Antifungal Combination Therapy for Invasive Aspergillosis

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The outcome of invasive aspergillosis (IA) continues to be associated with significant attributable mortality, especially in patients with hematological malignancies and in hematopoietic stem cell transplant recipients. In this context, antifungal combined therapy (ACT) has become an emerging strategy against IA. In an attempt to evaluate the benefits of ACT, a large number of experimental studies, clinical series, and randomized trials have been performed, with varying results. In addition, several controlled trials have been registered; however, in most cases, their final results have not been made available. In summary, there is an imbalance between the lack of published evidence regarding the benefits of ACT and its extensive and increasing use in current clinical practice, despite its associated cost. Here, we present a critical analysis of the available information regarding ACT for the treatment of IA as well as the authors’ opinion with respect to its use.

Keywords. antifungal combined therapy; invasive aspergillosis; salvage therapy.

Invasive aspergillosis (IA) is a life-threatening infection, especially in patients with hematological malignancies. Although survival has increased in recent years due to advances in diagnosis and the availability of an arsenal of new antifungals [1], the outcome of IA remains suboptimal, with an attributable mortality of up to 42%–64% [2, 3]. In this context, the combination of different antifungal drugs is attractive, making it possible to achieve potential synergistic effects due to the action of the drugs on different targets or inhibition of different steps of the same pathway; achieve broader antifungal coverage for empiric or targeted therapy in centers where there are high rates of resistance; and potentially reduce acquired resistance [4, 5]. The clinical results that would be expected if antifungal combined therapy (ACT) provided an added benefit are an increased response rate and improved survival. However, it should be noted that ACT could also lead to antagonism for some antifungal combinations, enhanced toxicity, and significant additional costs. The scientific evidence supporting the hypothesis that combination therapy could be more effective than monotherapy is mainly based on in vitro and experimental studies [5], whereas the studies performed in clinical settings are numerous and quite heterogeneous [5–7].

Here, we critically review the available information regarding ACT for IA in an attempt to answer the following question: Is ACT more effective and as safe as monotherapy for IA? To answer this question, we comprehensively evaluated the information available regarding ACT in the treatment of IA from published preclinical studies to current clinical trials that are not yet published in the scientific literature.

EFFICACY AND SAFETY OF ANTIFUNGAL COMBINED THERAPY

Preclinical Studies

There are numerous experimental studies on combinations of different antifungals for the treatment of
IA. These include both in vitro studies evaluating antifungal susceptibility and interactions between antifungals as well as studies in animal models [5].

Some studies have tested combinations of azoles and amphotericin (AMB) in vitro and shown variable effects from synergy to antagonistic activity [6]. However, the true efficacy of these combinations cannot be established due to the lack of a standardized Clinical and Laboratory Standards Institute (CLSI) protocol for in vitro synergy testing of fungi [8] and the lack of correlation between in vitro synergy data and clinical outcomes, which may indeed make results difficult to interpret.

The most significant results supporting ACT in animal models have been reported when combinations of triazoles with echinocandins were tested. A positive effect on survival in animal models of IA was shown with the combinations caspofungin plus voriconazole and micafungin plus itraconazole [9, 10] when compared with echinocandin alone, although survival was no better than with triazole monotherapy [11–13]. The combination of anidulafungin with voriconazole resulted in improved survival compared with either monotherapy in some animal models [14, 15], whereas other studies using the same combination have shown no differences in outcome regarding either of the monotherapy regimens [16]. In general, these contradictory results suggest that the addition of triazoles to echinocandin results in a trend toward improved survival compared with monotherapy with echinocandins, but there were no measurable benefit compared with monotherapy with voriconazole.

In summary, one must be aware of inherent problems associated with in vitro combination assays and the different animal models used to characterize these treatment regimens before translating these results into clinical practice.

Clinical Studies

Clinical Series

In recent years, there have been many clinical studies characterizing ACT for IA in which the authors attempted to determine if ACT offers an advantage in terms of successful response and/or survival. The main characteristics of these studies [1, 3, 17–24] are shown in Table 1.

The most important drawback of these studies is the limited scientific quality of their design, since they fail to establish the true efficacy of ACT, a fact that makes generalization of study conclusions challenging. Most of these studies have a retrospective and monocentric design [1, 3, 18, 20]. Two of these studies were carried out at the same institution over long periods of time during which different antifungal combinations were analyzed, and both studies involved unbalanced study arms in terms of the number of patients included [3, 18]. Only 1 study had a prospective and multicenter design, although the size of the study arm group for ACT was unbalanced, with micafungin vs monotherapy for salvage treatment and a small number of patients in primary therapy. Furthermore, this study had a noncomparative design, and analyzed heterogeneous populations and multiple micafungin-based combinations that were determined based on the treating clinician’s discretion [22]. Two additional studies had a case-control design; however, the control group data were retrospectively collected in both studies [17, 19]. Moreover, 1 of these studies was designed to evaluate posaconazole as salvage therapy and not to analyze the efficacy of ACT [17].

Therefore, none of the studies were randomized, and the decision to initiate ACT was left to the discretion of the treating physicians in 5 of 7 studies, both primary and/or salvage studies [1, 3, 17, 18, 22]. The selection of different combinations of antifungal drugs (caspofungin or micafungin plus a miscellaneous in 2 studies [21, 22], and amphotericin plus itraconazole in one [18]) in primary and/or salvage therapy and the absence of a predefined criteria for initiating ACT indication made it difficult to interpret the results of these studies.

In addition to the lack of appropriate design, the major challenge associated with studies that evaluated ACT was the absence of an appropriate measure of efficacy. Most studies evaluated the satisfactory response (including partial and complete resolution of clinical, radiological, and microbiological data) and overall survival [3, 17, 19, 21], whereas others analyzed only overall survival, crude and attributable mortality [1, 20], or only satisfactory response [18]. The difficulty of accessing clinical data and being able to determine the point where a satisfactory response can be measured, especially when using retrospective data, further complicated the interpretation of results. In addition, attributable mortality may be difficult to assess since the contribution of IA to death in hematological patients may not be properly established in the absence of autopsy because of the complexity of treating these patients. For these reasons, the impact of ACT on overall survival compared with monotherapy may be the most appropriate measure for efficacy when comparing clinical studies. Indeed, of the 6 studies evaluating overall survival, 3 found differences between treatment groups. In the first study [19], which compared ACT with voriconazole and caspofungin vs AMB for primary therapy of IA in solid organ transplant recipients, there was a significant difference in survival of patients with renal failure or infection due to Aspergillus fumigatus. This is an important finding since the comparator in monotherapy is AMB, which has known higher associated nephrotoxicity and lower efficacy than voriconazole (included in the combination therapy) for IA [25]. The second study [17] compared high doses of AMB plus caspofungin vs posaconazole for salvage therapy of IA and found higher overall survival in patients who received monotherapy. This result is not surprising since the study evaluated the compassionate use of posaconazole and used a historic control group that received high doses of AMB, which has been
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Design</th>
<th>Indication</th>
<th>Therapy Category</th>
<th>Satisfactory Response ACT vs MT</th>
<th>Crude Mortality ACT vs MT</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raad et al 2008</td>
<td>Cases and controls</td>
<td>Salvage</td>
<td>HD LF-AMB + Caspofungin (N = 38)</td>
<td>11% vs 40%, ( P &lt; .01 )</td>
<td>74% vs 43%, ( P = .02 )</td>
<td>Nontrial design Nonrandomized Retrospective control groups ACT group is control group Different time span between cases and control groups Groups with different sample sizes Single center ACT initiation at discretion of clinicians</td>
</tr>
<tr>
<td>Mihu et al 2010</td>
<td>Retrospective</td>
<td>Salvage</td>
<td>AMB + Echinocandin (N = 71)</td>
<td>21% vs 28%, ( P = .04 )</td>
<td>62% vs 67%, ( P = .78 )</td>
<td>Nontrial design Nonrandomized Retrospective 15-Year time span of inclusion period Groups with different sample sizes Single center ACT initiation at discretion of clinicians</td>
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<td></td>
<td>(1993–2008)</td>
<td></td>
<td>AMB (N = 70)</td>
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<tr>
<td>Kontoyiannis et al 2005</td>
<td>Retrospective</td>
<td>Primary</td>
<td>LF-AMB + Itraconazole (N = 11)</td>
<td>0% vs 10%, ( P = n.s. )</td>
<td>. . .</td>
<td>Nontrial design Nonrandomized Retrospective 10-Year time span of inclusion period Groups with different sample sizes Noncomparative Single center ACT initiation at discretion of clinicians</td>
</tr>
<tr>
<td>Singh et al 2006</td>
<td>Cases: prospective</td>
<td>Primary</td>
<td>Caspofungin + Voriconazole (N = 40)</td>
<td>70% vs 51%, ( P = .08 )</td>
<td>67.5% vs 51%, ( P = .11 )</td>
<td>Nontrial design Nonrandomized Prospective cases and retrospective historical control Small sample size</td>
</tr>
<tr>
<td>Reference</td>
<td>Study Design</td>
<td>Indication</td>
<td>Therapy Category</td>
<td>ACT (N)</td>
<td>MT (N)</td>
<td>Satisfactory Response ACT vs MT</td>
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<tr>
<td>Upton et al 2007 [1]</td>
<td>Retrospective</td>
<td>Primary</td>
<td>Voriconazole + Caspofungin (N = 33)</td>
<td>AMB (N = 131) L-AMB (N = 80) Voriconazole (N = 25)</td>
<td>. . .</td>
<td>. . .</td>
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<tr>
<td>Marr et al 2004 [20]</td>
<td>Retrospective</td>
<td>Salvage</td>
<td>Voriconazole + Caspofungin (n = 16)</td>
<td>Voriconazole (N = 31)</td>
<td>. . .</td>
<td>62.25% vs 67.7%; HR, 0.27 (0.09–0.78), P = .008</td>
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<tr>
<td>Cesaro et al 2007 [21]</td>
<td>Retrospective</td>
<td>Primary</td>
<td>Caspofungin + other antifungal class (N = 20 primary indication; N = 20 in salvage indication)</td>
<td>. . .</td>
<td>Primary group: 60% Salvage group: 45%</td>
<td>Primary group: 30% Salvage group: 30%, nontrial design</td>
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<tr>
<td>Denning et al 2006 [22]</td>
<td>Prospective</td>
<td>Primary</td>
<td>Micafungin + other antifungal class (N = 17 primary indication; N = 174 salvage indication)</td>
<td>Micafungin (N = 12 primary indication; N = 22 salvage indication)</td>
<td>Primary group: 29.4% vs 50% Salvage group: 34.5% vs 40.9%</td>
<td>. . .</td>
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<tr>
<td>Maertens et al 2006 [24]</td>
<td>Prospective</td>
<td>Salvage</td>
<td>Caspofungin + L-AMB (N = 16) Caspofungin + Triazoles (N = 37)</td>
<td>. . .</td>
<td>50% (8/16) 48.6% (17/35)</td>
<td>. . .</td>
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shown to be more toxic and no more effective than standard doses [26]. Another study, which compared voriconazole plus caspofungin vs voriconazole for salvage therapy, reported longer than 3-month survival for the ACT group [20]. However, the study had a retrospective design, with a small and unbalanced number of patients in the treatment groups. In addition, the time periods during which treatment groups were compared differed (the monotherapy group was treated between 1997 and 2001, whereas than the ACT group was treated only in 2001). ACT was administered only in the last year of the study, therefore, changes in clinical care during that period would not be detectable because of the observational design of the study and could have affected differences in survival.

An important issue relating to the combination of antifungal drugs is toxicity. However, only 3 of 8 studies included a comparison of toxicity between monotherapy and ACT [3, 17, 20]. Overall, the results showed that polyenes used alone or in combination with an echinocandin added to renal and hepatic toxicity [3, 17], especially when high doses were used [17]. However, there were no significant differences in hepatic or renal toxicity between ACT and monotherapy when the combination did not include a polyene [20].

Published Clinical Trials

In comparison with clinical trials that evaluated monotherapy, the design of trials that evaluated combination therapy presented additional complexities and challenges. Careful consideration should be given to the definition of combination therapy, study design (ie, superiority vs inferiority), selection of patient populations, control regimens, and endpoints. The 2 clinical trials evaluating ACT reported to date are detailed in Table 1. Both studies, which had different designs, had a small sample size, and their statistical design was not appropriate for determining if ACT is superior or equivalent (no calculation of delta factor) to monotherapy, leading to underpowered results. The first study [24] was an open-label, noncomparative, noncontrolled clinical trial that evaluated the efficacy and safety of caspofungin-based combinations for salvage therapy of IA. No differences in the endpoint (favorable response) in both combination groups were found, but these results were not compared with monotherapy. The second study [23] was a pilot, randomized, comparative study, but the monotherapy arm consisted of high-dose Liposomal-AMB (L-AMB). Taking into account that monotherapy with high-dose L-AMB is known to be more toxic and without additional benefits in terms of efficacy than standard doses [26], this was not the optimal comparator. As expected, a higher rate of drug-related adverse events were detected in this group. In this sense, the results that showed a higher favorable response rate in the ACT group may be debatable due to the use of an inappropriate comparator and the effect of the reduced sample size. In summary, it is not
possible to draw definite conclusions regarding the efficacy and safety of ACT from these clinical trials since their results are limited by small sample sizes and inappropriate design.

**International Guidelines From Scientific Societies**
Since guidelines published by scientific societies [27–29] are commonly used in clinical practice, it is essential to determine how the available scientific evidence, which is based on preclinical and clinical data, impacts the different international societies’ recommendations regarding ACT for IA. It is noteworthy that scientific societies differ in their recommendations as well as in the given strength of the evidence provided to support the use of ACT in both primary and salvage therapy (Table 2), in spite of the fact that these guidelines were published during the same time period (2008–2011) with the availability of similar scientific information. This fact underlines the subjectivity of these guidelines due to the weakness of the data that support them.

**Nonpublished Clinical Trials**
A global vision of this topic should include both published studies and data from clinical trials that, once started, never resulted in publication of the final results (Table 3). In addition to clinical trials that are withdrawn for different reasons without obtaining results, the common practice of stopping clinical trials prematurely may overestimate the real effect of the intervention. The results of 7 of the 9 clinical trials evaluating ACT that are registered on the National Institutes of Health’s website, www.clinicaltrial.gov, have not been published. Five have been withdrawn—3 before the enrollment of patients and 2 because of insufficient recruitment. The remaining 2 studies have been completed. The results of clinical trial NCT00037206, completed in February 2003, are neither available nor published. The other study, identified as NCT00531479, has an optimal design as it is a randomized, double-blind, clinical trial comparing voriconazole and anidulafungin vs voriconazole monotherapy for primary treatment of IA. The preliminary results, which were reported at the 22nd European Congress of Clinical Microbiology and Infectious Diseases after enrollment of the first 277 patients, showed a significant reduction in the 6-week crude mortality rate when cases of possible aspergillosis were excluded [30]. The study was completed with 454 treated patients. Pending final peer review, the results in the whole population, which are posted on the website clinicaltrial.gov (dated: 3 April 2012), do not seem to confirm the preliminary results, assuming that no significant differences are noted.

**ECONOMIC ASPECTS**
A crucial aspect to consider regarding antifungal use is its elevated cost. In recent years, antifungal consumption has increased, often due to misuse [31]. The widespread use of ACT may have contributed to this fact, together with the rising incidence of IA and the use of newer and more expensive antifungal drugs. Thus, antifungal drugs account for a high portion of drug expenditures in hospital settings [32], especially in the hematological setting where the cost ranges from 36% to 57% of total expenditures [33, 34]. This has prompted the need for optimization of antifungal use in the hospital settings [32–35]. To our knowledge, only 1 study evaluated the cost effectiveness of ACT vs monotherapy for salvage therapy of IA in hematopoietic stem cell transplant (HSCT) recipients [36], concluding that salvage monotherapy with caspofungin was the most cost-effective strategy (dominant strategy) in terms of life-years gained. Although salvage ACT with L-AMB plus caspofungin was

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**Table 2. Recommendations of International Scientific Societies for the Indication of Antifungal Therapy for Treatment of Invasive Aspergillosis**

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<tbody>
<tr>
<td><strong>Primary therapy</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Monotherapy</td>
<td>A-I&lt;sup&gt;a&lt;/sup&gt;</td>
<td>A-I</td>
<td>A-I</td>
</tr>
<tr>
<td>Combined therapy</td>
<td>B-II</td>
<td>C-I&lt;sup&gt;c&lt;/sup&gt;/C-III&lt;sup&gt;c&lt;/sup&gt;</td>
<td>C-III</td>
</tr>
<tr>
<td><strong>Salvage therapy</strong></td>
<td></td>
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</tr>
<tr>
<td>Monotherapy</td>
<td>A-II&lt;sup&gt;d&lt;/sup&gt;</td>
<td>B-II&lt;sup&gt;a&lt;/sup&gt;</td>
<td>A-II</td>
</tr>
<tr>
<td>Combined therapy</td>
<td>B-II&lt;sup&gt;f&lt;/sup&gt;</td>
<td>B-II</td>
<td>B-III</td>
</tr>
</tbody>
</table>

<sup>a</sup> A-I: Good evidence (from ≥1 properly randomized controlled trials) to recommend its use.

<sup>b</sup> C-I: provisional and for the combination of voriconazole plus anidulafungin.

<sup>c</sup> C-III: for other combinations.

<sup>d</sup> A-II: Good evidence (from ≥1 nonrandomized trials or observational studies) to recommend its use.

<sup>e</sup> B-II: Moderate evidence (from opinions, clinical experience, descriptive studies or reports of experts committees) to recommend its use.

<sup>f</sup> B-II: Moderate evidence (from ≥1 nonrandomized trials or observational studies) to recommend its use.
Table 3. Antifungal Combined Therapy Trials Registered on ClinicaTrial.gov

<table>
<thead>
<tr>
<th>Identifier</th>
<th>Year</th>
<th>Status</th>
<th>Study</th>
<th>Design</th>
<th>Condition</th>
<th>Intervention (Antifungal Agents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT00076869</td>
<td>2004</td>
<td>Completed; published results (Maertens et al 2006 [24])a</td>
<td>MK0991 in Combination With Standard Antifungal Agent(s) for the Treatment of Salvage Invasive Aspergillosis (0991–037)</td>
<td>Nonrandomized; open label</td>
<td>Aspergillosis</td>
<td>MK0991: Caspofungin, Amphotericin B or Liposomal Amphotericin B and/or Azoles</td>
</tr>
<tr>
<td>NCT00620074</td>
<td>2008</td>
<td>Closed due to insufficient recruitment; results not published</td>
<td>Study to Test the Combination of Voriconazole and Anidulafungin in Patients Who Have, or Are Thought to Have, Invasive Aspergillosis and Who Are Unable to Take a Common Antifungal Therapy (Polyene)</td>
<td>Nonrandomized; open label</td>
<td>Aspergillosis</td>
<td>Voriconazole</td>
</tr>
<tr>
<td>NCT00047827</td>
<td>2002</td>
<td>Closed due to insufficient recruitment; results not published</td>
<td>Trial of Micafungin (FK463) in Combination With Liposomal Amphotericin B (AmBisome) for Aspergillosis</td>
<td>Nonrandomized; open label</td>
<td>Aspergillosis</td>
<td>Noncomparative</td>
</tr>
<tr>
<td>NCT01207128</td>
<td>2010</td>
<td>Withdrawn prior to enrollment</td>
<td>Trial of Combination Antifungal Therapy (Vori + Mica vs Vori + Placebo) in Invasive Aspergillosis</td>
<td>Randomized; double blind</td>
<td>Invasive aspergilosis</td>
<td>Voriconazole + Placebo</td>
</tr>
<tr>
<td>NCT00334412</td>
<td>2006</td>
<td>Completed; published results (Caillot et al 2007 [23])a</td>
<td>COMBISTRAT: AmBisome in Combination With Caspofungin for the Treatment of Invasive Aspergillosis</td>
<td>Randomized; open label</td>
<td>Invasive aspergilosis</td>
<td>Ambisome</td>
</tr>
<tr>
<td>NCT00531479</td>
<td>2007</td>
<td>Completed; results not published but posted on ClinicalTrials.gov</td>
<td>Anidulafungin Plus Voriconazole vs Voriconazole for the treatment of Invasive Aspergillosis</td>
<td>Randomized; double blind</td>
<td>Aspergillosis</td>
<td>Voriconazole</td>
</tr>
<tr>
<td>NCT00423163</td>
<td>2007</td>
<td>Withdrawn prior to enrollment</td>
<td>Study to Evaluate the Effectiveness of Voriconazole + Micafungin vs Voriconazole Alone for Invasive Aspergillosis</td>
<td>Randomized; double blind</td>
<td>Aspergillosis</td>
<td>Voriconazole</td>
</tr>
<tr>
<td>NCT00037206</td>
<td>2002</td>
<td>Completed; results not published</td>
<td>A Safety &amp; Effectiveness Study of Intravenous Anidulafungin With AmBisome for Treatment of Invasive Aspergillosis (IA).</td>
<td>Nonrandomized; open label</td>
<td>Aspergillosis</td>
<td>Noncomparative</td>
</tr>
<tr>
<td>NCT01188759</td>
<td>2010</td>
<td>Withdrawn prior to enrollment</td>
<td>Voriconazole And Anidulafungin Combination for Invasive Aspergillosis In Pediatric Subjects</td>
<td>Randomized; open label</td>
<td>Invasive aspergilosis</td>
<td>Voriconazole</td>
</tr>
</tbody>
</table>

* Published clinical trials are listed in Table 1.
the most effective strategy, the financial requirements needed to gain 1 life-year exceeded the ceiling ratio usually used to make decisions. Thus, although only this study has analyzed the pharmacoeconomics of ACT and its results are limited by the paucity of published data (including a theoretical population of HSCT recipients derived from studies with a time frame of 12 weeks), our conclusion is that the increased costs associated with ACT may not be compensated for by its effectiveness.

CONCLUSION

From our point of view, the use of ACT has not proven to be more effective than monotherapy and is not justified for the treatment of IA. Although there are no data suggesting increased toxicity when standard doses are used, the increase in cost may be difficult to assume by healthcare systems in the current economic context. Other approaches, including prompt diagnosis that would allow for the early initiation of therapy and the optimization of current antifungal therapy by therapeutic drug monitoring, may be more effective in improving the outcome of patients with IA.

Notes

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