Granulocyte Macrophage Colony-Stimulating Factor in 66 Patients with Myeloid or Lymphoid Neoplasms and Recipients of Hematopoietic Stem Cell Transplantation with Invasive Fungal Disease

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Key Words

Invasive fungal disease • Granulocyte macrophage colony-stimulating factor • Stem cell transplant • Leukemia • Combination antifungal agents • Immune suppression

Abstract

Background/Aims: Adding granulocyte macrophage colony-stimulating factor (GM-CSF) may improve the response to antifungal therapy in immunosuppressed patients with invasive fungal disease (IFD). **Methods:** We retrospectively assessed 66 patients in whom GM-CSF was given during antifungal therapy. **Results:** Severe neutropenia (77%) and refractory/relapsed cancer (65%) were common in the group. Prior to GM-CSF therapy, 15% of patients received high-dose corticosteroids for a median of 30 ± 16 days [median cumulative dose (c.d.) 1,184 ± 1,019 mg], and 9 received steroids during GM-CSF therapy for a median of 16 ± 12 days (median c.d. 230 ± 1,314 mg). Mild toxic effects were noted in 9% of patients; there were no cases of cardiopulmonary toxicity. All-cause deaths were observed in 68% of patients and 48% died of progressive IFD. High-dose corticosteroids prior to GM-CSF (OR 24; 95% CI 2.21–264.9; $p \leq 0.009$), GM-CSF started in the intensive care unit (OR 10; 95% CI 1.66–63.8;

$p \leq 0.01$), concurrent granulocyte transfusions (OR 5; 95% CI 1.27–16.8; $p \leq 0.02$) and proven/probable IFD (OR 4; 95% CI 1–16.2; $p \leq 0.05$) predicted antifungal treatment failure.

Conclusions: GM-CSF adjuvant therapy was tolerated without serious toxicity and antifungal treatment failure remained a challenge in patients treated with high-dose systemic corticosteroids.

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Introduction

Treatment of invasive fungal disease (IFD) remains difficult in severely immunosuppressed patients with leukemia or lymphoma [1] and in patients following allogeneic hematopoietic stem cell transplantation [2]. To improve treatment, researchers have explored the use of recombinant cytokines such as recombinant human granulocyte macrophage colony-stimulating factor (GM-CSF) and interferon-gamma (IFN- γ) to improve the hosts' immune control of these life-threatening opportunistic infections

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[3]. In immunosuppressed animals, GM-CSF alone or in combination with IFN- γ has been shown to enhance the fungicidal activity of innate phagocytic cells [4, 5]. Furthermore, treating human neutrophils ex vivo with GM-CSF with or without IFN- γ has been shown to promote fungicidal activity against *Rhizopus* and *Absidia* species hyphae [6]; whereas without such an immune 'boost,' neutrophils are largely ineffective against germinating *Rhizopus* hyphae, a prominent agent of human mucormycosis [6]. Interestingly, IFN- γ alone was recently shown to have a modest impact in an experimental model of disseminated *Rhizopus* species infection and only when GM-CSF was added to IFN- γ , a prolonged survival and reduced fungal tissue burden occurred in mice [7]. Improved immune control following cytokine therapy has also been demonstrated for the emerging black molds such as *Scedosporium apiospermum* [8, 9] and *Scedosporium proliferans*. A differential efficacy in the optimal duration of GM-CSF exposure for promoting fungicidal activity of neutrophils against these two species of *Scedosporium* was an interesting finding in this study [10]; however, the clinical relevance of this observation remains uncertain.

Clinical data on the use of GM-CSF as adjuvant antifungal therapy are scarce; occasional case reports [11] or small case series of 2–8 patients, mostly with drug-refractory invasive aspergillosis, fusariosis, zygomycosis or *Scedosporium* species infection, have been published but provide limited information [12–15]. Large case series are critically needed to assess the role of therapeutic safety and more importantly efficacy of GM-CSF in patients with established invasive fungal disease before feasibility of prospective trial(s) can be considered.

We previously showed that large GM-CSF doses (median 4,750 μ g) can be safely administered to cancer patients with refractory neutropenia who are also receiving healthy-donor granulocyte transfusions plus IFN- γ for drug-refractory IFD. More than half of these patients had improvement and/or stabilization of life-threatening fungal disease [16]. Here, we present a comprehensive analysis of a substantial cohort of patients with cancer and/or allogeneic hematopoietic stem cell transplantation who developed refractory IFD and received adjuvant GM-CSF.

Materials and Methods

Study Design

We retrospectively analyzed all consecutive patients who had hematological malignancies and/or had undergone allogeneic hematopoietic stem cell transplantation and who had received GM-

CSF (sargramostim) as adjuvant immune enhancement therapy for IFD at the University of Texas MD Anderson Cancer Center (Houston, Tex., USA) between July 2000 and January 2007. The study was undertaken after obtaining approval from the institutional review board; the requirement for written informed consent was waived. Patients were followed until early 2010 for delayed GM-CSF adverse events, infection recurrence and all-cause mortality. Patients were identified using the institutional pharmacy database and data were collected from electronic medical records. These helped to identify patients in whom fungal disease occurred during GM-CSF prophylaxis and in whom GM-CSF was added as salvage adjuvant with progressive fungal disease. Data collected included demographic information, underlying cancer, comorbid conditions, history and type of stem cell transplantation, types of infections and outcomes.

The treatment response was determined by the following: (a) treating primary hematologist or bone marrow transplant faculty; (b) consulting infectious diseases and pulmonary faculty, when present; (c) reports from the institutional radiology department and microbiology laboratory, and (d) all treatment responses including deaths associated with progressive invasive fungal disease were independently reassessed by the study team and the principle investigator.

Definitions

IFD was defined as proven, probable or possible according to the guidelines of the Invasive Fungal Infections Cooperative Group of the European Organization for Research and Treatment of Cancer/Mycoses Study Group of the National Institute of Allergy and Infectious Diseases [17]. For probable and possible IFD, we considered host factors and major and minor criteria in determining the strength of the evidence supporting the diagnosis. Definite IFD required the demonstration of tissue-invasive mold on histological examination of tissue samples or the presence of fungemia in patients with clinical and/or radiographic evidence of deep-tissue infection. Isolation of *Aspergillus* and *Penicillium* species from a blood culture alone with no evidence of deep-tissue infection was regarded as pseudofungemia [18]. Adverse events were considered to be due to GM-CSF if attributed to the agent in notes by the treating physician, consulting physician, a mid-level healthcare provider and/or rounding pharmacy staff, or if discontinuation resulted in prompt resolution of symptoms. Patients were stratified into two groups: those who achieved complete or partial resolution of IFD and those in whom IFD did not improve or who showed clinical and/or radiographic progression. Infection-related death was defined as death within 12 weeks of the last positive culture among patients with microbiologically documented filamentous mold infections in the absence of known noninfectious causes of death. For patients with clinical and/or radiographic diagnosis of infection, infection-associated deaths were defined according to established guidelines [19].

Statistical Analysis

We calculated descriptive statistics for all patients and for those who did and who did not have a response to antifungal plus GM-CSF therapy. Differences in categorical data were analyzed using the χ^2 test or the Fisher exact test, and differences in discrete variables were analyzed using Student's two-tailed t test; a p value <0.05 considered to be statistically significant. A backward logis-

tic regression analysis model was used to identify factors predictive of treatment failure. We used PASW Statistics 18 software (2009; IBM® SPSS® software, Somers, N.Y., USA) for all statistical analyses.

Results

Sixty-six patients with IFD received adjuvant GM-CSF during the period studied. Patient, disease and treatment characteristics are summarized in table 1. Ten patients had received high-dose systemic corticosteroids before GM-CSF therapy; the median cumulative dose (c.d.) $\pm$ standard deviation was $1,184 \pm 1,019$ mg (range 338–3,540 mg), and the median duration was 30 ± 16 days (range 13–71 days). Nine patients received high-dose systemic corticosteroids during GM-CSF therapy; the median c.d. was $230 \pm 1,314$ mg (range 75–4,200 mg), and the median duration was 16 ± 12 days (range 2–30). The daily dose of GM-CSF was 500 μ g in 97% of patients; GM-CSF was given via subcutaneous injection in all cases. Thirty-five patients (53%) had a response, whereas IFD-related deaths were noted mostly in 29 (94%) of 31 patients who failed GM-CSF plus systemic antifungal therapy.

Table 2 summarizes the characteristics of infections among patients who did and did not have a response to antifungal plus adjuvant GM-CSF therapy. Causes of death are also given. It is interesting that 2 of 11 patients with proven zygomycosis and 2 of 7 patients with proven cutaneous and/or disseminated *Fusarium* spp. infection survived on long-term follow up; whereas all 4 with invasive aspergillosis had died.

Six patients had adverse events attributable to GM-CSF administration: 3 patients had myalgia and individual patients had fever, localized skin rash and bone pain, respectively. In all these patients symptoms resolved following discontinuation of GM-CSF. There were no cases of pulmonary capillary leak syndrome or unexplained noncardiogenic pulmonary edema during GM-CSF treatment or during the following 12 weeks. All-cause deaths were observed in 44 patients (68%) and 32 (48%) patients died of progressive IFD.

Univariate Analysis

Patients in whom antifungal drug plus GM-CSF therapy failed were compared to those in whom a complete or partial response was observed (table 1). In patients who had comorbid medical conditions such as diabetes mellitus or renal failure, antifungal plus GM-CSF therapy

was significantly more likely to fail (74 vs. 49%; $p \leq 0.04$). Similarly, high-dose corticosteroid therapy prior to GM-CSF use was significantly associated with treatment failure (29 vs. 3%; $p \leq 0.004$). Patients with proven/probable IFD (84 vs. 54%; $p \leq 0.01$) and those in whom GM-CSF therapy commenced during an intensive care unit stay (35 vs. 6%; $p \leq 0.04$) were significantly more likely to have treatment failure. Furthermore, patients in whom therapy failed required longer hospitalization (median 35 ± 27 vs. 25 ± 18 days; $p \leq 0.03$) and were more likely to die of any cause (100 vs. 37%; $p \leq 0.0001$) or of IFD (94 vs. 9%; $p \leq 0.0001$). Patients who had a favorable response were more likely to have received antineoplastic chemotherapy prior to GM-CSF use (86 vs. 61%; $p \leq 0.02$) and less likely to have received granulocyte transfusions (31 vs. 58%; $p \leq 0.03$). There were no differences between responders and nonresponders for other factors typically associated with poor treatment outcomes, including refractory cancer, allogeneic stem cell transplant, severe neutropenia, lymphocytopenia, high APACHE II score or concurrent therapy with granulocyte colony-stimulating factor and/or IFN- γ (table 1).

Multivariate Analysis

The results of logistic regression analysis for the factors associated with therapeutic failure are shown in table 3. The use of high-dose systemic corticosteroids prior to GM-CSF therapy (OR 24; 95% CI 2.21–264.9; $p \leq 0.009$), GM-CSF therapy initiated during an intensive care unit stay (OR 10; 95% CI 1.66–63.8; $p \leq 0.01$) and proven or probable IFD (OR 4; 95% CI 1–16.2; $p \leq 0.05$) were independently predictive of treatment failure. In addition, a five-fold higher probability of treatment failure was seen among the 29 patients who had received healthy-donor granulocyte transfusions (OR 5; 95% CI 1.27–16.8; $p \leq 0.02$); this finding was unexpected.

Discussion

In this analysis, GM-CSF was well tolerated without serious toxic effects in severely immunosuppressed patients who often received prolonged therapy (up to 57 doses). A complete or partial response occurred in more than half of the patients despite recent treatment with antineoplastic therapy. Initiation of GM-CSF therapy in critically ill patients did not improve outcomes significantly. Similarly, patients with medical comorbidities treated with GM-CSF plus antifungal drugs had higher rates of treatment failure.

Table 1. Characteristics of patients treated with adjuvant GM-CSF for IFD, by response to antifungal treatment

Characteristic	All patients (n = 66)	Complete or partial re- sponders (n = 35; 53%)	Nonresponders (n = 31; 47%)	p value
Age, years	54 ± 18 (8–80)	54 ± 18 (8–80)	52 ± 19 (8–78)	0.8
65 years or older	57 (86)	30 (86)	27 (87)	≥1.0
Sex, male	38 (58)	21 (60)	17 (55)	0.8
Underlying disease				
Acute leukemia	49 (74)	27 (77)	22 (71)	≥1.0
Chronic leukemia	5 (8)	2 (6)	3 (10)	0.7
Lymphoma	12 (18)	6 (17)	6 (19)	NS
Refractory or relapsed cancer	43 (65)	24 (69)	19 (61)	0.6
Comorbidities	40 (61)	17 (49)	23 (74)	0.04
Diabetes mellitus	8 (12)	4 (11)	4 (13)	≥1.0
Renal failure	4 (6)	1 (3)	3 (10)	0.3
Chemotherapy prior to GM-CSF	49 (74)	30 (86)	19 (61)	0.02
Stem cell transplantation				
Allogeneic graft recipients	25 (38)	12 (34)	13 (42)	0.3
Prior to GM-CSF therapy	30 (45)	16 (46)	14 (45)	≥1.0
Interval from transplantation to GM-CSF, days	346 ± 490 (16–2,091)	477 ± 455 (16–1,564)	267 ± 522 (2–365)	0.3
High-dose corticosteroids (>600 mg prednisone equivalent)				
Prior to GM-CSF	10 (15)	1 (3)	9 (29)	0.004
During GM-CSF	9 (14)	2 (6)	7 (23)	0.07
Neutropenia, <500 cells/ml				
Start of GM-CSF	51 (77)	30 (86)	21 (68)	0.1
During GM-CSF	30 (45)	19 (54)	11 (35)	0.1
End of GM-CSF	22 (33)	11 (31)	11 (35)	0.8
Lymphocyte count at start of GM-CSF, cells/ml	200 ± 98	200 ± 85	200 ± 111	0.3
Neutrophil count at start of GM-CSF, cells/ml	440 ± 3,429	255 ± 1,658	1,150 ± 4369	0.1
Duration of hospitalization, days	29 ± 23 (11–121)	25 ± 18 (14–96)	35 ± 27 (11–121)	0.03
Fungal disease status				
Probable or proven	45 (68)	19 (54)	26 (84)	0.01
Possible	21 (32)	16 (46)	5 (16)	
APACHE score	15 ± 4 (7–32)	15 ± 3 (7–22)	16 ± 5 (9–32)	0.1
APACHE score >16	29 (44)	12 (34)	17 (55)	0.1
Intensive care unit stay				
GM-CSF therapy	13 (20)	2 (6)	11 (35)	0.004
ICU stay prior to GM-CSF, days	3 ± 3 (1–11)	6 ± 1 (5–6)	3 ± 3 (1–11)	0.4
GM-CSF therapy				
Number of doses	13 ± 10 (7–57)	13 ± 11 (7–57)	13 ± 9 (7–36)	0.8
Therapy for <14 doses	34 (52)	19 (54)	15 (48)	0.8
Number of treatment days	13 ± 25 (7–177)	13 ± 32 (7–177)	13 ± 9 (7–36)	0.5
Interval from IFD diagnosis to start of GM-CSF, days	10 ± 18 (0–81)	9 ± 20 (1–81)	12 ± 15 (1–75)	0.9
Adverse events	6 (9)	3 (9)	3 (10)	≥1.0
Concurrent G-CSF	53 (80)	28 (80)	25 (81)	≥1.0
Number of doses	21 ± 17 (1–84)	21 ± 15 (4–63)	22 ± 19 (1–84)	0.9
Concurrent granulocyte transfusions	29 (44)	11 (31)	18 (58)	0.03
Number of transfusions	8 ± 5 (1–21)	5 ± 6 (1–18)	10 ± 5 (1–21)	0.2
Concurrent IFN-γ therapy	13 (20)	6 (17)	7 (23)	0.8
Number of doses	6 ± 5 (2–19)	9 ± 6 (3–19)	5 ± 3 (2–11)	0.2
Deaths due to all causes	44 (67)	13 (37)	31 (100)	0.0001
IFD-related deaths	32 (48)	3 (9)	29 (94)	0.0001
Interval between stopping GM-CSF and death, days	12 ± 23 (1–88)	46 ± 24 (2–88)	3 ± 11 (1–40)	0.0001

Data are presented as number of patients (%) or median value ± standard deviation (range). G-CSF = Granulocyte colony-stimulating factor.

Table 2. Characteristics of infections, treatment outcomes and results of extended follow-up in patients treated with adjuvant GM-CSF for IFD

Infection characteristics	Complete or partial responders (n = 35)	Nonresponders (n = 31)	Cause of death (n = 44)
Possible fungal infection	16 (46)	5 (16)	10 (23)
Fungal pneumonia	13 (37)	4 (13)	Relapsed malignancy and/or progressive infection
Fungal pneumonia and parainfluenza 3 pneumonitis	1 (3)	0	Adult respiratory distress syndrome
Fungal pneumonia and alpha <i>Streptococcus</i> bacteremia	1 (3)	0	Relapsed, progressive cancer
Fungal pneumonia, MRSA bacteremia and parainfluenza 3 upper respiratory tract infection	0	1 (3)	Graft-versus-host disease and progressive pneumonia
Fungal pneumonia and RSV upper respiratory tract infection	1 (3)	0	
Probable fungal infection	8 (23)	10 (32)	11 (25)
Fungal pneumonia	2 (6)	1 (3)	Relapsed malignancy and progressive infection
Fungal and MRSA pneumonia	1 (3)	0	Relapsed, progressive cancer
<i>Aspergillus</i> spp. pneumonia	0	1 (3)	Relapsed cancer and progressive infection
<i>Aspergillus terreus</i> pneumonia	1 (3)	0	
<i>Aspergillus flavus</i> pneumonia	0	1 (3)	Progressive fungal pneumonia
<i>Aspergillus</i> spp. pneumonia, disseminated adenovirus infection to lungs and bone marrow	0	1 (3)	Relapsed malignancy and progressive infection
<i>Aspergillus flavus</i> and <i>Stenotrophomonas maltophilia</i> pneumonia	0	1 (3)	Progressive polymicrobial pneumonia
<i>Aspergillus niger</i> pneumonia, <i>Enterococcus faecalis</i> pneumonia and bacteremia, and CoNS bacteremia	0	1 (3)	Progressive polymicrobial pneumonia
<i>Aspergillus fumigatus</i> pneumonia and parainfluenza 3 pneumonia	1 (3)	0	
<i>Aspergillus fumigatus</i> brain abscess; MRSA, <i>Stenotrophomonas maltophilia</i> and <i>Pseudomonas aeruginosa</i> pneumonia	0	1 (3)	Graft-versus-host disease and progressive pneumonia
<i>Aspergillus terreus</i> and <i>Enterococcus</i> spp. pneumonia	0	1 (3)	Graft failure and progressive pneumonia
<i>Aspergillus fumigatus</i> , <i>Aspergillus terreus</i> , <i>Aspergillus versicolor</i> , <i>Cladosporium</i> spp., <i>Mycobacterium gordonae</i> , and <i>Stenotrophomonas maltophilia</i> pneumonia	1 (3)	0	
<i>Aspergillus versicolor</i> and <i>Enterococcus</i> spp. pneumonia	0	1 (3)	Progressive pneumonia
<i>Alternaria</i> spp. and <i>Curvularia</i> spp. sinusitis	1 (3)	0	
<i>Mucor</i> spp. pneumonia	1 (3)	0	
<i>Mucor</i> spp. and <i>Enterococcus</i> spp. pneumonia	0	1 (3)	Progressive fungal pneumonia
Proven fungal infection	11 (31)	16 (52)	23 (52)
<i>Candida krusei</i> fungemia and RSV pneumonitis	0	1 (3)	Refractory RSV pneumonitis
<i>Fusarium</i> spp. sinusitis and <i>Aspergillus flavus</i> fungemia	0	1 (3)	Relapsed malignancy and progressive infection
<i>Aspergillus fumigatus</i> cerebral abscess, pulmonary zygomycosis, adenovirus cystitis	0	1 (3)	Graft failure and progressive fungal disease
<i>Aspergillus fumigatus</i> pneumonia, <i>Alternaria</i> spp. sinusitis, VRE pneumonia	0	1 (3)	Graft-versus-host disease and progressive fungal disease
<i>Aspergillus terreus</i> pneumonia	1 (3)	1 (3)	Relapsed malignancy plus progressive infection

Table 2 (continued)

Infection characteristics	Complete or partial responders (n = 35)	Nonresponders (n = 31)	Cause of death (n = 44)
<i>Candida tropicalis</i> disseminated disease, <i>Roseomonas</i> spp., CoNS, <i>Stomatococcus</i> spp., and alpha Streptococcus bacteremia	1 (3)	0	
Cutaneous fungal lesions ¹	1 (3)	0	Relapsed malignancy plus progressive infection
Cutaneous zygomycosis	0	1 (3)	Relapsed malignancy plus progressive infection
<i>Rhizopus</i> spp. skin infection	1 (3)	0	Relapsed, progressive cancer
<i>Candida tropicalis</i> fungemia	0	1 (3)	Relapsed malignancy plus progressive infection
<i>Fusarium</i> spp. fungemia	1 (3)	0	
<i>Scedosporium prolificans</i> fungemia, lung and skin infection	0	1 (3)	Progressive disseminated fungal disease
<i>Candida tropicalis</i> fungemia; <i>Enterobacter</i> spp. and <i>Pseudomonas aeruginosa</i> bacteremia	1 (3)	0	
<i>Fusarium</i> spp. fungemia, sinus and skin infection	1 (3)	0	Relapsed, progressive cancer
<i>Fusarium</i> spp. fungemia, lung and skin infection	1 (3)	0	
<i>Fusarium</i> spp. sinusitis	0	1 (3)	Progressive fusariosis with CNS involvement
<i>Fusarium</i> spp. sinusitis and skin infection	0	1 (3)	Relapsed, progressive malignancy and disseminated fusariosis
<i>Fusarium</i> spp. skin infection	1 (3)	0	Subarachnoid hemorrhage
<i>Rhizomucor</i> pneumonia	1 (3)	0	
<i>Rhizomucor</i> rhinocerebral disease	0	1 (3)	Relapsed, progressive cancer and rhinocerebral zygomycosis
<i>Rhizopus</i> rhinocerebral disease	0	1 (3)	Graft-versus-host disease and progressive fungal disease
<i>Rhizopus</i> and <i>Enterococcus faecium</i> skin infection and <i>Enterococcus faecalis</i> bacteremia	0	1 (3)	Relapsed, progressive cancer and polymicrobial infection
<i>Rhizomucor</i> sinusitis	0	1 (3)	Progressive fungal disease
Pulmonary zygomycosis	0	1 (3)	Relapsed, progressive malignancy and pulmonary zygomycosis
Pulmonary zygomycosis and <i>Stenotrophomonas maltophilia</i> sinusitis	0	1 (3)	Relapsed, progressive malignancy and polymicrobial infection
Pulmonary and skin zygomycosis	1 (3)	0	

Data are number of patients (%). CoNS = Coagulase-negative *Staphylococcus*; CNS = central nervous system; MRSA = methicillin-resistant *Staphylococcus aureus*; VRE = vancomycin-resistant *Enterococcus*; RSV = respiratory syncytial virus.

¹ Histologically confirmed invasive fungal disease while skin biopsy sample fungal cultures remained sterile.

Patients in whom antifungal plus GM-CSF therapy failed received significantly fewer doses of GM-CSF. This may have occurred for a number of reasons: because patients with refractory IFD died early; GM-CSF was discontinued owing to a lack of response; the patients experienced GM-CSF intolerance or toxicity, and/or the pri-

mary and/or infectious diseases consulting physicians may have changed, resulting in therapy changes.

Patients who received granulocyte transfusions had a five-fold increase in the probability of antifungal plus GM-CSF treatment failure. This finding might be attributable to the use of granulocyte transfusions in more se-

Table 3. Multivariate analysis of factors predictive of treatment failure in patients who received adjuvant GM-CSF for IFD

Factor associated with failure	OR	95% CI	p value
Steroid use prior to GM-CSF	24	2.21–264.9	0.009
Intensive care unit at start of GM-CSF	10	1.66–63.8	0.01
Granulocyte transfusion during GM-CSF	5	1.27–16.8	0.02
Proven or probable IFD	4	1–16.2	0.05

riously ill patients with prolonged, severe neutropenia [20] or unappreciated adverse events from the granulocyte transfusions, and possible reactivation of opportunistic herpes viruses such as human herpes virus 6, which was recently shown to be associated with granulocyte transfusions [21].

Significantly better outcome in patients presented in this study who recently received antineoplastic therapy may in part reflect early myeloid recovery associated with recombinant myeloid growth factor [22]. In a large multicenter trial, patients who were randomized to receive GM-CSF supportive therapy exhibited a median overall survival advantage of nearly 6 months. Patients treated with adjuvant GM-CSF also demonstrated an earlier neutrophil recovery, had fewer episodes of serious infections including fungal infections, and, importantly, experienced fewer infection-associated deaths in comparison with patients with no myeloid recovery support [22]. However, this benefit was not seen in a subsequent, albeit smaller, single-center trial [23]. More recently, in a matched-cohort study of nearly 2,000 health insurance claims in the USA from 2000 to 2007, GM-CSF recipients demonstrated a 56% lower risk of infection-related hospitalizations compared to patients treated with short- or long-acting granulocyte colony-stimulating factor [24].

In animal experiments, GM-CSF mitigates corticosteroid-induced suppression of proinflammatory cytokines such as interleukin-1 and tumor necrosis factor- α and chemotactic chemokines such as macrophage inflammatory protein-1- α by bronchoalveolar macrophages that are pivotal in early innate defenses against disease-causing *Aspergillus* microconidia [25]. Reversal of corticosteroid-induced macrophage suppression following GM-CSF exposure has been demonstrated to last for a week or longer in animal experiments [25]. Dexamethasone promotes κ B inhibitor levels in the cytoplasm of macrophages; this blocks nuclear factor- κ B translocation to the nucleus. GM-CSF reduces the cytoplasmic concentration of inhibitor κ B, resulting in high NF- κ B

concentrations in macrophage nuclei thereby augmenting innate microbicidal activity [26]. The clinical relevance of these findings gathered from experiments in nonprimate vertebrates remains unclear. In our study, 15% of patients had been exposed to high-dose systemic corticosteroids prior to and 14% during GM-CSF therapy. It was expected from the above findings that GM-CSF adjuvant therapy would improve outcomes for this subgroup of patients with fungal disease; however, on multivariate analysis, exposure to high-dose steroids remained the most prominent predictor of treatment failure despite GM-CSF therapy (table 3). This observation was unexpected and requires a further assessment of alternative mechanism(s) for GM-CSF-based immune modulation in patients with IFD. Impact on GM-CSF on other innate effector cells such as natural killer (NK) cells had also been demonstrated to promote fungicidal activity and reverse the immunosuppressive effect of fungi on NK cells [27]. A thorough analysis of innate immune function following GM-CSF therapy in relationship to IFD outcome is needed.

Alternative routes of cytokine administration have been studied to reduce systemic exposure and potential of unintended consequences such as leukemia stimulation and exacerbation of graft-versus-host disease. As the majority of fungal infections involve the lungs, aerosolized drug delivery has been considered an attractive platform for drug administration. In a recent study, high drug concentrations were achieved in lung tissue and undesired systemic exposure was prevented with aerosol GM-CSF delivery, resulting in low potential drug toxicity and drug-drug interactions [28]. In other experiments, intranasal GM-CSF resulted in a six-fold decrease in fungal burden among immunosuppressed mice in an experimental model of pulmonary necrotizing aspergillosis [29]. The safety and feasibility of aerosolized GM-CSF for metastatic disease have been evaluated in patients with respiratory tract malignancies [30] but need further evaluation for the treatment of pulmonary mycosis.

As with all retrospective studies, heterogeneity introduced by patient selection, dose and route of administration, various levels of immunosuppression due to underlying disease and type and number of antineoplastic therapies, various preparatory regimens for allogeneic stem cell transplantation, and the use of other myeloid growth factors (e.g. granulocyte colony-stimulating factor, IFN- γ) and a large cohort of patients with possible rather than probable or definite IFD may influence outcomes. However, in the cohort presented, the subgroups of patients who did and did not have a response showed no significant differences in the distribution of refractory cancer, allogeneic stem cell transplantation, presence of severe neutropenia or lymphocytopenia, severity of illness and multiorgan dysfunction as measured by APACHE II score, or concurrent use of other cytokines to promote neutrophil recovery or cellular immune responses (table 1). Furthermore, this is the largest study reported so far on GM-CSF use in patients with established IFD.

In summary, this large patient cohort provides comprehensive information regarding the tolerability of

modern recombinant GM-CSF preparations (sargramostim) and the feasibility of administering the agent to high-risk cancer patients and stem cell transplant recipients with IFD. It was unexpected that, despite the addition of GM-CSF, high-dose systemic corticosteroid therapy continued to remain a prominent predictor of treatment failure. A complete or partial response occurred in more than half of the patients treated with GM-CSF despite recent treatment with antineoplastic therapy and the presence of other predictors of poor outcome. Further prospective studies to assess GM-CSF efficacy in the treatment of established fungal disease are needed.

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