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Clinical Applications of G-CSF and GM-CSF in the Treatment of Infectious Diseases

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1. INTRODUCTION

Infectious diseases remain a major cause of morbidity and mortality throughout the world, including the United States. The immune system represents the primary host defense against pathogenic bacteria, fungi, and parasites. The first line of defense is comprised primarily of polymorphnuclear granulocytes (PMNs), macrophages, natural killer (NK) cells, and cytotoxic lymphocytes. Upon activation, these cells can destroy and eliminate pathogenic microorganisms. The possibility of augmenting host defense has increased dramatically during the past two decades with the discovery and development of cytokines. The immunomodulatory effects of colony-stimulating factors (CSFs) on selected leukocyte populations have received increasing attention recently.

This chapter will focus on the properties of two cytokines, namely granulocyte colony-stimulating factor (G-CSF) and granulocyte–macrophage colony-stimulating factor (GM-CSF). Both have been subjects of significant investigation as therapeutic immunomodulatory agents to upregulate phagocyte function during infection. Despite overlapping biologic activities, the two factors do not appear to be derivatives of a common evolutionary ancestral regulatory molecule based on their respective amino acid sequence structures *(1)*. Both cytokines are now available commercially in recombinant forms for clinical use worldwide.

2. G-CSF

2.1. Overview

Granulocyte-CSF is produced primarily by monocytes/macrophages, fibroblasts, and endothelial cells *(2)*. Two different genes encoding the amino acid sequence of G-CSF were initially isolated independently from different tissue sources. Nagata et al. isolated cDNA for G-CSF, which encoded a predicted amino acid sequence of 177 amino acids from a squamous cell line *(3)*. Because of alternative mRNA splicing sites, Souza et al. isolated a different cDNA for G-CSF that coded for a polypeptide of 174 amino acids from a bladder carcinoma cell line *(4)*. The G-CSF gene is located on chromosome 17 q21–22 near other genes involved in the development of neutrophilic granulocytes, whereas the G-CSF receptor is encoded by a single gene on chromosome 1 p35–p34.3 *(5)*. In its native form, the G-CSF protein is O-glycosylated with a molecular mass of approximately 20 kDa. Structurally, it is composed of four helices connected by amino acid loops, which contribute importantly to the molecule's three-dimensional structure *(6)*. Approved pharmaceutical forms of G-CSF

From: *Cytokines and Chemokines in Infectious Diseases Handbook*
Edited by: M. Kotb and T. Calandra © Humana Press Inc., Totowa, NJ

for human use include a recombinant nonglycosylated protein expressed in *Escherichia coli* (Filgrastim; Amgen, Thousand Oaks, CA) and a glycosylated form expressed in Chinese hamster ovary cells (Lenograstim; Chugai Pharmaceuticals, Tokyo, Japan). Glycosylation stabilizes the molecule in vitro by suppressing polymerization and conformational changes *(7)*. Furthermore, it confers resistance to degradation by proteases in human serum *(8)*. Both forms have similar biological activities and bioavailability following subcutaneous or intravenous administration.

In healthy persons, serum levels of G-CSF are 25 ± 19.7 pg/mL. During the acute stage of an infection, serum levels of G-CSF increase by an average 30-fold; in endotoxin-induced shock, levels rise to approx 200 ng/mL *(9)*. This rise is mediated by bacterial products and inflammatory mediators, such as tumor necrosis factor-α (TNF-α) *(10)*. G-CSF may also be a negative feedback signal for the release of TNF-α; in rats, G-CSF suppresses sepsis-induced production of TNF-α *(11)*.

The major target cells of G-CSF are neutrophil precursors and mature neutrophils *(2)*. The essential role of G-CSF in the normal regulation of neutrophil development was demonstrated in the G-CSF "knockout" mouse. Mice rendered G-CSF deficient by targeted disruption of the G-CSF gene in embryonic stem cells developed chronic neutropenia, associated with a 50% reduction of granulocyte precursor cells in the bone marrow. G-CSF knockout mice exhibited a markedly impaired ability to control infection by *Listeria monocytogenes* and failed to developed sepsis-related neutrophilia *(12)*.

In addition to the critical role of G-CSF in the regulation of granulopoiesis, interest has focused on qualitative improvements in the host immune response, including specific neutrophil functional responses and cytokine production, mediated by G-CSF in vitro and in vivo. Administration of G-CSF has been shown to enhance chemotaxis, respiratory (oxidative) burst, phagocytosis, and bactericidal activity of neutrophils, as well as to stimulate surface expression of CD11b/CD18, FcγRII (CD32) and FcγRIII (CD64) on neutrophils *(13–15)*. These effects are apparent at a G-CSF dose of 0.5–1 μg/kg *(16)*. To evaluate the effects of G-CSF on neutrophil function, Allen et al. treated healthy human volunteers with G-CSF daily for 2 wk and employed a chemiluminescence system for differential measurement of oxidase and myeloperoxidase (MPO) dioxygenation activities in whole blood *(17)*. Opsonin-receptor-mediated phagocyte functions were also measured with this system. G-CSF induced a dose-dependent neutrophil leukocytosis and a proportional increase in oxidase activity per volume of blood. The specific oxidase activity per neutrophil was only mildly increased in response to G-CSF treatment and remained relatively constant throughout the treatment period. In contrast, G-CSF treatment caused large, time-dependent increases in phagocyte-mediated MPO-dependent responses *(17)*.

2.2. *Effects of G-CSF in Animal Models of Infection*

In an experimental animal model, pretreatment with G-CSF has been shown to prevent sepsis-related mortality in mice *(18)*. In various other neutropenic and non-neutropenic animal studies involving severe burns, intramuscular abscesses, streptococcal infections, pneumonias, viral infections, peritonitis, and sepsis, administration of G-CSF has been associated with reduced mortality (overview in refs. *19* and *20*). Rabbits with pneumonia induced 24 hours after transtracheal inoculation of *Pasteurella multocida* were treated with penicillin and either G-CSF (5–8 μg/kg) or placebo for 5 d *(21)*. Although overall survival was only slightly improved (G-CSF: 77%; placebo: 67%), G-CSF treatment was associated with significantly increased survival in a subgroup of animals with sepsis-induced neutropenia (G-CSF: 57%; placebo: 39%). Because the survival benefit occurred mostly within the first 24 h of treatment before significant differences in the mean white blood cell (WBC) count between the two groups existed, leukocytosis was an unlikely explanation for improved survival in the G-CSF-treated animals .

More pronounced survival benefits were observed in other studies in which G-CSF was administered earlier in the course of infection and at higher dosages (50–100 μg/kg equivalent to 5–10 μg/kg in humans). In a hemorrhage mouse model designed to examine the high risk of pneumonia in patients with severe hemorrhage, mortality induced by *Pseudomonas aeruginosa* pneumonia was reduced from 92% to 62% by prophylactic treatment with G-CSF *(22)*. A similar treatment scheme also

Table 1
Animal Models Reporting Efficacy of G-CSF for Treatment of Infection

Authors	Animal	Infection and treatment	Outcome in G-CSF animals
Smith et al. *(22)*	Rabbit	Pneumonia, penicillin/G-CSF vs penicillin/placebo in neutropenic animals	Significant increase in survival
Abraham et al. *(23)*	Mouse	Prophylactic G-CSF in pneumonia after hemorrhage	Mortality reduced from 92% to 62%
Dunne et al. *(25)*	Rat	Prophylactic G-CSF in *E. coli* peritonitis	Increased survival from 38% to 78%
Cairo et al. *(26)*	Rat, neonatal	G-CSF 72 h after inducing group B streptococcal sepsis	Significant increase in survival with G-CSF plus antibiotics
Lundblad et al. *(11)*	Rat	G-CSF 4 h after inducing intra-abdominal sepsis	Reduced mortality rate from 96% to 42%; no neutrophil-mediated tissue damage

reduced the mortality of pneumonia caused by *Klebsiella pneumonia* in rats suffering from acute ethanol intoxication from 90% to 10% *(23)*. In this study, G-CSF enhanced granulocyte recruitment to the lungs, which was inhibited by alcohol intake. Similar G-CSF-mediated effects were observed following experimental *Streptococcus pneumoniae* pneumonia in splenectomized mice with impaired bacterial clearance, where G-CSF improved bacterial clearance and decreased the number of viable pneumococci in tracheobronchial lymph nodes compared with saline-treated controls *(24)*.

Prophylactic administration of G-CSF also increased survival from 38% to 78% in non-neutropenic rodents with *E. coli* peritonitis *(25)*. Bactericidal activity was significantly increased in neutrophils recovered from the peritoneal cavities of G-CSF-treated animals compared to neutrophils recovered from control animals. These observations suggested that enhancement of the cellular arm of the immune response by G-CSF improved survival *(25)*.

In a model of neonatal sepsis, newborn rats were inoculated with group B streptococcus and simultaneously treated with G-CSF or placebo *(26)*. Treatment with appropriate antibiotics was initiated in some animals 24 h following inoculation with bacteria. Seventy-two hours after inoculation, animals that received neither antibiotics nor G-CSF had a survival rate of 4%. Animals treated with antibiotics alone had a 28% survival rate, whereas 91% of the rats treated with antibiotics and G-CSF survived.

Significant synergistic effects on survival by treatment with G-CSF plus antibiotics were confirmed by other studies in septic animals *(27)*. Although granulocyte-mediated lung injury was enhanced by G-CSF in animals administered HCl intratracheally or cyclophosphamide systemically *(28)*, treatment with G-CSF was not associated with increased neutrophil-related tissue damage in animal models of sepsis *(17)*.

Table 1 summarizes studies reporting efficacy of G-CSF in animal models of infection. On the basis of the above physiologic and animal studies, many clinical trials have analyzed the effects of G-CSF as an adjuvant to antibiotics in the treatment of human infections in both neutropenic and non-neutropenic patients.

2.3. *Clinical Studies of G-CSF for the Prevention or Treatment of Infection*

2.3.1. *Therapeutic Use of G-CSF in Neutropenic Patients*

Currently, G-CSF is widely employed therapeutically for the treatment of clinical neutropenia, including neutropenia secondary to chemotherapy, radiotherapy, or myelosuppressive drugs, as well as idiopathic neutropenia, leukemia associated with neutropenia, and aplastic anemia *(29–31)*. Based

on randomized trials conducted in patients with chemotherapy-induced neutropenia, G-CSF has been approved to accelerate marrow recovery after standard-dose therapy for solid tumors and hematological malignancies *(32,33)*. Accelerated reversal of neutropenia leads to a decrease in the incidence of infection after standard regimens and fewer days with infectious and febrile neutropenic episodes during recovery from bone marrow transplantation. The decrease in morbidity is associated with shorter hospitalization times and reduced administration of parenteral agents *(34)*. Many ongoing studies are now focused on the use of G-CSF to accelerate myeloid recovery following dose-intensive chemotherapy, with the goal of improving overall survival for patients with various malignancies. Furthermore, many studies have investigated the mobilization of CD34+ hematopoietic stem cells from the marrow to the peripheral blood (PBSCs) for use in hematopoietic transplantation, in lieu of bone marrow cells (reviewed in ref. *35*). The results of these investigations have led to major changes in clinical practice, including an overall reduction in the duration of hospitalization as a result of the more rapid repopulation of marrow and recovery of blood counts in most patients receiving PBSC transplantation compared to patients receiving conventional bone marrow transplantation. However, it has yet to be established whether dose intensification of chemotherapy facilitated by the use of G-CSF or stem cell support affects remission rates or survival *(34)*.

Patients with congenital, idiopathic, or cyclic neutropenia have frequent and severe infections as a result of diminished neutrophil production and chronic neutropenia *(36)*. Prior to the availability of human growth factors, no predictably effective therapy was available for these disorders (review in ref. *37*). It is now known that more than 95% of these patients will respond promptly to G-CSF treatment *(37)*. In general, toxic and adverse events are not clinically troublesome and seldom necessitate discontinuing therapy *(38)*. However, data from the Severe Chronic Neutropenia International Registry have identified 23 of 249 patients with congenital neutropenia treated with G-CSF who have developed myelodysplasia or acute myelogenous leukemia within an average follow-up period of 4.5 yr *(38)*. In contrast, no malignant transformation has been observed in patients with cyclic or idiopathic neutropenia who were treated with G-CSF. However, the possibility of malignant transformation and other bone marrow alterations requires continued careful monitoring.

2.3.2. *Use of G-CSF in Granulocyte Transfusion Therapy*

Because neutropenia remains the major risk factor for the development of severe bacterial and fungal infections in patients undergoing hematopoietic stem cell transplantation and intensive chemotherapy of malignant diseases, the transfusion of normal neutrophils has long been considered as a logical approach to the treatment of such infections *(39)*. The infusion of granulocytes to restore host defense has been studied for more than 60 yr. Beginning early in this decade, several research groups began using hematopoietic growth factors to study their effects on neutrophil formation and function in normal subjects. It was quickly learned that G-CSF will rapidly and dramatically elevate the circulating neutrophil count in normal individuals. Administration of G-CSF to normal subjects allows for collection of $(40–80) \times 10^9$ neutrophils, a sufficient quantity of cells to increase the peripheral blood neutrophil counts to normal levels when transfused in severely neutropenic patients *(40)*. Moreover, neutrophil levels are maintained at or near normal levels for many hours after transfusion of cells from G-CSF-stimulated donors, possibly because G-CSF promotes cell survival by exerting an antiapoptotic effect on neutrophils *(41)*.

Recently, the feasibility of a community blood bank granulocyte transfusion program utilizing community donors stimulated with a single-dose regimen of subcutaneous G-CSF plus oral dexamethasone was examined *(42)*. The recipients of these transfusions were neutropenic hematopoietic stem cell transplantation patients with severe bacterial or fungal infections. Nineteen patients received 165 transfusions (mean: 8.6 transfusions/patients; range: 1–25). Ninety-four percent of the 175 donors providing transfusions were community donors, whereas only 6% of donors were relatives of the transfusion recipients. Sixty percent of the community donors initially contacted agreed to participate, and 98% of these individuals indicated a willingness to participate again. Adverse donor side

effects, such as bone pain and headache, likely attributable to G-CSF and/or dexamethasone, were relatively common but usually no more than mild to moderate in degree. Bone pain, headache, and insomnia, most likely an effect of dexamethasone, occurred in 41%, 30%, and 30% of the donors, respectively. Transfusion of ($81.9 \pm 2.3 \times 10^9$ neutrophils (mean ± SD) resulted in a mean 1-h posttransfusion neutrophil increment of (2.6 ± 2.6) × 109/μL and restored the peripheral neutrophil count to the normal range in 17 of the 19 patients. The buccal neutrophil response, a measure of the capacity of neutrophils to migrate to tissue sites in vivo, was restored to normal in most patients following transfusion. Chills, fever, and arterial oxygen desaturation of ≥ 3% occurred in 7% of the transfusions, but these changes were not sufficient to limit therapy. Infection resolved in 8 of 11 patients with invasive bacterial infections or candidemia, indicating that transfusion of neutrophils can restore a severely neutropenic patient's blood neutrophil supply and neutrophil inflammation response, thereby reducing the risk of lethal infections.

2.3.3. Therapeutic Use of G-CSF in Neonatal Sepsis

Because of the immaturity of the immune system, neonates, especially those who are premature or have low birth weight, are predisposed to severe infections with bacterial pathogens—in particular, group B streptococci *(43)*. Neutropenia resulting from an exhaustion of the neutrophil reserves is believed to be a major pathogenic mechanism predisposing to such infection *(44)*. A randomized trial was performed in 42 newborns (26–40 wk of age) with presumed bacterial sepsis within the first 3 d of life *(45)*. The patients were randomized to receive either placebo (9 patients) or varying doses of G-CSF (1, 5, or 10 μg/kg every 24 h (27 patients), or 5 or 10 μg/kg every 12 h (6 patients) on d 1, 2, and 3). Intravenous G-CSF was well tolerated, with no acute toxicity recognized. A significant increase in the peripheral blood neutrophil count was observed within 24 h in patients receiving 5 or 10 μg doses every 12 and 24 h. This response was sustained through 96 h. An increase in the neutrophil storage pool was observed at these dosages, as well as an increase in expression of CD11b/CD18, a neutrophil phagocytic and adhesion receptor, at 24 h in patients receiving 10 μg/kg of G-CSF every 24 h. These studies demonstrate that administration of G-CSF to neonates is safe, and larger controlled clinical trials are underway to examine the effects of G-CSF on infectious morbidity and mortality in the newborn.

2.3.4. Use of G-CSF in Fungal Infections

The rates of opportunistic fungal infections have increased substantially in both Europe and North America *(46)*. Because neutrophils constitute the main mechanism of host defense against fungi, including *Candida* and *Aspergillus* species, these infections occur predominately in patients with neutropenia or impaired neutrophil function *(47)*. Because G-CSF not only increases neutrophil number but also modulates various physiological properties of the neutrophils, interest has been directed toward the potential use of G-CSF in the therapeutic approach to fungal infections. The administration of G-CSF (300 μg/d subcutaneously) to five healthy volunteers for 6 d significantly enhanced neutrophil-mediated damage of *Candida albicans* pseudohyphae by 33% ($p = 0.007$) on d 2 and by 44% ($p = 0.04$) on d 6 *(48)*. However, fungicidal activity against hyphae from either *Fusarium solani* or *Aspergillus fumigatus* did not significantly change during the study period. In a mouse model of subacute or chronic disseminated *Candida* infection, G-CSF was less effective, indicating that neutrophil recruitment and activation are crucial in acute candidiasis, whereas other host defense mechanisms control the outcome of less overwhelming *Candida* infection *(49)*. Controlled clinical trials would be necessary to establish a role for G-CSF as an adjunctive immunomodulatory agent in fungal infections.

2.3.5. Therapeutic Use of G-CSF in Non-Neutropenic Patients with Infections

The safety and survival data from animal models of infection, combined with the favorable toxicity profile in humans, have led to several clinical trials of G-CSF as adjunctive therapy in the treatment of infections in non-neutropenic patients. The rationale for these studies has been to enhance

the number and functional activities of preformed neutrophils in order to promote recovery from or prevention of local or systemic infections. Sepsis prophylaxis is a promising indication for the use of G-CSF in non-neutropenic patients where the time-point for risk of infection is known or can be anticipated (surgery, trauma, burn, local infection). To date, information is available from several studies in patients with pneumonia and patients with insulin-dependent diabetes mellitus and foot infections, as well as small trials in a variety of other conditions (reviewed in ref. *50*).

2.3.5.1. Pneumonia

Pneumonia continues to be a leading cause of death in the United States, and resolution of infection is dependent on neutrophil function *(51)*. In a double-blind, controlled, multi-center trial with community-acquired bacterial pneumonia, 756 patients were enrolled to receive intravenous antibiotics plus either G-CSF (300 µg/d for up to 10 d; $n = 380$) or placebo ($n = 376$) *(52)*. Outcome measures included time to resolution of morbidity (TRM), 28-d mortality, length of stay, and adverse events. A microbial cause for pneumonia was identified in 56% of patients, and antimicrobial use was judged to be appropriate in 98% of the patients by an independent review group. Administration of G-CSF increased the peripheral blood neutrophil count threefold, but TRM, mortality, and length of hospitalization were not affected. However, the time to resolution of infiltrates was significantly more rapid in the G-CSF-treated group, and only one patient developed empyema as compared to six patients in the placebo group ($p = 0.068$). Not only was the administration of G-CSF safe and well tolerated, but also the development of sepsis-related organ failure acute respiratory distress syndrome (ARDS) and disseminated intravascular coagulopathy (DIC) was significantly ($p < 0.017$ and $p < 0.007$, respectively) reduced in the G-CSF recipients. The clinical benefits of G-CSF therapy appeared to be more pronounced in the subgroup of patients with multilobar pneumonia. In two recent studies, administration of G-CSF to patients with severe community-acquired pneumonia or nosocomial pneumonia did not alter morbidity or mortality *(53,54)*.

2.3.5.2. Diabetic Foot Infection

Foot infections are an important problem in diabetic patients. Extensive surgery and antibiotic therapy are often necessary to achieve resolution of infection. However, because of a lack of neutrophilia in diabetes and impaired superoxide generation in neutrophils from diabetic patients, G-CSF would appear to be a reasonable candidate for adjuvant therapy in the treatment of severe foot infections. Gough et al. conducted a randomized double-blind, placebo-controlled trial with G-CSF in 40 insulin-dependent diabetic patients with foot infections *(55)*. On admission, patients were randomly assigned to receive G-CSF ($n = 20$) or placebo ($n = 20$) for 7 d. Both groups were treated with similar antibiotic and insulin regimens. G-CSF treatment was associated with significantly earlier eradication of pathogens (median: 4 vs 8 d), quicker resolution of cellulitis (median: 7 vs 12 d), a shorter hospital stay (median: 10 vs 17.5 d), and a shorter duration of antibiotic treatment (median: 8.5 vs 14.4 d). No G-CSF-treated patient required surgery, whereas two placebo recipients underwent amputations and two required extensive debridement under anesthesia. G-CSF therapy was generally well tolerated. Neutrophil superoxide production was also measured and found to be low in the diabetic patients compared with normal controls at the start of therapy. After 7 d of G-CSF treatment, neutrophil superoxide production was significantly greater in the G-CSF group than in the placebo group. These encouraging results provide support for further studies of G-CSF in diabetic patients with severe infections.

2.3.5.3. HIV Infection

Neutropenia has been identified as an important independent risk factor for the development of infectious complications in human immunodeficiency virus (HIV)-infected individuals *(56)*. The administration of G-CSF has reversed or prevented neutropenia even during periods of full-dose myelotoxic therapy in HIV patients *(57)*. Furthermore, G-CSF has improved defective neutrophil function in vitro and in vivo in the setting of HIV infection *(57)*. In a study of 258 moderately neutro-

penic HIV-infected patients, G-CSF treatment significantly reduced the incidence of severe neutropenia and bacterial infections *(58)*. G-CSF-treated patients also had 54% fewer severe bacterial infections, and 45% fewer days of hospitalization were required for the management of any bacterial infection. In another clinical study, 30 acquired immunodeficiency syndrome (AIDS) patients were randomized to receive 5 d of treatment with rifabutin, G-CSF, or both *(59)*. The capacity to kill *Mycobacterium avium* was assessed in neutrophils harvested from each patient before intervention, during therapy (d 4), and after therapy (d 7). A 90% reduction in *M. avium* growth was observed after therapy for patients treated with G-CSF alone ($p = 0.01$), whereas a reduction in growth of 59% and 11% were observed for patients treated with both agents ($p = 0.06$) and with rifabutin alone ($p = 0.84$), respectively.

Interleukin-2 (IL-2) production in HIV-infected patients is depressed and may contribute to decreased lymphocyte proliferation and reduced generation of cytotoxic T-lymphocytes *(60)*. Administration of G-CSF to patients with advanced HIV infection has been shown to improve the production of IL-2 in whole blood ex vivo *(60)*.

2.3.5.4. Trauma and Surgical Prophylaxis

Various other smaller studies have shown beneficial effects of G-CSF therapy in non-neutropenic infectious conditions. In a pilot study with 20 polytraumatized patients and patients undergoing major surgery with a high risk of developing sepsis, prophylactic administration of G-CSF improved the neutrophil oxidative burst and reduced the number of episodes of sepsis (G-CSF: 0; placebo: 3) *(61)*. Thirty-seven patients who received G-CSF beginning the first day after liver transplantation had significantly reduced rejection, sepsis, and sepsis-related mortality compared with grafted patients who did not receive G-CSF *(62)*. Endo et al. treated 24 patients with sepsis-induced granulocytopenia who had failed to respond to antibiotics with low-dose G-CSF (75 µg/d) for 5 d *(63)*. Leukocyte counts increased ninefold in 19 survivors, whereas 5 nonresponders died. Thus, G-CSF may favorably modulate the host immune response during the "immune paralysis" of late sepsis.

Because esophagectomy for patients with esophageal carcinoma is associated with substantial infectious complications, the use of G-CSF to reduce the number of postoperative infections in this patient population was investigated *(64)*. Nineteen patients were treated perioperatively by daily administration of G-CSF for 10 d beginning 2 d before surgery. Their outcome was compared historically with 77 patients with esophageal cancer who did not receive G-CSF. Within the first 10 d after surgery, 23 untreated patients but none of the G-CSF-treated patients developed infections (29.9% vs 0%; $p = 0.005$). Following cessation of G-CSF therapy, two cases of infection developed in the G-CSF-treated group compared to six additional cases of infection in the untreated group (10.5% vs 37.7%; $p < 0.05$). The rate of fatal hospital infections was reduced in the G-CSF group, but not significantly (G-CSF patients: 0%; historical controls: 9%). During G-CSF treatment, the investigators found an increase in neutrophil phagocytosis, neutrophil oxidative burst, and protective serum cytokines, respectively, which might explain the reduced infection rate.

Table 2 provides an overview of studies examining the use of G-CSF for the treatment of clinical infections. To date, clinical studies indicate a potential use of G-CSF to reduce neutropenic and non-neutropenic infectious complications. However, only large controlled clinical trials will define the impact of G-CSF on the prevention and treatment of specific infections, particular in patients with compromised granulocyte reserves or function.

3. GM-CSF

3.1. Overview

The principal cellular sources of GM-CSF are monocytes/macrophages, fibroblasts and endothelial cells *(2)*. GM-CSF promotes growth and differentiation of multipotential hematopoietic progenitor cells and stimulates physiologic activity of neutrophils, eosinophils, and monocytes/macrophages

Table 2
Clinical Studies of G-CSF for the Prevention or Treatment of Infection.

Authors	Study population	Treatment	Outcome in G-CSF patients
Trillet-Lenoir et al. *(33)*	130 patients with small-cell lung CDE[a] chemotherapy	G-CSF vs placebo on d 4–17 cancer receiving	Significant decrease in neutropenic fever, parenteral antibiotics, infection-related hospitalization and delay of next cycle
Gillan et al. *(45)*	42 newborns with presumed bacterial sepsis	Prophylactic G-CSF versus placebo	Significant increase in blood neutrophils and functional activation of neutrophils in a subgroup of patients; no acute toxicity
Nelson et al. *(52)*	756 patients with community-acquired bacterial pneumonia	Antibiotics plus G-CSF or placebo	Significant acceleration of radiologic improvement; significant reduction of sepsis-related organ failures; no differences in TRM and mortality
Mitsuyasu *(58)*	258 HIV-infected patients	Prophylactic G-CSF	Significant decrease in severe neutropenia and bacterial infections
Gough et al. *(55)*	40 insulin-dependent diabetic patients with foot infections	Antibiotics plus G-CSF versus placebo	Significant improvement in eradication of pathogens, resolution of cellulitis, hospital stay and intravenous antibi otic treatment

[a]CDE: cyclophosphamide, doxorubicin, etoposide.

(2). However, GM-CSF appears not to play an essential role in normal neutrophil development, as shown by studies in the GM-CSF "knockout" mouse *(65)*. Mice lacking GM-CSF have impaired pulmonary homeostasis, manifested as alveolar proteinosis, and increased splenic hematopoietic progenitors. However, steady-state hematopoiesis is not impaired *(65)*. Murine GM-CSF was cloned in 1984, followed by the cloning of human GM-CSF in 1985 *(66)*. The single copy of the gene-encoding human GM-CSF is located on chromosome 5, region 5q21–5q32 *(67)*. Other genes in this area include those for M-CSF, interleukin (IL)-3, IL-4, and IL-5. In knockout mice lacking functional IL-3, GM-CSF, and IL-5, no hematological defect other than a reduced number of eosinophils was found, indicating a functional overlap with other cytokine systems for hematopoiesis *(68)*. Deletions in this region have been reported in therapy-related myelodysplastic syndromes, acute leukemias, and refractory anemia with morphologic abnormalities of the megakaryocytes *(69)*.

The initial GM-CSF polypeptide consists of 144 amino acids that undergoes cleavage of a 17-amino-acid segment from the amino terminus, resulting in a mature protein of 127 amino acids with 4 α-helices and two β-sheets in a bilobed configuration *(70)*. Because of glycosylation of two serine residues near the amino terminus and the variable N-linked addition of complex carbohydrate at two sides, the molecular weight varies between 14.5 and 35 kDa *(71)*. Commercial, nonglycosylated GM-CSF is expressed in *E. coli* (Molgramostim; Sandoz Ltd, Basel, Switzerland), and the commercial glycosylated form is a yeast-derived GM-CSF (Sargramostim; Immunex Corp., Seattle, WA). The lack or presence of glycosylation does not appear to affect the primary actions of the molecule but may alter its pharmakokinetics. In a study conducted in healthy volunteers, the serum concentration of the nonglycosylated form reached a higher level more rapidly, which also decreased more quickly than the glycosylated form *(72)*. Therefore, the profile of the side effects of both molecules are different. The glycosylated form has a lower incidence of side effects in humans than does the form derived from bacteria *(73)*.

To clarify the effects of glycosylated GM-CSF on neutrophil and monocyte function and the mechanisms of neutrophilia caused by this cytokine, recombinant human GM-CSF was administered

to seven normal volunteers for periods of up to 14 d *(74)*. Each subject received GM-CSF at a dose of 250 $\mu g/m^2/d$ subcutaneously. This schedule of daily injection caused symptoms in all subjects. Itching and redness at the injection side, bone pain, and headache were the most common symptoms. In addition, all individuals developed eosinophilia. During treatment, blood neutrophil counts rose gradually to peak at a level 3.5-fold greater than baseline by d 14. Marrow aspirates on d 5 of GM-CSF treatment showed a statistically significant increase in the proportion of promyelocytes and myelocytes, accompanied by a significant decrease in band and segmented neutrophils. The transit time through the postmitotic marrow pool accelerated (normal = 6.4 d, GM-CSF = 3.9 d; $p < 0.01$). Treatment caused minimal effects on either the blood neutrophil half-life or the neutrophil turnover rate. The migration of neutrophils to skin chambers was significantly decreased by GM-CSF. The buccal neutrophil response was reduced during GM-CSF treatment in four of five subjects, but the responses were quite variable and the differences were not significant. Treatment increased expression of CD11b/CD18 but not Fcγ receptors. Treatment also stimulated the in vitro neutrophil respiratory burst in response to a variety of agonists, and this enhancement persisted for the duration of treatment.

3.2. *Effects of GM-CSF in Animal Models of Infection*

The protein sequence homology between human and murine GM-CSF is only 60% *(66)*. Even monkeys have developed antibodies against GM-CSF following administration of human GM-CSF. Thus, animal models to study recombinant GM-CSF have been relatively limited *(1,66)*.

Neutrophil production and function are compromised in neonatal rats during infection. The prophylactic intraperitoneal administration of murine GM-CSF to neonatal rats 6 h prior to a 90% lethal dose challenge of *Staphylococcus aureus* significantly improved survival *(75)*. In another study in which human GM-CSF was given after infection to neonatal rats, GM-CSF-treated animals had a higher survival rate than control animals *(76)*. In group B streptococcal infection, the administration of penicillin plus GM-CSF decreased the mortality rate substantially as compared to antibiotics alone *(77)*. However, in a rat model of intraperitoneal infection by cecal ligation and puncture, GM-CSF failed to enhance survival above that of control animals *(78)*. Moreover, the mortality rate was higher and animals died earlier after receiving GM-CSF. Inhibition of leukosequestration in the peritoneal cavity was also observed. The liver of GM-CSF-treated rats showed centrilobular degeneration and necrotic changes. These conflicting data raise questions concerning the immunomodulatory role of GM-CSF. In infection, no systemic GM-CSF levels can be detected, so it is likely that endogenous GM-CSF plays its major physiological role in the immediate vicinity of the cells by which it is secreted *(79)*. Exposure of macrophages to GM-CSF primes them for enhanced release of inflammatory cytokines when triggered by lipopolysaccharides (LPS), including IL-1, TNF, and IL-6 *(80)*. After intravenous challenge with endotoxin, mice pretreated with GM-CSF at a dose that did not induce neutrophilia exhibited a significant increase in TNF-α, and a nonlethal dose of endotoxin became lethal *(81)*. In contrast, pretreatment with G-CSF in the same study raised the neutrophil count by 78% but did not enhance TNF-α expression.

A critical role for GM-CSF in pulmonary host defense was recently demonstrated in mice rendered deficient in GM-CSF by targeted gene disruption *(82)*. In these mice, susceptibility to group B streptococcal pneumonia was increased. Additionally, administration of GM-CSF was therapeutically beneficial in a mouse model of disseminated *M. avium* complex (MAC) infection *(83)*. MAC synthesizes superoxide dismutase, which can inactivate macrophage-derived superoxide anions as well as enzymes which can hinder superoxide production. However, stimulation of MAC-infected macrophages by GM-CSF was shown to increase production of reactive oxygen species and enhance mycobacteriostatic/mycobactericidal activity in vitro and in vivo. In this study, a significant reduction in the number of viable bacteria was observed in the blood, liver, and spleen of mice treated with a combination of GM-CSF and antimicrobial agents compared with control mice and those treated with GM-CSF or antimicrobials alone.

Table 3
Animal Studies Reporting Efficacy of GM-CSF for Treatment of Infection

Authors	Animal	Infection and treatment	Outcome in GM-CSF animals
Frenck et al. *(75)*	Rat, neonatal	Prophylactic GM-CSF prior to a 90% lethal dose of *S. aureus*	Significant increase in survival
Givner et al. *(77)*	Rat, neonatal	Penicillin alone versus penicillin + G-CSF	Significant decrease in mortality rate
Toda et al. *(75)*	Rat	GM-CSF in peritonitis	No enhanced survival; higher mortality rate compared to controls
Bermudez et al. *(83)*	Mouse	Antibiotics alone versus antibiotics + GM-CSF in disseminated *M. avium* complex infection	Significant reduction of bacteria in the blood, liver, and spleen
Liehl et al. *(84)*	Mouse	GM-CSF in *C. albicans* infection	Significant increase in survival and clearance of pathogen from the liver and spleen, but not from the kidney
Mayer et al. (85)	Mouse	Prophylactic GM-CSF in neutropenic mice with *C. albicans*, *P. aeruginosa*, or *S. aureus* infection	Protection against lethal infection

Considerable research efforts have been devoted toward the potential use of GM-CSF as an adjunctive treatment for fungal infection. In one study in mice, the use of GM-CSF led to significantly enhanced survival during 15 d associated with clearing of *C. albicans* from the liver and spleen, but not from the kidney *(84)*. In a neutropenic mouse model, GM-CSF was administered prophylactically prior to experimental *C. albicans*, *P. aeruginosa*, or *S. aureus* infection *(85)*. Prophylactic GM-CSF protected against lethal infection, resulting in increased numbers of survivors. In another study, GM-CSF was given to mice for 7 or 14 d beginning 4 wk after CD4+ T-lymphocyte depletion and infection with *P. carinii (86)*. As compared to a control group that did not receive GM-CSF treatment, the investigators found a significant decrease in the intensity of infection by histological examination of lung tissue and a reduced inflammation score (which did not reach statistical significance). Alveolar macrophages from mice treated with GM-CSF released significantly more TNF-α than cells from control mice after in vitro stimulation with LPS alone or with LPS plus murine recombinant interferon-γ (IFN-γ).

Table 3 provides an overview of animal studies examining the use of GM-CSF for treatment of infections. Overall, data from animal models of acute infection support a protective role for GM-CSF when administered prophylactically. However, the protective actions of GM-CSF may be accompanied and counterbalanced by an exaggerated systemic inflammatory cytokine response. In certain circumstances, especially when severe infection is already present, GM-CSF may have detrimental effects if administered alone, although it may be useful as an adjuvant to antibiotic therapy.

3.3. Studies of GM-CSF for the Prevention and Treatment of Infection

3.3.1. Therapeutic Use of GM-CSF in Neutropenic Patients

Similar to G-CSF, the predominant clinical use of GM-CSF is in the treatment of neutropenia. Its efficacy was evaluated in patients with cancer chemotherapy-induced myelosuppression *(87)*, patients who had been accidentally exposed to cesium-137 *(70)*, and patients undergoing bone marrow or peripheral hematopoietic stem cell transplantation (reviewed in ref. *88*). Moreover, considerable interest has focused recently on the use of GM-CSF for ex vivo expansion of hematopoietic stem and progenitor cells for a variety of applications, including in vitro tumor cell purging and the reduction in the volume of blood required for processing leukapheresis *(88)*.

In a retrospective study, the incidence of infection in patients receiving GM-CSF following autologous bone marrow transplantation was compared to a group of patients who did not receive GM-CSF treatment *(89)*. From the day of transplantation to 28 d posttransplantation, when both groups had severe neutropenia, 40% (38 of 95) of control patients developed infection compared to only 13% (6 of 46) of GM-CSF-treated patients ($p = 0.001$). In GM-CSF-treated patients, there was a trend toward fewer fungal infections, Gram-negative bacterial infections, and pulmonary infections. Patients who did not receive GM-CSF experienced more days of treatment with amphotericin B ($p = 0.0305$) and intravenous antibiotics (not significant). Two control patients, but no GM-CSF-treated patients, died because of infection before d 28. Along with other findings, these results demonstrate that prophylactic GM-CSF can reduce infectious complications in specific clinical settings.

Similar to G-CSF, GM-CSF has the ability to support engraftment following bone marrow or hematopoietic stem cell transplantation and to induce the mobilization of hematopoietic progenitor cells into the peripheral blood for collection and use in subsequent autotransplantation *(84)*. The latter procedure has facilitated further intensification of standard chemotherapy protocols in order to enhance tumor response to cytotoxic agents. Patients receiving hematopoietic stem cells collected following GM-CSF-induced mobilization experience faster recoveries of neutrophil counts and platelet counts, thereby reducing the costs for antibiotic therapy and platelet transfusions *(90)*. In leukemic patients receiving T-cell-depleted allogeneic bone marrow transplantation, GM-CSF may induce antileukemic mechanisms in monocytes, such as the secretion of pro-inflammatory mediators or the stimulation of antibody-dependent cellular cytotoxicity *(91)*. The use of GM-CSF in such patients may minimize the incidence of tumor relapse and accelerate engraftment.

As mentioned previously, GM-CSF exerts effects on a large population of target cells. Therefore, it is not surprising that investigators have tried administration of this growth factor to patients with aplastic anemia. Although GM-CSF treatment may be beneficial in the early stages of aplastic anemia, clinical results have been disappointing in later stages of disease associated with progressive hypocellularity of the bone marrow *(92)*. A greater benefit was evident when administration of GM-CSF was combined with other cytokines, such as IL-1 or IL-3, which act at earlier stages of hematopoietic development *(93)*.

Yoshida et al. examined the effects of long-term treatment with GM-CSF in 61 patients with myelodysplastic syndrome (MDS) who were randomized to receive GM-CSF on a daily schedule at a dose of 60 $\mu g/m^2$, 125 $\mu g/m^2$, or 250 $\mu g/m^2$ for 8 wk *(94)*. In all three groups, neutrophil counts and eosinophil counts increased within 1 wk after the start of treatment. There were no consistent changes in other cell lineages, including monocytes, lymphocytes, reticulocytes, or platelets, but peak levels of these cells were significantly higher as compared with the baseline levels. Infectious complications were reduced in the groups of patients receiving higher doses. In this trial, GM-CSF therapy was generally well tolerated, and no serious side effects were observed. However, as reported by Lieschke and Burgess, patients with MDS who have $\geq 14\%$ marrow blasts before GM-CSF therapy are at risk of developing acute myeloid leukemia (AML) during GM-CSF treatment *(95)*.

3.3.2. Use of GM-CSF in Fungal Infections

Candida and *Aspergillus* species have been consistently noted as the most important opportunistic fungal pathogens in cancer patients *(96)*. GM-CSF has been shown to inhibit fungal growth in a number of systems. Specifically, GM-CSF increased the cytotoxicity of human monocytes and macrophages isolated from the lamina propria of the intestine against *C. albicans (97)*. Furthermore, the use of GM-CSF and pentoxifylline, a xanthine derivative that may preserve neutrophil migration during GM-CSF administration, was reported to be effective for treatment of a patient with invasive candidiasis resulting from *C. albicans (98)*.

The ability of proinflammatory cytokines to enhance the host immune response during fungal infection was evaluated in isolated neutrophils and buffy coat cells from healthy donors *(99)*. The study compared the differential effects of IFN-γ, G-CSF, and GM-CSF in vitro on hyphal damage of *A. fumigatus*, *F. solani*, and *C. albicans*. IFN-γ significantly enhanced neutrophil-mediated damage

to the hyphal and pseudophyphal forms of the three opportunistic fungi studied. Additionally, IFN-γ also significantly enhanced the antifungal activity of buffy coat cells against all three fungi. The colony-stimulating factors G-CSF and GM-CSF were less effective. G-CSF increased hyphal damage mediated by both neutrophils and buffy coat cells against *F. solani*, and GM-CSF augmented the antifungal activity of buffy coat cells against hyphal forms of both *F. solani* and *C. albicans*. The observations provide further experimental support for the use of cytokines as an adjunct to conventional antifungal therapy in the treatment of infections because of opportunistic fungal pathogens.

3.3.3. Overview of Clinical Studies Investigating Administration of GM-CSF for Various Conditions

In vitro, GM-CSF potentiated the recruitment of blast cells into active phases of the cell cycle, thereby increasing their sensitivity to cell-dependent cytotoxic drugs like cytarabine *(90)*. This property has been part of the rationale for the use of GM-CSF in patients with AML *(100)*. Leukemic progenitor cells, which express GM-CSF receptors on their cell surface, can be triggered into S-phase by GM-CSF stimulation. However, based on current knowledge, GM-CSF should be used only in a subset of AML patients at high risk of infection and in those patients who relapse or are resistant to induction therapy (reviewed in ref. *101*). The risk of stimulating the leukemic clone with GM-CSF should be kept in mind, although the balance of evidence indicates that GM-CSF therapy does not affect relapse rates, frequency of remissions, or patient life expectancy *(101)*.

The possible induction of tumor cell cytotoxicity in leukocytes by GM-CSF may also help to destroy cytokeratin-positive cells, such as in micrometastases. In a pilot study of patients with gastric cancer, administration of GM-CSF activated monocytes, leading to a decrease in the number of cytokeratin-positive cells in the bone marrow *(102)*. GM-CSF treatment was also associated with a reduction in the risk of relapse *(102)*.

Oral mucositis in patients treated with intensive cancer chemotherapy or radiotherapy is a major cause of dose reduction or treatment delay. Mucosal damage can be caused directly by cytotoxic effects and indirectly by sustained neutropenia after cytostatic therapy. An impaired mucosal barrier predisposes to life-threatening infectious complications and sepsis. Therefore, there is a great need for effective prophylaxis and treatment of oral mucositis. Several studies have demonstrated the efficacy of GM-CSF therapy in the treatment of oral mucositis after chemotherapy or radiotherapy. Chi et al. evaluated the effects of GM-CSF on chemotherapy-induced oral mucositis in 20 patients with stage IV squamous cell carcinoma of the head and neck *(103)*. Patients were randomized to receive either 4 μg/kg/d GM-CSF subcutaneously from d 5 to 14 or no therapy. Administration of GM-CSF during the first cycle of chemotherapy significantly reduced the incidence, mean duration, and mean area under the curve of severe oral gross mucositis. These beneficial clinical effects continued when patients were crossed over to not receive GM-CSF in the second cycle of chemotherapy. Furthermore, GM-CSF administered in the second cycle to patients who did not receive GM-CSF during the first cycle reduced the incidence of severe mucositis. In a small phase II study, 14 patients undergoing surgery for oropharyngeal tumors received adjuvant radiotherapy at a dose of 2 Gy/d/wk to a total dose of 64 Gy *(104)*. All patients received GM-CSF treatment at the appearance of the mucositis at 30 Gy. Five patients who had already presented mucositis at 30 Gy improved their symptoms significantly during treatment with GM-CSF (from stage III to I or 0). Six patients had an improvement from stage III to II or I, whereas three patients had no measurable benefit by the treatment. A randomized phase III multicenter study of this treatment modality is in progress.

Beneficial modulation of host defense by GM-CSF has been demonstrated in a variety of clinical studies of infectious diseases. It has been well documented that GM-CSF can inhibit the intracellular replication of bacteria or protozoa. Macrophages, which provide a protected environment for microorganisms against extracellular concentrations of antibiotics because of limitations of endocytosis and membrane permeability, are activated by GM-CSF to allow higher intracellular concentrations of certain antimicrobial agents *(105)*. Quantitative and qualitative defects in leukocytes of HIV-infected

Table 4
Clinical Studies of GM-CSF for the Prevention or Treatment of Infection.

Authors	Study population	Treatment	Outcome in GM-CSF patients
Nemunaitis et al. *(89)*	141 neutropenic patients following autologous bone marrow transplantation	Prophylactic GM-CSF versus placebo amphotericin B	Significant decrease in infection and use of
Yoshida et al. *(94)*	61 patients with MDS[a]	Daily GM-CSF at doses of 60, 125, or 250 μg/m^2 for 8 wk	Significant increase in neutrophils and eosinophils; reduced infections in higher dose groups
Chi et al. *(103)*	20 patients with chemotherapy-induced mucositis	Prophylactic GM-CSF versus no therapy	Significant decrease in incidence and mean duration of severe mu cositis
Skowron et al. *(106)*	20 HIV-infected patients with antiretroviral therapy	GM-CSF three times/week for 8 wk versus placebo	Increase of CD4+ count; trend toward decreased HIV RNA
Badaro et al. *(107)*	20 neutropenic patients with leishmaniasis	Supportive treatment with GM-CSF versus placebo for 10 d	Significant increase in neutrophils, eosinophils, monocytes, and plate lets; significant decrease in secondary infections

[a]MDS myelodysplastic syndrome

patients, which may be exacerbated by antiviral therapy, contribute to the high incidence of opportunistic infections and neoplasms *(90)*. Therapy with GM-CSF has been shown to increase both the number and the function of neutrophils, monocytes, and eosinophils in HIV-infected individuals, as well as to enhance the activity of certain antiretroviral drugs. In a randomized, double-blind study of 20 patients with AIDS receiving antiretroviral regimens, either GM-CSF or placebo was administered three times a week for 8 wk *(106)*. GM-CSF was well tolerated and increased the CD4+ lymphocyte count. Furthermore, a trend toward decreased HIV RNA (i.e., "viral load") was noted in patients receiving GM-CSF. Inflammatory cytokines and surrogate markers of disease progression, such as IL-10 and soluble TNF receptors, remained stable during the course of GM-CSF treatment.

In acute visceral leishmaniasis, patients experience suppression of the immune system, associated with abnormal production of cytokines, such as IL-2, IL-3, IFN-γ, and GM-CSF. This altered regulation of cytokine production has been hypothesized to contribute to downregulation of the host antileishmanial response *(107)*. Administration of exogenous GM-CSF might increase inhibition of leishmania proliferation in macrophages by altering the immune balance.

Table 4 provides an overview of reported clinical studies examining the use of GM-CSF for treatment of infectious diseases.

4. COMPARISON OF G-CSF AND GM-CSF TOXICITIES

The clinical use of the hematopoietic growth factors G-CSF and GM-CSF may be summarized as follows: (1) to increase the proliferation the normal hematopoiesis in marrow disorders, such as aplastic anemia, cyclic neutropenia, and myelodysplastic syndromes; (2) to reduce infectious complications by increasing the absolute neutrophil count and the neutrophil function in cancer patients after radiotherapy, conventional chemotherapy, or bone marrow or stem cell transplantation; (3) to enhance the number of circulating stem cells and neutrophils to allow stem cell transplantation and granulocyte transfusion; and (4) to recruit tumor cells into the S-phase of the cell cycle to increase their susceptibility to cytotoxic agents.

The clinical indications for the use of G-CSF and GM-CSF are very similar, and both growth factors have been shown to play a valuable therapeutic role. However, the reason that G-CSF is currently more widely employed clinically than GM-CSF is not only a result of marketing strategies but also the spectrum of potential side effects. Extensive experience has been gained worldwide with G-CSF therapy, and the most commonly reported side effect is mild to moderate bone and/or muskulosceletal pain, seen in 20–30% of recipients *(16,50)*. Aside from this effect, the safety profile of G-CSF appears to be fairly innocuous, even after years of administration in patients with severe chronic neutropenia. Aside from bone pain, the most common adverse effects have been anemia, splenomegaly, thrombocytopenia, and injection-side reactions *(108)*. Allergic reactions have been rarely reported. In clinical trials, up to 2% of the patients developed skin reactions consistent with a clinical diagnosis of cutaneous vasculitis (Sweet's syndrome) *(108)*. G-CSF has not produced dose-limiting side effects, and adverse events decrease following discontinuation of treatment.

In contrast to the anti-inflammatory characteristics of G-CSF, GM-CSF treatment has been associated with a greater number of more severe side effects because of its proinflammatory nature. The most frequently reported side effect is fever, which occurs in >20% of patients and can serve as a confounding factor in the evaluation of responses to the treatment of infection *(109)*. Fever is often accompanied by myalgias and a flulike syndrome. First-dose reactions, consisting of flushing, tachycardia, hypotension, musculoskeletal pain, dyspnea, nausea, vomiting, and arterial oxygen desaturation, have been reported in 5% of recipients *(110)*. High doses (>15 μg/kg) have been reported to cause a generalized capillary leak syndrome *(111)*.

Although there is no convincing evidence to date that either G-CSF or GM-CSF has caused malignant transformation or worsened the course of malignant diseases, careful evaluation of the clinical application of either agent is recommended. In a human skin carcinoma model, tumor progression to high-grade malignancy has been associated with constitutive expression and secretion of G-CSF and GM-CSF, both in vitro and in vivo *(112)*. The carcinoma cells expressed both G-CSF and GM-CSF receptors, and both G-CSF and GM-CSF stimulated cell proliferation and migration in vitro. Additionally, both proliferation and migration were inhibited by neutralizing antibodies to G-CSF and GM-CSF, respectively.

5. SUMMARY

In summary, both G-CSF and GM-CSF have the ability to upregulate phagocyte function. These cytokines play critical roles in the host defense response during infection. As demonstrated in experimental animal models of infection, G-CSF and GM-CSF can potentially be administered therapeutically to enhance pathogen eradication and decrease morbidity and/or mortality. However, this therapeutic potential must be substantiated in controlled clinical trials, which are required to define the proper role of G-CSF and GM-CSF therapy in the clinical management of specific infections.

ACKNOWLEDGMENT

This work was supported in part by a grant from Deutsche Krebshilfe, Germany.

REFERENCES

1. Metcalf, D. (1986) The molecular biology and functions of the granulocyte–macrophage colony-stimulating factors. *Blood* **67,** 257–267.
2. Liles, W.C. and Van Voorhis, W.C. (1995) Nomenclature and biologic significance of cytokines involved in inflammation and the host immune response. *J. Infect. Dis.* **172,** 1573–1580.
3. Nagata, S., Tsuchiya, M., Asano, S., Kaziro, Y., Yamazaki, T., Yamamoto, O., et al. (1986) Molecular cloning and expression of cDNA for human granulocyte colony-stimulating factor. *Nature* **319,** 415–418.
4. Souza, L.M., Boone, T.C., Gabrilove, J., Lai, P.H., Zsebo, K.M., Murdock, D.C., et al. (1986) Recombinant human granulocyte colony-stimulating factor: effects on normal and leukemic myeloid cells. *Science* **232,** 61–65.
5. Gabrilove, J.L. and Jakubowski, A. (1990) Hematopoietic growth factors: biology and clinical application. *J. Natl. Cancer Inst. Monogr.* **10,** 73–77.

6. Hill, C.P., Osslund, T.D., and Eisenberg, D.S. (1993) The structure of granulocyte colony-stimulating factor (r-hu-G-CSF) and its relationship to other growth factors. *Proc. Natl. Acad. Sci. USA* **90,** 5167–5171.
7. Oh-eda, M., Hasegawa, M., Hattori, K., Kuboniwa, H., Kojima, T., Orita, T., et al. (1990) O-linked sugar chain of human granulocyte colony-stimulating factor protects it against polymerization and denaturation allowing it to retain its biological activity. *J. Biol. Chem.* **265,** 11,432–11,435.
8. Nissen, C., Carbonare, V.D., and Moser, Y. (1994) In vitro comparison of the biological potency of glycosylated versus nonglycosylated rG-CSF. *Drug. Invest.* **7,** 346–352.
9. Kawakami, M., Tsursumi, H., Kumakawa, T., Abe, H., Hirai, M., Kurossawa, S., et al. (1990) Levels of serum granulocyte colony-stimulating factor in patients with infections. *Blood* **76,** 1962–1964.
10. Koeffler, H.P., Gasson, J., Ranyard, J., Souza, L., Shepard, M., and Munker, R. (1987) Recombinant human TNF-(stimulates production of granulocyte colony-stimulating factor. *Blood* **70,** 55–59.
11. Lundblad, R., Nesland, J.M., and Giercksky, K.E. (1996) Granulocyte colony-stimulating factor improves survival rate and reduces concentrations of bacteria, endotoxin, tumor necrosis factor, and endothelin-1 in fulminant intra-abdominal sepsis in rats. *Crit. Care Med.* **24,** 820–826.
12. Lieschke, G.J., Grail, D., Hodgson, G., Metcalf, D., Stanley, E., Cheers, C., et al. (1994) Mice lacking granulocyte colony-stimulating factor have chronic neutropenia, granulocyte and macrophage progenitor cell deficiency, and impaired neutrophil mobilization. *Blood* **84,** 1737–1746.
13. Carulli, G., Minnucci, S., Azzara, A., Angiolini, C., Sbrana, S., Caracciolo, F., et al. (1995) Granulocyte colony-stimulating factor (G-CSF) administration increases PMN CD32 (FcRII) expression and FcR-related functions. *Haematologica* **80,** 150–154.
14. Nathan, C.F. (1989) Respiratory burst in adherent human neutrophils: triggering by colony-stimulating factors CSF-GM and CSF-G. *Blood* **73,** 301–306.
15. Roilides, E., Walsh, T.J., Pizzo, P.A., and Rubin, M. (1991) Granulocyte colony-stimulating factor enhances the phagocytic and bactericidal activity of normal and defective human neutrophils. *J. Infect. Dis.* **163,** 579–583.
16. Itoh, Y., Kuratsuji, T., Tsunawaki, S., Aizawa, S., and Toyama, K. (1991) In vivo effects of recombinant human granulocyte colony-stimulating factor on normal neutrophil function and membrane effector molecule expression. *Int. J. Hematol.* **54,** 463–469.
17. Allen, R.C., Stevens, P.R., Price, T.H., Chatta, G.S., and Dale, D.C. (1997) In vivo effects of recombinant human granulocyte colony-stimulating factor on neutrophil oxidative functions in normal human volunteers. *J. Infect. Dis.* **175,** 1184–1192.
18. Gorgen, I., Hartung, T., Leist, M., Niehorster, M., Tiegs, G., Uhlig, S., et al. (1992) Granulocyte colony-stimulating factor treatment protects rodents against lipopolysaccharide-induced toxicity via suppression of systemic tumor necrosis factor-α. *J. Immunol.* **149,** 918–924.
19. Dale, D.C., Liles, W.C., Summer, W.R., and Nelson, S. (1995) Granulocyte colony-stimulating factor—role and relationships in infectious diseases. *J. Infect. Dis.* **172,** 1061–1075.
20. Nelson, S. (1995) Role of granulocyte colony-stimulating factor in the immune response to acute bacterial infection in the nonneutropenic host: an overview. *Clin. Infect. Dis.* **18,** 197–204.
21. Smith, W.S., Sumnicht, G.E., Sharpe, R.W., Samuelson, D., and Millard, F.E. (1995) Granulocyte colony-stimulating factor versus placebo in addition to penicillin G in a randomized blinded study of gram-negative pneumonia sepsis: analysis of survival and multisystem organ failure. *Blood* **86,** 1301–1309.
22. Abraham, E. and Stevens, P. (1992) Effects of granulocyte colony-stimulating factor in modifying mortality from *Pseudomonas aeruginosa* pneumonia after hemorrhage. *Crit. Care Med.* **20,** 1127–1133.
23. Nelson, S., Summer, W., Bagby, G., Nakamura, C., Steward, L., Lipscornb, G., et al. (1991) Granulocyte colony-stimulating factor enhances pulmonary host defences in normal and ethanol-treated rats. *J. Infect. Dis.* **164,** 901–906.
24. Hebert, J.C., O'Reilly, M., and Gamelli, R.L. (1990) Protective effect of recombinant human granulocyte colony-stimulating factor against pneumococcal infections in splenectomized mice. *Arch. Surg.* **125,** 1075–1078.
25. Dunne, J.R., Dunkin, B.J., Nelson, S., and White, J.C. (1996) Effects of granulocyte colony-stimulating factor in a non-neutropenic rodent model of *Escherichia coli* peritonitis. *J. Surg. Res.* **61,** 348–354.
26. Cairo, M.S., Mauss, D., Kommareddy, S., Norris, K., van de Ven, C., and Modanlou, H. (1990) Prophylactic or simultaneous administration of recombinant human granulocyte colony stimulating factor in the treatment of group B streptococcal sepsis in neonatal rats. *Pediatr. Res.* **27,** 612–616.
27. Nelson, S., Daifuku, R., and Andresen, J. (1994) Use of Filgrastim (r-metHuG-CSF) in infectious diseases, in *Filgrastim (r-metHuG-CSF) in Clinical Practice* (Morstyn, G. and Dexter, T.M., eds.), Marcel Dekker, New York, pp. 253–266.
28. King, J., Deboisblanc, B.P., Mason, C.M., Onofrio, J.M., Lipscomb, G., Mercante, D.E., et al. (1995) Effect of granulocyte colony-stimulating factor on acute lung injury in the rat. *Am. J. Respir. Crit. Care Med.* **151,** 302–309.
29. Hollingshead, L.M. and Goa, K.L. (1991) Recombinant granulocyte colony-stimulating factor (rG-CSF). A review of its pharmacological properties and prospective role in neutropenic conditions. *Drugs* **42,** 300–330.
30. Steward, W.P. (1993) Granulocyte and granulocyte–macrophage colony-stimulating factors. *Lancet* **342,** 153–157.
31. Dale, D.C. (1994) Potential role of colony-stimulating factors in the prevention and treatment of infectious diseases. *Clin. Infect. Dis.* **18(Suppl 2),** S180–S188.
32. Crawfort, J., Ozer, H., Stoller, R., Johnson, D., Lyman, G., Tabbara, I., et al. (1991) Reduction by granulocyte colony-stimulating factor of fever and neutropenia induced by chemotherapy in patients with small cell lung cancer. *N. Engl. J. Med.* **325,** 164–170.
33. Trillet-Lenoir, V., Green, J., Manegold, C., Von Pawel, J., Gatzemeier, U., Lebebau, B., et al. (1993) Recombinant granulocyte colony-stimulating factor reduces the infectious complications of cytotoxic chemotherapy. *Eur. J. Cancer.* **29A,** 319–324.

34. Frampton, J.E., Yarker, Y.E., and Goa, K.L. (1995) Lenograstim. A review of its pharmacological properties and therapeutic efficacy in neutropenia and related clinical settings. *Drugs* **49,** 767–793.
35. Welte, K., Gabrilove, J., Bronchud, M.H., Platzer, E., and Morstyn, G. (1996) Filgrastim (r-metHuG-CSF): the first 10 years. *Blood* **88,** 1907–1929.
36. Dale, D.C. (1998) The discovery, development and clinical applications of granulocyte colony-stimulating factor. *Trans. Am. Clin. Climatol. Assoc.* **109,** 27–36.
37. Welte, K. and Boxer, L.A. (1997) Severe chronic neutropenia: pathophysiology and therapy. *Semin. Hematol.* **34,** 267–278.
38. Freedman, M.H. (1997) Safety of long-term administration of granulocyte colony-stimulating factor for severe chronic neutropenia. *Curr. Opin. Hematol.* **4,** 217–224.
39. Pizzo, P.A. (1993) Management of fever in patients with cancer and treatment-induced neutropenia. *N. Engl. J. Med.* **328,** 1323–1332.
40. Dale, D.C., Liles, W.C., Llewellyn, C., Rodger, E., Bowden, R., and Price, T.H. (1998) Neutrophil transfusion therapy: characteristics and kinetics of cells from donors treated with a combination of G-CSF and dexamethasone. *Transfusion* **38,** 713–721.
41. Price, T.H., Bowden, R.A., Boeckh, M., Liles, W.C., Llewellyn, C., Fuller, F., et al. (1997) Neutrophil collection from community apheresis donors after granulocyte colony-stimulating factor and dexamethasone stimulation: feasibility and efficacy. *Blood* **90,** 137b (abstract).
42. Price, T.H., Bowden, R.A., Boeckh, M., Bux, J., Nelson, K., Liles, W.C., et al. (2000) Phase I/II trial of neutrophil transfusion from donors stimulated with G-CSF and dexamethasone for treatment of infections in hematopoietic stem cell transplantation patients. *Blood* **95,** 3302–3304.
43. Ferrieri, P. (1990) Neonatal susceptibility and immunity to major bacterial pathogens. *Rev. Infect. Dis.* **12(Suppl.),** S394–400.
44. Berger, M. (1990) Complement deficiency and neutrophil dysfunction as risk factors for bacterial infection in newborns and the role of granulocyte transfusion in therapy. *Rev. Infect. Dis.* **12 (Suppl),** S401–409.
45. Gillan, E.R., Christensen, R.D., Suen, Y., Ellis, R., van de Ven, C., and Cairo, M.S. (1994) A randomized, placebo-controlled trial of recombinant human granulocyte colony-stimulating factor administration in newborn infants with presumed sepsis: significant induction of peripheral and bone marrow neutrophilia. *Blood* **84,** 1427–1233.
46. Bohme, A. and Karthaus, M. (1999) Systemic fungal infections in patients with hematologic malignancies: indications and limitations of the antifungal armamentarium. *Chemotherapy* **45,** 315–324.
47. Diamond, R.D. (1993) Interactions of phagocytic cells with Candida and other opportunistic fungi. *Arch. Med. Res.* **24,** 361–369.
48. Gaviria, J.M., van Burk, J.-A.H., Dale, D.C., Root, R.K., and Liles, W.C. (1999) Modulation of neutrophil-mediated activity against the pseudohyphal form of *Candida albicans* by granulocyte colony-stimulating factor (G-CSF) administered in vivo. *J. Infect. Dis.* **179,** 1301–1304.
49. Kullberg, B.J., Netea, M.G., Vonk, A.G., and van der Meer, J.W. (1999) Modulation of neutrophil function in host defense against disseminated *Candida albicans* infection in mice. *FEMS Immunol. Med. Microbiol.* **26,** 299–307.
50. Root, R.K. and Dale, D.C. (1999) Granulocyte colony-stimualting factor and granulocyte–macrophage colony stimulating factor: comparisons and potential for use in the treatment of infections in nonneutropenic patients. *J. Infect. Dis.* **179 (Suppl 2),** S342–S352.
51. Smith, J.A. (1994) Neutrophils, host defense, and inflammation: a double-edged sword. *J. Leukocyte Biol.* **56,** 672–686.
52. Nelson, S., Belknap, S.M., Carlson, R.W., Dale, D., DeBoisblanc, B., Farkas, S., et al. (1998) A randomized controlled trial of filgrastim as an adjunct to antibiotics for treatment of hospitalized patients with community-acquired pneumonia. CAP study group. *J. Infect. Dis.* **178,** 1075–1080.
53. Nelson, S., Heyder, A.M., Stone, J., Bergeron, M.G., Daugherty, S., Peterson, G., et al. (2000) A randomized controlled trial of Filgrastim for the treatment of hospitalized patients with multilabor pneumonia. *J. Infect. Dis.* **182,** 970–973.
54. Root, R., Nelson, S., Dale, D., Martin, T., and Welch, W. (2000) A multicenter, double-blind, placebo-controlled study of Filgrastim (r-methuG-CSF) in the treatment of patients with multilobar community-acquired pneumonia (MLCAP). *Am. J. Respir. Crit. Care Med.* **161,** A91 (abstract).
55. Gough, A., Clapperton, M., Rolando, N., Foster, A.V., Philpott-Howard, J., and Edmonds, M.E. (1997) Randomized placebo-controlled trial of granulocyte-colony stimulating factor in diabetic foot infection. *Lancet* **350,** 855–859.
56. Hermans, P. (1999) HIV disease-related neutropenia: an independent risk factor for severe infections. *AIDS* **13 (Suppl 2),** S11–S17.
57. Frumkin, L.R. (1997) Role of granulocyte colony-stimulating factor and granulocyte–macrophage colony-stimulating factor in the treatment of patients with HIV infection. *Curr. Opin. Hematol.* **4,** 200–206.
58. Mitsuyasu, R. (1999) Prevention of bacterial infections in patients with advanced HIV infection. *AIDS* **13(Suppl. 2),** S19–S23.
59. George, S., Coffey, M., Cinti, S., Collins, J., Brown, M., and Kazanjian, P. (1998) Neutrophils from AIDS patients treated with granulocyte colony-stimulating factor demonstrate enhanced killing of *Mycobacterium avium*. *J. Infect. Dis.* **178,** 1530–1533.
60. Hartung, T., Pitrak, D.L., Foote, M., Shatzen, E.M., Verral, S.C., and Wendel, A. (1998) Filgrastim restores interleukin-2 production in blood from patients with advanced human immunodeficiency virus infection. *J. Infect. Dis.* **178,** 686–692.
61. Weiss, M., Gross-Weege, W., Schneider, M., Neidhardt, H., Liebert, S., Mirow, N., et al. (1995) Enhancement of neutrophil function by in vivo filgrastim treatment for prophylaxis of sepsis in surgical intensive care patients. *J. Crit. Care* **10,** 21–26.

62. Foster, P.F., Mital, D., Sankary, H.N., McChesney, L.P., Marcon, J., Koukoulis, G., et al. (1995) The use of granulocyte colony-stimulating factor after liver transplantation. *Transplantation* **59,** 1557–1563.
63. Endo, S., Inada, K., Inoue, Y., Yamada, Y., Takakuwa, T., Kasai, T., et al. (1994) Evaluation of recombinant human granulocyte colony-stimulating factor (rhG-CSF) therapy in granulopoietic patients complicated with sepsis. *Curr. Med. Res. Opin.* **13,** 233–241.
64. Schafer, H., Hubel, K., Bohlen, H., Mansmann, G., Hegener, K., Richarz, B., et al. (2000) Perioperative treatment with granulocyte colony-stimulating factor (G-CSF) in patients with esophageal cancer stimulates granulocyte function and reduces infectious complications after esophagectomy. *Ann. Hematol.* **79,** 143–151.
65. Seymour, J.F., Lieschke, G.J., Grail, D., Quilici, C., Hodgson, G., and Dunn, A.R. (1997) Mice lacking both granulocyte colony-stimulating factor (CSF) and granulocyte–macrophage CSF have impaired reproductive capacity, pertubed neonatal granulopoiesis, lung disease, amyloidosis, and reduced long-term survival. *Blood* **90,** 3037–3049.
66. Wong, G.G., Witek, J.S., Temple, P.A., Wilkens, K.M., Leary, A.C., Luxenberg, D.P., et al. (1985) Human GM-CSF: molecular cloning of the complementary DNA and purification of the natural and recombinant proteins. *Science* **228,** 810–815.
67. Huebner, K., Isobe, M., Croce, C.M., Golde, D.W., Kaufmann, S.E., and Gasson, J.C. (1985) The human gene encoding GM-CSF is at 5q21–q32, the chromosome region deleted in the 5q- anomaly. *Science* **230,** 1282–1285.
68. Hara, T. and Miyajima, A. (1996) Function and signal transduction mediated by the interleukin 3 receptor system in hematopoiesis. *Stem Cells* **14,** 605–618.
69. Gasson, J.C. (1991) Molecular physiology of granulocyte–macrophage colony-stimulating factor. *Blood* **77,** 1131–1145.
70. Ruef, C. and Coleman, D.L. (1990) Granulocyte–macrophage colony-stimulating factor: pleiotropic cytokine with potential clinical usefulness. *Rev. Infect. Dis.* **12,** 41–62.
71. Clark, S.C. (1988) Biological activities of human granulocyte–macrophage colony-stimulating factor. *Int. J. Cell. Cloning* **6,** 365–377.
72. Denzlinger, C., Tetzloff, W., Gerhartz, H.H., Pokorny, R., Sagebiel, S., Haberl, C., et al. (1993) Differential activation of the endogenous leukotriene biosynthesis by two different preparations of granulocyte–macrophage colony-stimulating factor in healthy volunteers. *Blood* **81,** 2007–2013.
73. Dorr, R.T. (1993) Clinical properties of yeast-derived granulocyte–macrophage colony stimulating factor. *Clin. Ther.* **15,** 19–29.
74. Dale, D.C., Liles, W.C., Llewellyn, C., and Price, T.H. (1998) Effects of granulocyte–macrophage colony-stimulating factor (GM-CSF) on neutrophil kinetics and function in normal human volunteers. *Am. J. Hematol.* **57,** 7–15.
75. Frenck, R.W., Sarman, G., Harper, T.E., and Buescher, E.S. (1990) The ability of recombinant murine granulocyte–macrophage colony-stimulating factor to protect neonatal rats from septic death due to *Staphylococcus aureus*. *J. Infect. Dis.* **162,** 109–114.
76. Wheeler, J.G. and Givner, L.B. (1992) Therapeutic use of recombinant human granulocyte–macrophage colony-stimulating factor in neonatal rats with type III group B streptococcal sepsis. *J. Infect. Dis.* **165,** 938–941.
77. Givner, L.B. and Nagaraj, S.K. (1993) Hyperimmune human IgG or recombinant human granulocyte–macrophage colony-stimulating factor as adjunctive therapy for group B streptococcal sepsis in newborn rats. *J. Pediatr.* **122,** 774–779.
78. Toda, H., Murata, A., Oka, Y., Uda, K., Tanaka, N., Ohashi, I., et al. (1994) Effect of granulocyte–macrophage colony-stimulating factor on sepsis-induced organ injury in rats. *Blood* **83,** 2893–2898.
79. Freund, M. and Kleine, H.D. (1992) The role of GM-CSF in infection. *Infection* **20(Suppl 2),** S84–S92.
80. Heidenreich, S., Gong, J.H., Schmidt, A., Nain, M., and Gemsa, D. (1989) Macrophage activation by granulocyte/macrophage colony-stimulating factor. *J. Immunol.* **143,** 1198–1205.
81. Tiegs, G., Barsig, J., Matiba, B., Uhlig, S., and Wendel, A. (1994) Potentiation by granulocyte–macrophage colony-stimulating factor of lipopolysaccharide toxicity in mice. *J. Clin. Invest.* **93,** 2616–2622.
82. LeVine, A.M., Reed, J.A., Kurak, K.E., Cianciolo, E., and Whitsett, J.A. (1999) GM-CSF-deficient mice are susceptible to pulmonary group B streptococcal infection. *J. Clin. Invest.* **103,** 563–569.
83. Bermudez, L.E., Martinelli, J., Petrofsky, M., Kolonoski, P., and Young, L.S. (1994) Recombinant granulocyte–macrophage colony-stimulating factor enhances the effects of antibiotics against *Mycobacterium avium* complex infection in the beige mouse model. *J. Infect. Dis.* **169,** 575–580.
84. Liehl, E., Hildebrandt, J., Lam, C., and Mayer, P. (1994) Prediction of the role of granulocyte–macrophage colony-stimulating factor in animals and man from in vitro results. *Eur. J. Clin. Microbiol. Infect. Dis.* **13(Suppl 2),** S9–S17.
85. Mayer, P., Schutze, E., Lam, C., Kricek, F., and Liehl, E. (1991) Recombinant murine granulocyte–macrophage colony-stimulating factor augments neutrophil recovery and enhances resistance to infections in myelosuppressed mice. *J. Infect. Dis.* **163,** 584–590.
86. Mandujano, J.F., D'Souza, N.B., Nelson, S., Summer, W.R., Beckerman, R.C., and Shellito, J.E. (1995) Granulocyte–macrophage colony stimulating factor and Pneumocystis carinii pneumonia in mice. *Am. J. Respir. Crit. Care Med.* **151,** 1233–1238.
87. Antman, K.S., Griffin, J.D., Elias, A., Socinski, M.A., Ryan, L., Cannistra, S.A., et al. (1988) Effect of recombinant human granulocyte–macrophage colony-stimulating factor on chemotherapy-induced myelosuppression. *N. Engl. J. Med.* **319,** 593–598.
88. Avanzi, G.C., Gallicchio, M., and Saglio, G. (1998) Hematopoietic growth factors in autologous transplantation. *Biotherapy* **10,** 299–308.
89. Nemunaitis, J., Buckner, C.D., Dorsey, K.S., Willis, D., Meyer, W., and Appelbaum, F. (1998) Retrospective analysis of infectious disease in patients who received recombinant human granulocyte–macrophage colony-stimulating factor versus patients not receiving a cytokine who underwent autologous bone marrow transplantation for treatment of lymphoid cancer. *Am. J. Clin. Oncol.* **21,** 341–346.

90. Scarffe, J.H. (1991) Emerging clinical use for GM-CSF. *Eur. J. Cancer* **27,** 1493–1504.
91. De Witte, T., Vreugdenhil, A., and Schattenberg, A. (1995) The role of recombinant granulocyte–macrophage colony-stimulating factor (GM-CSF) after T-cell deplete allogenic bone marrow transplantation, in *Granulocyte–Macrophage Colony-Stimulating Factor (GM-CSF). Current Use and Future Applications* (Nissen, N.I. and Dexter, T.M., eds.), Pennine, Cheshire, pp. 30–34.
92. Ruef, C. and Colemann, D.L. (1991) GM-CSF and G-CSF: cytokines in clinical applications. *Schweiz. Med. Wochenschr.* **121,** 397–412.
93. Moore, M.A. (1991) The clinical use of colony stimulating factors. *Annu. Rev. Immunol.* **9,** 159–191.
94. Yoshida, Y., Nakahata, T., Shibata, A., Takahashi, M., Moriyama, Y., Kaku, K., et al. (1995) Effects of long-term treatment with recombinant human granuolocyte-macrophage colony-stimulating factor in patients with myelodysplastic syndrome. *Leukocyte Lymphoma* **18,** 457–463.
95. Lieschke, G.J. and Burgess, A.W. (1992) Granulocyte colony-stimulating factor and granulocyte–macrophage colony-stimulating factor. *N. Engl. J. Med.* **327,** 99–106.
96. Bodey, G., Bueltmann, B., Duguid, W., Gibbs, D., Hanak, H., Hotchi, M., et al. (1992) Fungal infections in cancer patients: an internatonal autopsy survey. *Eur. J. Clin. Microbiol. Infect. Dis.* **11,** 99–109.
97. Smith, P.D., Janoff, E.N. and Wahl, S.M. (1993) Granulocyte–macrophage colony-stimulating factor augmentation of human monocyte effector and accessory cell function, in *Hemopoietic Growth Factors and Mononuclear Phagocytes* (Van Furth, R., ed.), Karger, Basel, pp. 79–89.
98. Montgomery, B., Bianco, J.A., Jacobsen, A., and Singer, J.W. (1991) Localization of transfused neutrophils to site of infection during treatment with recombinant human granulocyte–macrophage colony-stimulating factor and pentoxyfylline. *Blood* **78,** 533–534.
99. Gaviria, J.M., van Burik, J.-A.H., Dale, D.C., Root, R.K., and Liles, W.C. (1999) Comparison of interferon-gamma, granulocyte colony-stimulating factor, and granulocyte–macrophage colony-stimulating factor for priming leukocyte-mediated hyphal damage of opportunistic fungal pathogens. *J. Infect. Dis.* **179,** 1038–1041.
100. Hast, R., Bernell, P., Kimby, E., and Petrescu, A. (1995) rhGM-CSF in combination with standard induction chemotherapy for the treatment of acute myeloid leukemia—an overview, in *Granulocyte–Macrophage Colony-Stimulating Factor (GM-CSF). Current Use and Future Applications* (Nissen, N.I. and Dexter, T.M., eds.), Pennine, Cheshire, pp. 24–26.
101. Lanza, F., Rigolin, G.M., Castagnari, B., Moretti, S., and Castoldi, G. (1997) Potential clinical applications of rhGM-CSF in acute myeloid leukemia based on its biologic activity and receptor interaction. *Haematologica* **82,** 239–245.
102. Schlimok, G., Fackler-Schwalbe, I., Lindemann, F., Gruber, R., Flieger, D., Heiss, M., et al. (1995) GM-CSF in gastric cancer: monitoring of micrometastatic tumour cells in bone marrow, in *Granulocyte–Macrophage Colony-Stimulating Factor (GM-CSF). Current Use and Future Applications* (Nissen, N.I. and Dexter, T.M., eds.), Pennine Cheshire, pp. 20–24.
103. Chi, K.H., Chen, C.H., Chan, W.K., Chow, K.C., Cher, S., Yen, S.H., et al. (1995) Effect of granulocyte–macrophage colony-stimulating factor on oral mucositis in head and neck cancer patients after cisplatin, fluorouracil and leucovorin chemotherapy. *J. Clin. Oncol.* **13,** 2620–2628.
104. Masucci, G. (1996) New clinical applications of granulocyte–macrophage colony-stimulating factor. *Med. Oncol.* **13,** 149–154.
105. Silverstein, S.C., Cao, C., Rudin, D., Gao, P., and Neu, H. (1993) Organic anion transporters promote the secretion of anionic antibiotics from cells of the J774 macrophage-like cell line, in *Hemopoietic Growth Factors and Mononuclear Phagocytes* (Van Furth, R., ed.), Karger, Basel, pp. 134–139.
106. Skowron, G., Stein, D., Drusano, G., Melbourne, K., Bilello, J., Mikolich, D., et al. (1999) The safety and efficacy of granulocyte–macrophage colony-stimulating factor (Sargramostin) added to indinavir- or ritonavir-based antiretroviral therapy: a randomized double-blind, placebo-controlled trial. *J. Infect. Dis.* **180,** 1064–1071.
107. Badaro, R., Nascimento, C., Carvalho, J.S., Badaro, F., Russo, D., Ho, J.L., et al. (1994) Granulocyte–macrophage colony-stimulating factor in combination with pentavalent antimony for the treatment of visceral leishmaniasis. *Eur. J. Clin. Microbiol. Infect. Dis.* **13 (Suppl. 2),** S23–S28.
108. Decoster, G., Rich, W., and Brown, S.L. (1994) Safety profile of Filgrastim (r-metHuG-CSF), in Filgrastim (r-metHuG-CSF) in *Clinical Practice* (Morstyn, G. and Dexter, T.M., eds.), Marcel Dekker, New York, pp. 267–290.
109. Peters, W.P., Shogan, J.S., Shpall, E.J., Jones, R.B., and Kim, C.S. (1988) Recombinant human granulocyte–macrophage colony-stimulating factor produces fever. [letter]. *Lancet* **1,** 950.
110. Lieschke, G.J., Cebon, J., and Morstyn, G. (1989) Characterization of the clinical effects after the first dose of bacterially synthesized recombinant granulocyte–macrophage colony-stimulating factor. *Blood* **74,** 2634–2643.
111. Arnig, M., Kliche, K.O., and Schneider, W. (1991) GM-CSF therapy and capillary-leak syndrome. [letter]. *Ann. Hematol.* **62,** 83.
112. Mueller, M.M. and Fusenig, N.E. (1999) Constitutive expression of G-CSF and GM-CSF in human skin carcinoma cells with functional consequence for tumor progression. *Int. J. Cancer* **83,** 780–789.