Difficulties with Fungal Infections in Acute Myelogenous Leukemia Patients: Immune Enhancement Strategies

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Abstract
Invasive fungal infection in severely immunosuppressed patients with acute myelogenous leukemia (AML) remains a serious challenge because (a) of the higher rates of non–drug susceptible fungal sinopulmonary disease; (b) despite advances in diagnostic fungal assays, the correct identification of causative organism(s) is difficult, and antifungal drug susceptibility data are seldom available during clinical decision making; and (c) the increasing frequencies of zygomycosis, scedosporiosis, and highly virulent Candida tropicalis infection have undermined the gains attributed to effective antifungal drug therapy. Recombinant cytokines, such as recombinant human (rh)GM-CSF and interferon (IFN)-γ, have been explored to augment host antifungal immune responses. These cytokines promote activation and recruitment of granulocyte and monocellular phagocytic effector cells. Prophylaxis with rhGM-CSF was associated with significantly fewer life-threatening and serious (grade ≥3) infections, especially in older patients undergoing treatment for AML. The limited experience with rhGM-CSF for the treatment of invasive fungal infections in combination with antifungal drug(s) was associated with a favorable outcome, and in contrast to Escherichia coli–derived rhGM-CSF, the new preparation (sargramostim) was well tolerated and rarely associated with serious systemic toxicities. Similarly, IFN-γ has been successfully used in patients with antimicrobial drug–refractory and/or disseminated fungal infection. Most patients tolerate the T-helper type 1 protagonist cytokine without serious adverse events. In difficult-to-treat fungal infections, the addition of cytokines appears to improve outcome and may be considered early in severely immunosuppressed patients with AML.

Introduction
Several challenges exist in the treatment of invasive fungal infections (IFI) in patients with acute myelogenous leukemia (AML). First, the epidemiology of fungal infections is changing as saprophytic fungi that are more drug resistant are increasingly being associated with human disease. Second, early and correct diagnosis of fungal infections is difficult, even with the new generation of enzymatic immunoassays. Third, monotherapy with an antifungal agent is often unsuccessful, and additional approaches are being investigated, including combination therapy and immune-enhancement strategies.

Changing Epidemiology of IFI
Mortality resulting from systemic fungal infections has increased markedly in recent decades [1]. The attributable-mortality rates from candidemia (~24%) and invasive aspergillosis (~58%) are higher in older patients with cancer [2, 3]. Furthermore, IFIs are now frequently being associated with serious systemic disease and sepsis [4]. The problem of the increasing number of infections is compounded by an emergence of infections with non-Aspergillus fungi that are resistant to standard antifungal agents, for example, dematiaceous or “black” molds, which tend to be intrinsically less susceptible to amphotericin B, as well as Scedosporium. 

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apiospernum, the asexual form of Pseudallescheria boydii [5]. Some fungal species that were historically seen only in South America or Africa, typically associated with mucormycosis, phaeohyphomycosis, or chromoblastomycosis, are also appearing more frequently in the U.S.

**Difficulties in Diagnosing IFIs**

An additional problem for physicians who treat patients with IFIs is the difficulty in appropriate and early diagnosis of the infection [6]. Probable IFIs can be identified by one of four means: (a) culture-based analysis; (b) radiographic determination of a disease compatible with the diagnosis of IFI; for example, the “halo sign” seen on high-resolution computed tomography scans is clinically significant during the acute/early phase of invasive pulmonary aspergillosis [7]; (3) enzymatic immunoassays (EIAs) that detect fungal antigen galactomannan (GM-EIA) or d-glucan; and (d) DNA-amplification assays using polymerase chain reaction technology [8]. While GM-EIA is now used at several major cancer centers, published data for both the sensitivity and specificity of the assay vary widely [8]. Best results are obtained in patients who have not been treated with a systemic antimal agent. Once patients receive antifungal medications active against the filamentous fungi, even prophylactically, the sensitivity of EIA drops sharply [8].

Despite the greater availability of EIAs, therefore, most fungal infections cannot be diagnosed definitively without tissue biopsies. These include IFIs in patients with AML, which are often presumed or probable fungal infections that are treated empirically, and in high-risk AML patients with prolonged severe neutropenia, antifungal treatment may be started preemptively. Most preemptive treatment is given in recipients of allogeneic stem cell transplants, especially those with a high risk for developing fungal infections.

**Treatment of IFIs with Single Agents**

The “gold standard” treatment for IFIs has been the use of either amphotericin B deoxycholate or lipid formulations of amphotericin B. However, data from two large meta-analyses indicated that invasive aspergillosis does not respond well to amphotericin B, even when lipid formulations are used [3, 9]. The response rate to amphotericin B was approximately 55% in patients with leukemia [3], with similar results in a combined group of patients with leukemia or lymphoma [3]. In particular, patients with a fungal infection of the central nervous system had high case fatality rates [3, 9].

Broad-spectrum triazoles, including voriconazole, have also been evaluated for the treatment of IFIs. An often-cited report comparing voriconazole (Vfend®; Pfizer Inc., New York) with amphotericin B deoxycholate concluded that voriconazole was more effective [10]. The partial response rate with voriconazole was 32%, compared with 15% for amphotericin B (Table 1). However, the intent-to-treat analysis revealed less impressive results for complete responses, which were nearly the same in each treatment arm (amphotericin B, 17%; voriconazole, 21%) [10]. Voriconazole has also been used extensively in patients with cancer or bone marrow transplants since it became available in 2001, and while this has been associated with a drop in invasive aspergillosis, the increased incidence of serious invasive zygomycosis in these immunosuppressed patients has been attributed to voriconazole use, to which these organisms are intrinsically resistant [11]. In a study from our institution, prior exposure to voriconazole increased the risk for zygomycosis 20-fold, while in patients with fungal sinusitis, the probability of zygomycosis was nearly 80-fold higher [11].

**Combination Antifungal Therapy**

Caspofungin (Cancidas®, Merck & Co., Inc., West Point, PA) has also been evaluated in a small number of patients with invasive aspergillosis who were refractory to or intolerant of amphotericin B formulations or triazoles [12]. Complete and partial responses to caspofungin occurred much more frequently in patients who were intolerant to conventional therapy (75% of patients) rather than in patients who were truly refractory to conventional treatment (39% of patients) [12].

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<tr>
<th>Table 1. Response rates for patients with invasive aspergillosis treated with either voriconazole or amphotericin B in a randomized trial [6]</th>
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drug; the study (a retrospective chart review) was not an evaluation of initial treatment with combination therapy. While the overall favorable response was 60%, most of the patients fell within the “possible” (n = 20) rather than the “probable” (n = 4) or “proven” (n = 6) IFI response group, which skewed the data to a more favorable impression of the combination treatment [13]. A second study evaluated the addition of caspofungin in patients with IFIs refractory to liposomal amphotericin B [14]. Nearly equal numbers of patients had documented versus possible fungal infections, which again skewed the response rates. While the overall response rate was 42%, the response rate was only 22% in those with documented IFIs compared with 60% in those with possible IFIs [14].

A third study of combination therapy evaluated the use of either voriconazole alone (n = 31) or voriconazole plus caspofungin (n = 16) in patients who developed invasive aspergillosis after receiving stem cell transplants (mostly allogeneic) or cytotoxic chemotherapy, and who had failed initial therapy with amphotericin B formulations [15]. Treatment with the combination was associated with a higher survival rate at 90 days compared with voriconazole monotherapy (p = .048) [15].

A study published in early 2007 compared a higher dose of caspofungin (100 mg/day, twice the maintenance dose) with standard-dose caspofungin (70 mg followed by 50 mg daily) in patients with hematologic malignancies and hematopoietic stem cell transplantation (HSCT) [16]. Thirty-four patients received the higher dose; 63 patients received standard-dose caspofungin. Twelve weeks after the beginning of treatment, 44% of patients in the higher-dose group exhibited a complete or partial response, compared with 29% in the standard-dose group (p = .1). A significant probability of a favorable outcome (a lower associated risk for death) at 12 weeks was noted by logistic regression analysis in patients who received high-dose caspofungin (odds ratio, 3.066; 95% confidence interval, 1.092–8.61; p = .033). The better response in the higher-dose group may be a result of, in part, the fact that more patients in the higher-dose group received immune enhancement with recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF)—in this case, the yeast-derived rhGM-CSF sargramostim (Leukine®; Bayer HealthCare Pharmaceuticals Inc., Wayne, NJ), 41% versus 14% in the standard-dose group (p = .04)—and/or recombinant human interferon-γ (rhIFN-γ; Actimmune®; InterMune, San Francisco, CA), 26% versus 5% in the standard-dose group (p = .003) [16].

### IMMUNE-ENHANCEMENT STRATEGIES

During the immune response to an IFI, alveolar macrophages recruit more neutrophils to neutralize microconidia, which in turn release proteases that trigger an adaptive immune response. Activated T-helper type 1 (Th1) lymphocytes migrate from the lymph nodes back to the site of infection. Several factors can inhibit a host’s immune response to an IFI, including hematologic malignancy, chemotherapy, radiation therapy, systemic corticosteroids, and treatment for graft-versus-host disease (GVHD) [8].

Immunomodulators may play a role in this immune recovery process by restoring effector cell numbers, enhancing effector cell function, and promoting a Th1 environment. Several options exist for providing immune enhancement, including granulocyte colony-stimulating factor (known as filgrastim when referring to the recombinant human product—Neupogen®; Amgen Inc., Thousand Oaks, CA), GM-CSF, IFN-γ, and transfusions of mobilized donor granulocytes (Table 2) [8].

### GM-CSF

GM-CSF, as the name implies, affects the function of macrophages, promoting the development of a Th1 environment that contributes to optimal protection against fungal infections. GM-CSF has been evaluated in several clinical trials in patients with AML or other leukemias [17–19]. In a study of GM-CSF in patients with AML published in 1991, 30 patients received yeast-derived rhGM-CSF (sargramostim) after chemotherapy, and their clinical outcomes were compared with those of 56 patients selected as historical controls [17]. Sargramostim therapy produced rapid neutrophil recovery and a lower early death rate (14% versus 39% in the control group; p = .009). No major adverse events occurred, and no clinically significant leukemic stimulation was observed [17].

A small pilot study of *Escherichia coli*–derived rhGM-CSF as adjuvant treatment in neutropenic patients with fungal infections included eight patients (six with acute leukemias) [18]. Researchers documented four complete responses and two partial responses with the use of rhGM-CSF plus amphotericin B [18].

A phase III trial of rhGM-CSF (sargramostim) was undertaken in a group of older adults (age, 55–70) with de novo AML to determine whether immune enhancement could shorten the period of neutropenia and decrease...
life-threatening or severe infections [19]. Of the eligible and evaluable patients, 60 received sargramostim and 57 received placebo. The median time to neutrophil recovery was shorter in the group receiving sargramostim, and the overall survival duration was longer (sargramostim, 10.6 months; placebo, 4.8 months; \( p = .048 \)) [19]. Furthermore, fewer serious infections occurred in patients receiving sargramostim (grade 4 or 5 infections, 9.6% versus 36.2%; \( p = .002 \)) [15, 16]. Fatal infections in 20 patients with grade 3 or 4 fungal infections were much lower in the sargramostim group as well (12.5% versus 75%; \( p = .02 \)) [20].

IFN-\( \gamma \)

IFN-\( \gamma \) is a Th1 cytokine that promotes the fungicidal activity of mononuclear cells, but several concerns have been noted about its use (Table 3) [21]. The primary concerns are drug toxicity and the lack of data on its therapeutic efficacy. An additional concern in patients with leukemia who have undergone transplantation is the possibility of exacerbated GVHD [21].

Data from 32 patients with fungal infections who received rhIFN-\( \gamma \)lB after HSCT formed the basis for a retrospective analysis to establish the safety of this adjuvant treatment [21]. While fever was common during treatment with rhIFN-\( \gamma \)lB (\( n = 9, 28\% \)), new-onset lymphocytopenia occurred in only 3% of patients and resolved after cytokine therapy was discontinued. No rhIFN-\( \gamma \)lB–related episodes of neutropenia, thrombocytopenia, anemia, or liver dysfunction were observed. Most importantly, rhIFN-\( \gamma \)lB therapy did not precipitate or exacerbate acute or chronic GVHD. Instead, improvement in GVHD occurred in 15% of patients and tachycardia in 5% of patients were improved. Twenty percent of patients developed fever, and 10% developed skin rashes. Reversible liver dysfunction in 15% of patients and tachycardia in 5% of patients were considered to be rhIFN-\( \gamma \)lB–associated adverse events. One patient (5%) had transient dyspnea that was attributed to granulocyte transfusion. Although the study focused primarily on safety, treatment responses were recorded 4 weeks after initiation of therapy. Nine patients (45%) had complete or partial resolution of infection and three (15%) had stable IFIs [23].

Most patients (85%) had neutropenia during granulocyte transfusion therapy [23]. An evaluation of adverse events revealed that 20% of patients developed fever, and 10% developed skin rashes. Reversible liver dysfunction in 15% of patients and tachycardia in 5% of patients were considered to be rhIFN-\( \gamma \)lB–associated adverse reactions. One patient (5%) had transient dyspnea that was attributed to granulocyte transfusion. Although the study focused primarily on safety, treatment responses were recorded 4 weeks after initiation of therapy. Nine patients (45%) had complete or partial resolution of infection and three (15%) had stable IFIs [23].

Table 3. Potential concerns about interferon-\( \gamma \) therapy in patients with hematologic malignancy and hematopoietic stem cell transplantation [17]

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<td>Relapse hematologic malignancy</td>
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<td>Lack of therapeutic efficacy</td>
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Conclusions

A multimodal approach is needed to improve outcome in patients with AML who have fungal infections. Early and accurate diagnosis is critical, but this still poses a significant problem, despite newer diagnostic assays. Current treatment approaches include the use of more effective antifungal drugs, including combination and/or high-dose therapy, as well as the use of agents to enhance the
immune response. Two approaches to immune enhancement that have shown promise in recent research studies are (a) the use of rhGM-CSF and rhIFN-γ and (b) the combination of donor granulocyte transfusion plus rhIFN-γ and/or rhGM-CSF. Adjunct immune therapy should be explored further in larger, prospective, randomized clinical trials with adequate statistical power to determine its true effectiveness.

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