspects of study design that are directly linked to evaluating treatment responses are discussed. In constructing these criteria, we have tried to address potential limitations in previously employed definitions. Reflecting the “bias” of our panel that is chiefly comprised of clinician investigators who care for immunocompromised patients, we have tried to construct definitions of study outcomes that the practicing physician would find most meaningful.

The proposed response criteria will be restricted to antifungal trials of established IFIs, and will not address prophylactic or empirical antifungal trials. The first section addresses general challenges and pitfalls in assessing responses in antifungal trials. In the second section,

we propose specific criteria to evaluate treatment responses to invasive disease due to *Candida* species, *Aspergillus* species, other moulds, *Cryptococcus neoformans*, histoplasmosis, and coccidioidomycosis. Our guidelines focus on defining treatment outcomes in definitive randomized trials rather than phase I and II trials in which efficacy is an exploratory endpoint that may be based primarily or entirely on laboratory criteria (e.g., sterilization of blood).

Challenges in evaluating clinical, radiological, and mycological responses

The criteria for assessing response to established IFIs generally encompass clinical, radiological, and microbiological criteria. Definitions of global responses are summarized in Table 1 and response criteria for specific IFIs are defined in Tables 2 to 5.

Assessment of clinical criteria of response entails some level of subjectivity by the treating physician. In the case of fungal pneumonia, improvements in cough or dyspnea are difficult to objectively quantify. Similarly, improvement of auscultatory findings (e.g., rales, wheezing) is non-specific and reports of these findings can vary among examiners. Even “objective” criteria such as fever and oxygen saturation can be affected by factors unrelated to IFI.

Because of the inherent ambiguities in assessing clinical criteria for response, trials appropriately require evidence of radiological or microbiological improvement to score a successful global response. However, even these “harder” criteria have limitations. Lesions followed by serial imaging may initially increase in size on therapy as a function of the natural course of infection or immune reconstitution as opposed to being a reflection of poorly controlled infection [5]. Serial microbiological assessment is difficult if it entails an invasive procedure, such as a lung biopsy. In specific IFIs, laboratory detection markers can be used as surrogates of mycological response. In clinical trials, measurement of specific markers should

ideally be performed at a single reference laboratory using the same protocol to avoid interlaboratory variability

Stable response

Most therapeutic studies require either a complete response (CR) or a partial response (PR) for a successful outcome. These assessment criteria were originally applied to tumor response to therapy [6], but have been adapted to assessing infections as well. If a CR or PR is the primary study endpoint, then patients with stable or indeterminate responses are scored in the same way as those who have failed therapy. In most IFIs, lack of improvement within a pre-specified period should be scored as failure. However, in some scenarios, a stable response may be considered a salutary outcome by both the treating physician and the patient. For example, an antifungal agent that stabilizes a previously progressive invasive mould infection in an allogeneic hematopoietic stem cell transplant (HSCT) recipient receiving intensive immunosuppressive therapy may demonstrate the efficacy of the new drug and be an acceptable short-term outcome.

Requirement for survival

The majority of panel members considered survival through at least the time of assessment of the primary endpoint to be necessary, though not sufficient, for a successful outcome. A minority of members reasonably argued that mortality may result from causes unrelated to the IFI (e.g., relapsed malignancy), and that overall survival is not an accurate reflection of drug effect; therefore, more direct markers of antifungal response (e.g., sterilization of infected sites or a reduction in a given laboratory marker) should be used as primary endpoints instead of overall survival. Pre-specified criteria for attributable mortality have been used in some antifungal studies [7, 8]. Anaissie [9] argued that in patients with invasive

aspergillosis (IA), death without autopsy evidence of persistent fungal infection should be equated with a successful outcome from the standpoint of antifungal therapy.

The majority of panel members favored using overall survival rather than absence of mortality attributed to IFI for the following reasons: First, attribution of mortality is difficult, and criteria are not standardized. Second, we likely do not understand the full effects of antifungal drugs. Drug toxicity may influence survival in ways that are not obvious to the investigator (e.g., a drug-drug interaction) [10]. In addition, the interaction of antifungal drugs with host immunity is an area of growing interest [11-16]; such interactions cannot be encapsulated solely based on fungal markers, and may influence survival in ways we don't understand. Thus, particularly for phase III clinical trials, overall survival within the pre-specified period of evaluation should be a requirement for a successful outcome. Randomization is expected to balance confounding variables (e.g., relapse of malignancy) that may affect survival independently of antifungal therapy among study arms.

Conflicting data

Evaluation of the global response is often difficult with either inadequate data or conflicting data. Protocols should ideally pre-specify a rank order of the weight given to specific categories of data should conflicting data occur. In general, objective data (e.g., tachypnea or oxygen saturation) should carry more weight than subjective data (e.g., dyspnea) and specific signs of fungal diseases (e.g., facial swelling in the case of invasive fungal sinusitis) should be given more weight than less specific signs (e.g., fever). For some diseases (e.g., candidemia and cryptococcal meningitis), culture clearance must occur for success, irrespective of clinical or radiological criteria. In other diseases in which repeat cultures may not be feasible or reliable, serial radiologic studies may be the most reliable and objective category of data, though

limitations exist. For example, in invasive pulmonary mould infections, 25% or greater reduction in the size of the lesion would provide strong evidence of a positive drug effect even if fever (which can result from multiple causes) persists. In Tables 2-5 and in the text, we suggest a hierarchy for categories of data used to assess response to specific fungal diseases.

Discordant clinical, radiologic, and/or mycologic data may result from an inadequate period of evaluation. In certain cases, it may take weeks or months to judge success or failure of an antifungal agent. It is therefore key to select time points for assessment of response following enrollment that make provision for the potential for early conflicting data. A competing concern is that a longer period of evaluation of response may increase the likelihood of seemingly unrelated events (such as relapse of malignancy) confounding the interpretation of response to antifungal therapy. Suggested minimum periods of observation for the major fungal diseases that attempt to balance these concerns are included in Tables 2 to 5.

Data Review Committee

The data review committee (DRC) is charged with assessing eligibility of patients enrolled in clinical trials and in assessing response to therapy. DRC panel members should have expertise in the diagnosis and management of IFIs and in conducting clinical trials in mycology. Radiologists should be included in the DRC panel to assess radiological responses.

In comparative trials, the DRC should be blinded to the assigned treatment group of study patients to avoid the potential for bias. In most studies, site investigators, who may or may not be blinded to the treatment regimen, are asked to provide their assessment of the global response of study patients enrolled at their site. Studies often pre-specify that the assessment by the DRC will be considered definitive. This approach has the important advantage of ensuring uniformity and avoiding bias in the case of non-blinded studies in assessing eligibility and

treatment outcomes. The major disadvantage is that case report forms reviewed by the DRC may lack important information that is available to the site investigator. For this reason, an opportunity should be given to the investigator to review his assessment and to provide additional information or to correct a potential mistake in material prior to DRC review. In addition, the investigator should be able to communicate through an intermediary (to avoid inadvertently unblinding the DRC) data needed by the DRC during the DRC review if the DRC requests it.

Candidemia and invasive candidiasis

A successful response in patients with candidemia or other forms of invasive candidiasis generally requires a CR or PR using clinical, radiological (if applicable) and microbiological criteria. It is possible to have discordance among these 3 criteria. For example, in chronic disseminated candidiasis, fever may resolve following initiation of antifungal therapy, but radiological lesions in visceral organs may persist for prolonged periods and do not *per se* indicate lack of control of infection. If radiological improvement does occur, it would be presumptive evidence of mycological improvement. Repeating a biopsy of these lesions solely to document sterilization in a patient who has clinically responded to antifungal therapy is not warranted. Therefore, in cases in which repeat sampling of cultures is not feasible, we may only be able to rely on clinical and radiological criteria, or clinical criteria alone.

In contrast, in patients with candidemia, sterilization of blood should be a requirement for a successful outcome. Even if symptoms and signs (e.g., fever) attributable to infection persist, they are non-specific and should not be equated with failure if blood cultures are sterilized. Removal of a central line may reduce the time to clearance of blood cultures in candidemia [17]. However, unless the protocol pre-specifies that intravenous catheters be

removed as a requirement for eligibility, the decision to remove intravenous catheters should not be considered in the assessment of outcome for individual patients. Follow-up sampling of easily accessible sites, such as cerebrospinal fluid in the case of *Candida* meningitis and persistent joint fluid in the case of *Candida* arthritis, should be required to evaluate response to therapy. If follow-up samples are not obtained, then the response should either be scored as indeterminate or failure if there are other signs of progressive or poorly controlled infection (e.g., multiorgan failure). Relapse of candidiasis either in the blood or at a distant site (e.g., endophthalmitis) during the pre-specified period of evaluation should be equated with failure.

Two recent randomized studies of invasive candidiasis provide instructive examples related to the importance of the time that the primary endpoint is assessed on study outcomes. Ruhnke et al. [18] assessed the primary efficacy endpoint at end of therapy, and showed 90% successful outcome in a per protocol population of patients with candidemia who survived the first 5 days of invasive candidiasis randomized to receive micafungin or liposomal amphotericin B. Kullberg et al. [19] assessed the primary endpoint at 12 weeks after end of therapy, and showed 41% successful outcome in patients with candidemia randomized to receive voriconazole or amphotericin B followed by fluconazole. This response rate appeared to be substantially worse than previously published randomized studies that used earlier time points. In both the studies of Ruhnke et al. and Kullberg et al., survival at 12 weeks post-treatment was ~ 60%, and the success rate in the MITT population was ~ 70%. This underscores the need to report outcomes in the ITT or MITT population as the primary analysis, since excluding patients with early failures by reporting per-protocol subgroups would lead to underreporting of up to 20% of patients failing therapy. While there were several differences in the design of these two studies, the time to assess the primary efficacy endpoint and the use of MITT is the primary

endpoint analysis were major factors accounting for the large difference in successful outcomes between the two studies.

The advantage of a primary endpoint that encompasses a large period of observation is that it captures late occurrences of persistence, relapse, adverse effects, or excess mortality of treatment groups. A potential disadvantage is that the overall response rate may be affected by variables unrelated to the study drug (such as the patient not being available for a 12-week assessment); the effect of introducing these unrelated variables, assuming they occur with equal frequency in the treatment arm evaluated, is to reduce the likelihood of detecting a clinically meaningful difference between therapies.

The time to assess primary outcomes in trials of candidemia should encompass not just sterilization of blood, but also be adequate to detect recrudescence of candidiasis and mortality directly or indirectly related to infection (Table 2). We suggest a period of observation of at least 4 weeks from the time of enrollment in trials of candidemia (Table 2).

End of therapy (EOT) responses should be avoided as a primary endpoint in the view of most of the panel members since there is subjectivity by an individual clinician as to when to stop study drug. Reporting end of study drug successes will not allow capture of persistent infections (while cultures are being taken during active therapy only), nor will it capture relapses after discontinuation of therapy. Recurrent candidemia after initial sterilization of blood cultures is uncommon and would suggest a persistent nidus of endovascular infection (e.g., infected catheter or endocarditis). Also, any difference between study arms in terms of late mortality due to adverse effects or interactions of study drugs will not be detected.

Invasive aspergillosis and other moulds

Evaluation of response to therapy in invasive mould infection is difficult for several reasons. Attributable symptoms and signs may be non-specific. In the highly immunocompromised patient, fever and localizing physical examination findings are often absent [20]. In addition, some of the clinical manifestations of IA may not necessarily indicate clinical deterioration. For example, hemoptysis is more frequent after neutrophil recovery [21], and may not signify refractory disease.

Evaluation of radiological responses, particularly at early time points, poses several challenges. Caillot et al. [5] performed sequential CT scans on patients with neutropenia and IA. Despite effective antifungal treatment leading to a positive clinical response in most patients, the median volume of lesions increased four-fold during the first week of therapy and remained stable during the second week. An increase in size of pulmonary lesions within the first week did not predict a negative response to therapy. Halo signs were very common at diagnosis but decreased during the first week of infection as the frequency of the air crescent sign increased. This study has implications in interpreting the results of salvage therapy or compassionate use studies in which neutropenic patients with IA could be enrolled after only 7 days of standard antifungal therapy if the size of pulmonary lesions were unchanged or increased [22-27]. There is a gap in knowledge about the radiological evolution of IA in non-neutropenic patients who respond to antifungal therapy.

An air-crescent sign and cavitation may be another finding that can be perceived as evidence of disease progression. As demonstrated by several reports, the appearance of air-crescent sign coincides with bone marrow recovery [5, 28, 29]. Cavitation is also associated

with reemergence of neutrophils [30]. Both findings may be incorrectly perceived as disease progression; an air-crescent sign may in fact correlate with clinical improvement.

Persistence of positive cultures from infected sites is in general equated with mycological failure. In many cases, repeat biopsies may not be warranted based on the risk of an invasive procedure (e.g., lung biopsy). In such situations, a radiological response can be equated with control of infection.

Other potential problems in assessing outcome are mixed fungal infections [20, 31], co-existent bacterial and fungal infections, and uncertainty about whether two infections or a non-infectious disease exist. For example, while receiving antifungal therapy for proven or probable IA, one pulmonary lesion may improve while a new lesion develops, raising a concern about two concurrent diseases. There is no easy solution to interpreting results in these highly complicated cases, and the outcome may need to be scored as indeterminate in the absence of an invasive procedure.

Surgery as a therapeutic modality poses an additional challenge for interpreting response to antifungal therapy. If a lung lesion known to be IA is resected, can we evaluate the effect of antifungal drug therapy? If cultures from the resected lesion are sterile, we would have presumptive evidence of drug effect. However, the final outcome must be considered the result of both drug and surgery. In the case of craniofacial mould infection, debridement plus antifungal therapy is the standard of care, and it would generally not be possible to judge the effect of drug alone. We suggest judging success or failure at the pre-specified time of analysis without considering whether surgery was performed. In a secondary analysis, patients treated with drug alone versus drug plus surgery may be analyzed separately.

The Platelia *Aspergillus* enzyme immunoassay (Bio-Rad Laboratories, Redmond, WA), a sensitive double sandwich ELISA that detects the fungal cell wall constituent galactomannan [32], has been approved for the diagnosis of IA. In addition to facilitating early diagnosis, serial determination of serum galactomannan levels is a useful tool for assessing prognosis of IA during treatment [8, 33]. In animal models of aspergillosis, the serum galactomannan index (GMI) correlated with the level of fungal tissue burden, and a reduction in the GMI correlated with a positive response to antifungal therapy [8, 34-36]. A number of studies have suggested that a rising serum GMI correlates with failure of antifungal therapy and decreasing GMI correlates with a positive outcome in patients with IA [8, 33, 37-39]. Boutboul et al. [33] serially evaluated serum GMI in 37 allogeneic HSCT recipients receiving therapy for IA. GMI levels significantly increased in patients who failed to respond to antifungal therapy, whereas no significant change occurred in patients with a complete or partial response to therapy. An increase in the GMI level of at least 1.0 over the baseline value during the first week of observation was predictive of treatment failure with a sensitivity of 44%, a specificity of 87%, and a positive predictive value of 94%. Maertens et al. [37] reported that all 24 patients with IA with persistent or rising serum GMI eventually died of or with IA [37]. Additional data presented in abstract form further demonstrate the utility of serial GMI testing as a predictor of outcome in patients with IA [40].

There are several limitations to these studies. In most of the studies, the primary goal was to assess serum GMI as a diagnostic adjunct for IA rather than a surrogate endpoint for response to therapy. Substantial variability existed among the different studies including when serum GMI was measured after the diagnosis of IA, the cut-off values for a positive versus negative GMI, the antifungal regimens used, the definitions of success or failure, and the time to

evaluate response to therapy. There are many causes of false positive galactomannan levels, including concomitant piperacillin/tazobactam therapy and potentially other semisynthetic B-lactams [41, 42].

Anaissie has cogently argued that serum GMI be used both in practice and in clinical trials as a surrogate of early response to therapy in IA [9]. The majority of panel members considered serial GMI measurements to be a highly promising surrogate of response to therapy, but felt that it was premature to adopt serum GMI as a mycological endpoint to define success or failure in clinical trials. The panel noted the research priority to conduct trials of IA in which correlation of serial GMI and potentially other laboratory surrogates (e.g., B-glucan, PCR) with clinical outcome is a pre-specified endpoint.

Taken together, there are substantial pitfalls in assessing response to mould infections because of a constellation of several factors: non-specific clinical findings; radiologic changes over time that often do not enable straightforward interpretation of control or worsening of infection, sites of infection that are often inaccessible to sampling for culture, and lack of validated markers for mycological response. Clinical, radiologic, and mycologic endpoints may conflict, particularly at early time points.

In the study by Herbrecht et al. [43] that compared voriconazole with amphotericin B as primary therapy for IA, the difference in successful outcomes was apparent by 6 weeks, although the primary endpoint for assessing outcomes was 12 weeks after study initiation. Most failures in the first 6 weeks were antifungal failures; most failures during the second 6 weeks were attributed to host factors (e.g., relapsed malignancy) (Drs. John Wingard and Haran Schlamm, personal communication).

These results suggest that assessment of the primary endpoint at 6 weeks would be acceptable for IA. However, results from two pooled trials (P041 and P02387) evaluating posaconazole as salvage therapy for invasive mould infections (aspergillosis, zygomycosis, and fusariosis) provide a cautionary note about early endpoints. As judged by the DRC, the overall rate of concordance between treatment responses assessed at 1 and 3 months was only 42% (Catherine Hardalo MD, Schering-Plough, personal communication). The concordance between 3 and 6 month assessments was substantially improved (76%). There are key differences in the design of these two trials -- primary versus salvage, randomization, inclusion of non-*Aspergillus* mould infections, antifungal agents used, and pre-specified duration of study drug – that may have affected the concordance between earlier and later time points.

For primary therapy trials of IA, some of the panel members considered 6 weeks after enrollment to be the minimum time to assess the primary outcome endpoint for IA. Other panel members preferred 12 weeks to encompass recrudescence infections and long-term drug toxicity. A time point of 12 weeks or greater should be included as a secondary endpoint. There is a gap in knowledge as to the optimal time to evaluate outcome for invasive non-*Aspergillus* mould infections. In salvage studies of invasive mould infections, a time point of 12 weeks or greater should be used for the primary endpoint analysis.

Cryptococcal meningitis

C. neoformans disease most commonly manifests as meningitis though other sites may be involved. The panel's recommendations focus on cryptococcal meningitis. Assessment of treatment response in cryptococcal meningitis relies on clinical and mycological criteria [44-46]. A successful clinical response generally involves improvement or resolution of attributable symptoms and signs, including fever, headache, and meningismus. The gold standard for

mycological response is sterilization of cerebrospinal fluid. For both cryptococcal and coccidioidal meningitis, CSF obtained by lumbar puncture is likely to be more sensitive for recovery of organisms than intraventricular collection of CSF; if the initial lumbar fluid was positive followed by negative ventricular fluid, no conclusion should be drawn.

Repeat sampling of CSF is a key component in assessing the overall response to therapy because the level of clinical symptoms may not correlate with the severity of infection. Systemic corticosteroids and other immunosuppressive agents may blunt symptoms and physical exam findings associated with meningitis. We therefore advise that a successful response to therapy require evidence of both a clinical and mycological response to therapy. If a repeat CSF sample is not obtained, then the outcome should be scored as “indeterminate” if a clinical response occurs, and as “failure” if clinical findings are unchanged or worsen.

Brouwer et al. [47], conducted a randomized study of combination antifungal therapies for AIDS-associated cryptococcal meningitis in which the primary endpoint was fungicidal activity, assessed by the rate of reduction in CSF colony-forming units (CFU) within the first 2 weeks of treatment. The study compared 4 treatment regimens, with 16 patients randomized to each regimen. Despite the low number of subjects, this study identified amphotericin B plus flucytosine as the most effective regimen in clearance of cryptococci. These findings were consistent with the greater fungicidal activity of amphotericin plus flucytosine compared with amphotericin B alone observed in the pivotal MSG trial [44]. In a separate study of patients with AIDS-associated cryptococcal meningitis there was no correlation between the rate of decline of CSF cryptococcal CFU counts and that of CSF cryptococcal antigen titers [48]. These results are in contrast with a prior study in which the CSF cryptococcal antigen titer was an independent predictor of failure to achieve negative CSF cultures by day 14 [49]. In phase

I/II studies in which patient accrual is limited, such quantitative mycologic endpoints provide a valuable tool to evaluate treatment regimens. However, definitive phase III trials should include longer term endpoints and be adequately powered to evaluate survival, persistent morbidity, and long-term drug toxicity.

In cases of concurrent extraneural *C. neoformans* infection, assessment of the global response will involve clinical, radiological, and mycological criteria. A mycological response involves sterilization of involved sites if repeat sampling is feasible (e.g., repeat sputum or bronchoalveolar lavage samples in cases of cryptococcal pneumonia). If the baseline blood culture is positive, repeat cultures should be obtained to document sterilization following initiation of therapy.

Histoplasmosis and coccidioidomycosis

Dimorphic fungi cause infections in both immunocompetent and immunocompromised patients although disease is often more extensive in patients with impaired cellular immunity. We have focused our guidelines on histoplasmosis and coccidioidomycosis because the clinical trial database and laboratory markers are the most developed for these two diseases. The general principles should be applicable to other dimorphic fungal diseases.

Dimorphic fungal infections range from asymptomatic to life-threatening disseminated disease. In cases of well-defined infections at specific anatomic sites global assessment is straightforward. In cases of fungal pneumonia, clinical and radiological improvement provides adequate evidence of control of infection. There are chronic fibrocavitary forms of pulmonary histoplasmosis and coccidioidomycosis that show little radiologic improvement with successful drug therapy. As the lesions heal by scarring, the fibrocavitary infiltrates and nodules shrink and emphysema may occur in the contiguous lung. The solitary cavity in coccidioidomycosis may

take years to shrink. For purposes of these guidelines, we will discuss only the disseminated forms. In cases of meningitis, clinical and mycological evidence of control of infection are requisites of a successful global response. Radiologic resolution of central nervous system fungal lesions is rarely complete, even after years of observation. Improvement of CT and MRI images is a more useful endpoint to judge success, with the caveat that improvement in edema can be due to corticosteroids.

Histoplasmosis

Clearance of blood cultures is the gold standard for mycological response in patients with histoplasmosis and positive blood cultures. Non-culture laboratory markers are useful adjuncts in monitoring the response to systemic histoplasmosis with the provision that these tests are conducted using the same method and ideally in the same reference lab. Although the Histoplasma antigen test has not been used as a study endpoint in clinical trials, changes in antigen have paralleled those of culture in patients with positive cultures [50-52]. In patients with histoplasmosis and positive blood cultures, clearance of fungemia is a better measure of antifungal effect than clearance of antigen [53]. However, reduction in antigen levels could be used as a mycological endpoint in patients with negative blood cultures, and as additional evidence for response in patients with positive cultures. Using a conservative measure, a fall in serum antigen by at least 50% during the first 3 months of therapy relative to the baseline level can be equated with a positive mycologic response. In patients whose antigen levels have fallen with therapy, subsequent increase of 20% or more raises concern about relapse [54]. One limitation of this database is that antigen levels were evaluated principally in AIDS-associated disseminated histoplasmosis; its predictive value of therapeutic response in other patient populations has not been established. Antigen levels in urine may not decline for several weeks

even with effective therapy [55]; persistence of antigenuria should therefore not be equated with failure of therapy.

Coccidioidomycosis

Several trials of coccidioidomycosis used a composite scoring system to assess the level of burden of infection at baseline and the response to therapy [56-60]. Points were assigned based on 1) symptoms; 2) physical examination; 3) quantitative complement fixation titers (baseline and follow-up titers measured in the same laboratory concurrently); and 4) cultures. Numerical values were assigned based on pre-specified rules and the sum of these values at reassessment was compared with baseline values, with an increasing score indicating deterioration. A successful response was defined as a greater than 50% reduction in baseline abnormalities within 8 months of therapy. Patients who did not have a satisfactory response were considered to have failed (equated with stable or progressive disease). Variations of this outcome score were used successfully in several trials of coccidioidomycosis; it's not possible to state which specific outcome score is best.

Coccidioidomycosis may result in a persistent or chronic disease. In CNS coccidioidomycosis, life-long suppressive antifungal therapy is the standard of care because of the high frequency of recrudescence if therapy is stopped [61, 62]. A composite numeric outcome score using clinical and laboratory abnormalities detected in baseline and subsequent evaluations has been applied to evaluate response to therapy in coccidioidal meningitis [63, 64]. In one study, a response was defined as 40% or greater reduction in abnormalities without subsequent relapse during fluconazole treatment [63]. After 8 months, a patient not achieving this level of improvement was considered to be a nonresponder. Chronic soft tissue, bone, and pulmonary disease are also characteristic of coccidioidomycosis. In fact, some of the original salvage therapy trials in mycoses were applied to persistent coccidioidomycosis [65].

Therefore, in clinical trials of certain forms of coccidioidomycosis, improvement of clinical and laboratory endpoints on therapy without eradication of disease may fulfill criteria for a successful outcome. A minority of patients with coccidioidomycosis may require 9 months or more to respond to antifungal therapy [66]; therefore extending the time to evaluate the primary endpoint to, say, 12 months is expected to change the outcome in this subset of patients.

Gaps in knowledge

The development of reproducible and validated quantitative criteria to assess responses to antifungal therapy is a key priority. Sterilization of infected sites is the gold standard when repeat sampling for culture is feasible, such as infections involving blood or CSF. When repeat sampling for culture may not be feasible or reliable (e.g., fungal pneumonia), there is a need for validated lab markers to assess response to therapy. This problem is most acute in invasive mould infections in which lab markers have been validated as diagnostic adjunct but not as surrogates of therapeutic response. To enhance trial efficiency, the U.S. Food and Drug Administration (FDA) has recommended the use of surrogate markers that can substitute for clinical events as tools to increase diagnosis specificity and provide objective outcome measures [67]. Future trials of invasive mould infections should include validation of these lab assays as predictive correlates of outcome. We also anticipate that the future development of sensitive non-culture based diagnostic assays (e.g., PCR) will facilitate both the early diagnosis of IFIs and assessment of response to therapy.

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Table 1. General criteria for global responses to antifungal therapy

Outcome	Response
Successful	<p>Complete response: Survival within the pre-specified period of observation, and resolution of all attributable symptoms and signs of infection and radiological abnormalities, and mycological evidence of eradication of infection.</p> <p>Partial response: Survival within the pre-specified period of observation, and improvement in attributable symptoms and signs of infection and radiological abnormalities, and evidence of sterilization of cultures or reduction of fungal burden assessed by a quantitative and validated laboratory marker</p>
Failure	<p>Stable response¹: Survival within the pre-specified period of observation and minor or no improvement in fungal disease, but no evidence of progression, based on a composite of clinical, radiologic, and mycologic criteria; or</p> <p>Progression of Fungal Disease: Evidence of progressive fungal disease based on a composite of clinical, radiologic, and mycologic criteria; or</p> <p>Death: Death during the pre-specified period of evaluation regardless of attribution</p>
Non-evaluable	Indeterminate²: Inability to assess global response. Potential reasons include inadequate diagnostic evaluation, conflicting clinical, radiographic,

	or mycological data, or presence of other factors such as an unrelated infection or relapse of malignancy that confound assessment of response to antifungal therapy.
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¹ In certain IFIs (e.g., invasive mould infections), stabilization of fungal disease during periods of severe immunocompromise provides evidence of efficacy of the treatment and may be a reasonable short-term therapeutic goal until immune reconstitution occurs (see text).

² Some studies pre-specify that “Indeterminate” responses will be scored as failures. If the pre-specified primary endpoint for comparison between two treatment regimens is “proportion with successful outcomes”, then Failure and Indeterminate outcomes are *de facto* treated as equivalent results. Such a scenario is particularly problematic if the proportion of “Indeterminate” outcomes is significantly different between study groups, emphasizing the need for well-defined criteria for treatment responses with adequate periods for evaluation of response. This is best handled by separate assessments in the intent-to-treat analysis and pre-specified evaluable subset of patients.

Completion of the assigned treatment regimen as one criterion for a successful outcome has been widely accepted among both clinicians and regulatory agencies. However, it is also reasonable to make provision for “success with modification” as was done in the trial by Herbrecht et al. [43] that compared voriconazole with amphotericin B as primary therapy for invasive aspergillosis; in this scenario, the protocol is, in effect, evaluating two different treatment strategies rather than two different drugs. One consequence of permitting success with

modification is to diminish the likelihood of detecting a difference between the initially assigned treatment groups.

Table 2. Responses to antifungal therapy in patients with candidemia or invasive candidiasis

Outcome	Response
Successful	<p>Complete response:</p> <ul style="list-style-type: none"> · Survival and resolution of all attributable symptoms and signs of infection; plus • Sterilization of blood in cases of candidemia; plus • Sterilization of infected sites that are accessible to repeat sampling (e.g., cerebrospinal fluid). • If repeat cultures are not feasible (e.g., in cases of candidiasis involving visceral organs), survival and resolution of all attributable symptoms and signs of infection and radiological resolution can be equated with a complete response. <p>Partial response:</p> <ul style="list-style-type: none"> · Survival and improvement of attributable symptoms and signs of infection¹; plus • Sterilization of blood in cases of candidemia; plus • Sterilization of infected sites that are accessible to repeat sampling (e.g., CSF). • If repeat cultures are not feasible, survival and resolution of attributable symptoms and signs of infection and radiological improvement or stabilization can be equated with a partial response.²
Failure	<p>Stable response:</p> <ul style="list-style-type: none"> • Survival and minor or no improvement in attributable symptoms and signs of infection; plus • Persistent isolation of <i>Candida</i> sp. from blood or other sterile sites; or

	<ul style="list-style-type: none"> • If repeat cultures are not feasible, radiological stabilization can be equated with a stable response. <p>Progression of infection:</p> <ul style="list-style-type: none"> • Persistent isolation of <i>Candida</i> sp. from blood or other sterile sites in association with worsening clinical symptoms or signs of infection (e.g., septic shock, progression of hematogenous cutaneous candidiasis); or • New sites of infection or worsening of pre-existing lesions radiologically (e.g., those observed in chronic disseminated candidiasis) in association with clinical deterioration <p>Death: Death during the pre-specified period of evaluation regardless of attribution</p>
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¹ Fever without localizing symptoms or physical exam findings is the most common manifestation of candidemia. However, because fever can result from multiple causes unrelated to candidemia, we suggest that more weight be given to sterilization of blood than to resolution of fever in the global assessment of response to therapy. Thus, the scenario of persistent or recurrent fever despite sterilization of blood should be globally assessed as at least a partial response.

²In visceral candidiasis (e.g., hepatosplenic candidiasis) with negative blood cultures at baseline, persistent fever may be the only attributable clinical sign of candidiasis and radiologic abnormalities can persist for prolonged periods. In such situations, resolution of fever and stable radiologic disease may be equated with a partial response. Laboratory markers such as PCR, anti-mannan antibodies, and the B-glucan assay have not been adequately validated as markers of response to therapy for invasive candidiasis.

Minimum Period of Observation: Four weeks after start of therapy. The rationale for this minimum period of evaluation is to detect relapses of infection. Relapse generally requires a positive culture from blood or another sterile site, and not simply recurrence of symptoms or signs (e.g., fever) that are generally non-specific. In the specific cases of visceral organ involvement (e.g., endocarditis, meningitis, retinitis, or chronic disseminated candidiasis), we suggest a period of observation of at least 12 weeks after start of therapy.

Table 3. Responses to antifungal therapy in patients with invasive mould infections

Outcome	Response
Successful	<p>Complete response:</p> <ul style="list-style-type: none"> · Survival and resolution of all attributable symptoms and signs of infection; plus • Resolution of radiological lesion(s). Persistence of only a scar or post-operative changes can be equated with a complete radiological response; plus • Sterilization of infected sites that are accessible to repeat sampling (e.g., mould infection involving the palate, sinuses, or cutaneous lesions) <p>Partial response:</p> <ul style="list-style-type: none"> · Survival and improvement of attributable symptoms and signs of infection¹; plus • At least 25% reduction in diameter of radiologic lesion (s); plus • Sterilization of infected sites that are accessible to repeat sampling (e.g., mould infection involving the palate, sinuses, or cutaneous lesions). • In cases of radiological <u>stabilization</u> (defined as 0 to 25% reduction in diameter of lesion), resolution of all attributable symptoms and signs of fungal disease can be equated with a partial response. • In cases of radiological <u>stabilization</u>, biopsy of an infected site (e.g., lung biopsy) showing no evidence of hyphae and negative cultures can be equated with a partial response.
Failure	Stable response:

	<ul style="list-style-type: none"> • Survival and minor or no improvement in attributable symptoms and signs of infection; plus • Radiologic stabilization (defined as 0 to 25% reduction in diameter of lesion); or • Persistent isolation of mould or presence of invasive hyphae histologically in infected sites <p>Progression of infection:</p> <ul style="list-style-type: none"> • Worsening clinical symptoms or signs of infection; plus • New sites of infection or worsening of pre-existing lesions radiologically; or • Persistent isolation of mould species from infected sites <p>Death: Death during the pre-specified period of evaluation regardless of attribution</p>
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¹Clear evidence of a radiologic response (reduction in diameter by at least 25% with no new sites of infection) should be given more weight than subjective, non-specific, or difficult to quantify symptoms or signs of infection. Thus, in the scenario of fungal pneumonia, we suggest that radiologic improvement but persistence of fever or cough should be scored as a partial response. Since radiologic improvement often lags behind clinical improvement, especially if a short-term period of evaluation is employed (see text), we suggest that radiologic stabilization and resolution of all attributable symptoms and signs of infection can also be equated with a partial response.

Minimum Period of Observation: The panel was unable to arrive at a consensus as to whether 6 or 12 weeks constituted the minimum period to evaluate the primary outcome endpoint in studies of primary therapy for invasive aspergillosis. All panel members agreed that 12-week data should be included in the analysis, either as primary or secondary endpoints. A gap in knowledge exists related to the optimal time to evaluate response to invasive non-*Aspergillus* mould infections. In salvage studies, the primary endpoint should be assessed at least 12 weeks after enrollment (see text).

Table 4. Responses to antifungal therapy in cryptococcal meningitis

Outcome	Response
Successful	<p>Complete response:</p> <ul style="list-style-type: none"> · Survival and resolution of all attributable symptoms and signs of infection; plus • Sterilization of cerebrospinal fluid (CSF); plus • Sterilization of blood in cases of bloodstream infection; plus • Sterilization of other sites of infection (if repeat cultures are obtained); plus • Resolution of radiological lesions if present (e.g. CNS cryptococcomas) <p>Partial response:</p> <ul style="list-style-type: none"> · Survival and improvement of attributable symptoms and signs of infection¹; plus • Sterilization of CSF; plus • Sterilization of blood in cases of bloodstream infection; plus • Sterilization of other sites of infection (if repeat cultures are obtained); plus • Improvement or stabilization in radiological lesions if present at baseline
Failure	<p>Stable response:</p> <ul style="list-style-type: none"> • Survival and minor or no improvement in attributable symptoms and signs of infection; plus • Persistent positive cultures from CSF or other infected sites <p>Progression of infection:</p> <ul style="list-style-type: none"> • Worsening clinical symptoms or signs of infection plus

	<ul style="list-style-type: none"> • Persistent positive cultures from CSF or other infected sites or • New sites of infection or worsening of pre-existing lesions radiologically <p>Death: Death during the pre-specified period of evaluation regardless of attribution.</p>
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¹In cryptococcal meningitis, sterilization of CSF typically precedes the expected reduction in antigen titers in patients with a positive response to antifungal therapy [44] and is the gold standard to evaluate mycologic response. Sterilization of CSF is given more weight than clinical criteria (e.g., fever, meningismus) in assessing the global response. Thus, sterilization of CSF but persistence of fever or headache should be equated with at least a partial response. In contrast, the occurrence of significant and irreversible CNS sequelae (e.g., persistent coma, blindness, or dementia) should be equated with failure even with CSF sterilization. Immune reconstitution inflammatory syndrome (IRIS), results from an exuberant inflammatory response towards previously diagnosed or incubating pathogens (e.g., mycobacterial and CMV disease). IRIS is well-described in AIDS-associated cryptococcal meningitis following initiation of effective antiretroviral therapy, and manifests with meningismus and elevated CSF opening pressures, glucose levels, and white blood cell counts [68, 69]. Repeat CSF cultures are required to distinguish IRIS from persistent or recrudescing cryptococcal disease. IRIS does not represent treatment failure.

Minimum Period of Observation: 10 weeks from time of initiation of study drug. The rationale for this minimum period of evaluation is that assessments of clinical and mycological responses may conflict at early time points.

Table 5. Responses to antifungal therapy in systemic histoplasmosis

Outcome	Response
Successful	<p>Complete response:</p> <ul style="list-style-type: none"> · Survival and resolution of all attributable symptoms and signs of infection; plus • Resolution of radiological lesion(s). Persistence of only a scar or post-operative changes can be equated with a complete radiological response; plus • Sterilization of infected sites that are accessible to repeat sampling, e.g., blood, cerebrospinal fluid (CSF). • If infected sites are not accessible to repeat sampling for cultures, clearance of Histoplasma antigen (HPA) from serum and urine (if detected at baseline) can be used as a mycologic criterion for complete response. <p>Partial response:</p> <ul style="list-style-type: none"> · Survival and improvement of attributable symptoms and signs of infection; plus • Improvement in radiological lesions; plus • Sterilization of infected sites that are accessible to repeat sampling, e.g., blood, CSF • If infected sites are not accessible to repeat sampling for cultures, a fall in serum HPA by at least 50% during the first 3 months of therapy relative to the baseline level can be equated with a partial mycologic response
Failure	<p>Stable response:</p> <ul style="list-style-type: none"> • Survival and minor or no improvement in attributable symptoms and signs of

	<p>infection; plus</p> <ul style="list-style-type: none"> • Radiological stabilization; or • Persistent positive cultures from infected sites ; or • If infected sites are not accessible to repeat sampling for cultures, lack of a fall in serum HPA by at least 50% after 3 months of therapy can be equated with a stable mycologic response <p>Progression of infection:</p> <ul style="list-style-type: none"> • Worsening clinical symptoms or signs of infection; plus • New sites of infection or worsening of pre-existing lesions radiologically or • Persistent positive cultures from infected sites or • If infected sites are not accessible to repeat sampling for cultures, an increase in serum HPA by > 20% can be a mycologic criterion for worsening of infection. <p>Death: Death during the pre-specified period of evaluation regardless of attribution.</p>
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Minimum Period of Observation: Three months from time of initiation of study drug is a suggested minimum period of observation for systemic histoplasmosis. Because some patients develop relapsed disease while on antifungal therapy, assessment of outcome at 12 months after initiation of study drug is suggested as a secondary endpoint.