Filgrastim (r-metHuG-CSF): The First 10 Years

By Karl Welte, Janice Gabrilove, Miguel H. Bronchud, Erich Platzer, and George Morstyn

IT HAS BEEN KNOWN for at least three decades that very specific factors control hematopoiesis, acting on early cells in the hematopoietic system to produce mature, functional cells. The isolation, purification, and cloning of these factors has lead to a new class of therapeutic agents, including the colony-stimulating factors and interleukins. This review is devoted to granulocyte colony-stimulating factor (G-CSF), specifically Filgrastim (r-metHuG-CSF), the bacterially synthesized recombinant protein form of G-CSF, that acts on neutrophils, the body’s major defense against infections.

The purification and molecular cloning of Filgrastim were performed between 1984 and 1986,1,2 and the clinical development of Filgrastim commenced in 1986, with approval for clinical use in cancer patients treated with chemotherapy3,4 obtained in the United States in February 1991. In the 5 years since its approval, 1.2 million patients have been treated with Filgrastim (Amgen [Thousand Oaks, CA], data on file).

Filgrastim was initially used as an adjunct to chemotherapy for ameliorating neutropenia, one of the major side effects of cancer chemotherapy. Its use has led to reduced infections and hospital admissions for patients with cancer. Besides chemotherapy-induced neutropenia, Filgrastim has been approved in more than 70 countries for the treatment of myelosuppression after bone marrow transplantation, severe chronic neutropenia (SCN), acute leukemia, aplastic anemia (AA), myelodysplastic syndromes (MDS), and mobilization of peripheral blood progenitor cells (PBPCs) for transplantation. The use of Filgrastim has made possible delivery of full-dose chemotherapy and high-dose chemotherapy and has benefited patients who are immunocompromised. A second form of rHuG-CSF (lenograstim) received approval in Europe in 1993. The biologic properties of Filgrastim and lenograstim are similar and are given in Table 1.

This review focuses on Filgrastim and reviews the first 10 years of clinical development and the first 5 years of postapproval use as well as additional potential clinical applications.

PHARMACOLOGY OF FILGRASTIM

The pharmacodynamics and pharmacokinetics of Filgrastim were studied in rodents,3,4 and primates6,7 and then in normal volunteers and in patients with malignant disease. The results of these studies indicated that Filgrastim had a consistent and predictable pharmacologic profile when administered either subcutaneously or intravenously over a wide dose range.9

Biologic Effects

Mice lacking endogenous G-CSF have chronic neutropenia and impaired neutrophil mobilization, indicating that G-CSF is indispensable for maintaining the normal quantitative balance of neutrophil production during steady-state granulopoiesis in vivo and suggesting that G-CSF has a role in emergency granulopoiesis during infections.10 In response to an infection or to neutropenia, the amount of circulating endogenous G-CSF in the blood increases.11,12

Secondary structure-prediction analyses show that Filgrastim has four antiparallel helices (Fig 1).13 G-CSF selectively and specifically stimulates the proliferation and differentiation of neutrophil precursors by binding to a specific cell-surface receptor (for a review of the receptor and its actions, see Demetri and Griffin14). This explains the selective action of G-CSF compared with other cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3). The number of human neutrophil receptors increases slightly as the cell matures. The binding of G-CSF to its receptors decreases the number of available surface receptors as the receptor complex is internalized and degraded. Native G-CSF is found on chromosome 17q21-22 and the human G-CSF receptor is encoded by a single gene on chromosome 1p35-p34.15 The protein tyrosine kinases JAK1 and JAK2 are phosphorylated on tyrosine after the interaction of G-CSF with its receptor, and the Ras/MAP kinase pathway has been shown to be activated in response to G-CSF.16-21

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Table 1. Biochemical Characteristics of Filgrastim and Lenograstim*  

<table>
<thead>
<tr>
<th>Chemical Moiety</th>
<th>Lenograstim (CHO)</th>
<th>Filgrastim (E. coli)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids (n)</td>
<td>174</td>
<td>175</td>
</tr>
<tr>
<td>N-terminal acid</td>
<td>Threonine</td>
<td>Methionine</td>
</tr>
<tr>
<td>Glycosylation</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>MW (kD)</td>
<td>21.6 (SDS)</td>
<td>18.7 (SDS)</td>
</tr>
<tr>
<td></td>
<td>23.5 (UC)</td>
<td>18.9 (GF)</td>
</tr>
</tbody>
</table>

Abbreviations: MW, molecular weight; SDS, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; UC, ultracentrifuge; GF, gel filtration.

G-CSF affects mature neutrophils as well as progenitor cells, enhances chemotaxis by increased binding of f-MLP, and enhances superoxide production in response to chemotactants. Filgrastim promotes the anti-Candida albicans activity of normal human polymorphonuclear (PMN) cells in vitro at amounts as small as 0.1 ng/mL and enhances f-MLP--stimulated superoxide anion production by PMN cells.

In vivo preclinical studies showed that, with a single subcutaneous dose of Filgrastim at 10 µg/kg, the serum concentration peaked at 2 to 4 hours, with an accompanying fivefold decrease in the absolute neutrophil count (ANC) and a 2.5-fold increase in chemiluminescence. Between 24 and 48 hours, there was a second sixfold neutrophil increase and a threefold chemiluminescence increase. In addition to elevating neutrophil levels, G-CSF also reduces the neutrophil maturation time from 5 days to 1 day, leading to a rapid release of neutrophils from the marrow into the circulation (Fig 2). The high-affinity receptor for Ig (CD64, Fc-γRI) was strongly expressed in Filgrastim-treated patients. These neutrophils exhibited an enhanced antitumor cytotoxicity in an antibody-dependent cell-mediated cytotoxicity (ADCC) setting.

Clinically, the efficacy of Filgrastim is related directly to its biologic effects as it acts selectively on cells of the neutrophil lineage. However, some data show that G-CSF acts on blast progenitor cells and on myeloid progenitor and mature cells. The predominant changes in the marrow of patients enrolled in phase 1 studies were dose-dependent increases in the proportion of early myeloid cells that yielded an increase in the early-to-late myeloid cell ratio. There were also twofold non--dose-dependent increases in lymphocytes and dose-dependent increases in monocytes. In other studies, there were small, but inconsistent, decreases in other cell types, specifically in the number of red blood cells and platelets in some patients. There was no consistent effect on hemoglobin or hematocrit.

A number of studies showed an initial transient decrease in the number of circulating neutrophils after intravenous or subcutaneous injection. This was followed consistently by a rapid and significant increase to a greater-than-normal count within 4 to 6 hours. The precise mechanism of this early depression was thought to be due to margination of neutrophils to endothelial cells. Because the increase in neutrophil counts occurs within 6 hours, it is possible that neutrophil demargination or prolongation of circulating neutrophil survival may contribute to the earliest observed neutrophil increase.

Filgrastim elicited neutrophil responses in both young (20 to 30 years of age) and elderly (70 to 80 years of age) healthy volunteers. Neutrophil counts, peak neutrophil counts, and the rate of attaining the peak neutrophil count were similar in both age groups at both 30 and 300 µg/d, and daily tests of neutrophil function showed nearly identical responses to several agonists for both age groups.

Because the effect of Filgrastim on neutrophils is readily measured, pharmacokinetics did not play as significant a role in defining dose and schedule as did the pharmacodynamic effects. Nevertheless, pharmacokinetics were measured in several studies by immunoassay or bioassay.

Pharmacokinetic Studies

Normal male volunteers receiving single doses of 3.45 µg/kg Filgrastim by 30-minute intravenous infusion had a mean serum concentration of 20.8 ng/mL 5 minutes after the end of infusion, but it is thought that the peak concentration was most likely underestimated (Fig 3). The mean ±

![Fig 1. Schematic representation of the structure of Filgrastim.](https://www.bloodjournal.org/bloodjournal)
SD elimination half-life was 163 ± 7.4 minutes and G-CSF levels returned to normal values within 14 to 18 hours. Cancer patients receiving Filgrastim at 11.5 µg/kg as a 30-minute intravenous infusion had a peak serum concentration of 384 ng/mL. When cancer patients received subcutaneous bolus or subcutaneous infusion doses, serum concentrations of G-CSF reflected a rapid absorption. A similar maximum increase in ANC was achieved with Filgrastim by all routes of administration tested. The bioavailability of Filgrastim may be influenced by the removal of the protein by the increased number of neutrophils it stimulates to be formed. Increased neutrophil levels were shown to be associated with increased clearance of Filgrastim when administered to patients with cancer, suggesting that this may be a negative feedback mechanism involved in maintaining neutrophil numbers at optimum values in normal and disease states. A cross-over study in neutropenic rats, using intravenous and subcutaneous routes of administration, showed that there were higher terminal half-lives for both routes compared with nonneutropenic control rats, with the apparent bioavailability increasing approximately 15% in the neutropenic rats. The bioavailability of Filgrastim was reported to be an average of 53% when administered to normal volunteers at very low doses (1 µg/kg), but, when administered at therapeutic doses, its bioavailability has been as high as 80%.

The pharmacokinetics of Filgrastim in children receiving myelosuppressive treatment for advanced neuroblastoma was studied by Stute et al. Filgrastim at 5, 10, or 15 µg/kg was administered for 10 days and blood samples were taken on days 1 and 10 at several timepoints. The serum concentration of Filgrastim began to increase shortly after administration and absorption continued over several hours, with the peak concentration reached between 4 and 12 hours.

**THERAPEUTIC USES**

**Standard-Dose Chemotherapy**

*Early Studies in Solid Tumors*

Filgrastim attracted the attention of hematologists and oncologists at a time when pessimism was beginning to prevail because of the relative lack of major therapeutic breakthroughs. The promising results of Filgrastim in a pilot study in patients with small-cell lung cancer, showing that the degree of neutropenia was substantially reduced after chemotherapy cycles in which Filgrastim was administered prophylactically when compared with cycles in which Filgrastim was not administered, formed the basis for the first phase 3 study of its use as an adjunct to chemotherapy in patients. This study, and a similar multicenter randomized study later performed in Europe, was designed to test whether Filgrastim could decrease the incidence of infection as manifested by febrile neutropenia and whether the use of this new substance in this patient population would lead to a reduction in the incidence of intravenous antibiotic use, hospitalization, and culture-confirmed infections.

It is well known that febrile neutropenia per se does not necessarily imply infection in an oncologic patient, but it remains a basic indication for intravenous antibiotics. It is important to stress that none of these studies was designed to show whether Filgrastim led to improved survival in patients with small-cell lung cancer. The American study enrolled 211 patients and a cross-over was allowed in the event of febrile neutropenia. The febrile neutropenia event rate was 57% in the placebo group during the first cycle of chemotherapy (with cyclophosphamide, doxorubicin, and etoposide), with a cumulative rate of 77% by the end of the six cycles of treatment, whereas only 28% of the Filgrastim-treated patients developed fever and neutropenia during their first cycle and a total of 40% over the six cycles of treatment (Fig 4).
In the European trial, also in small-cell lung cancer and with essentially the same combination chemotherapy, the overall rates of febrile neutropenic events and documented infections were somewhat lower than in the American study, but the reduction in febrile neutropenia was similar (ie, 26% vs 53% for Filgrastim and placebo patients, respectively). In these two studies, no statistically meaningful differences were detected in tumor response rates or survival between the groups receiving Filgrastim or placebo. Small-cell lung cancer has remained resistant to intensification of doses administered in the normal range, and the studies did not use the very high doses of chemotherapy that might be associated with improved survival. Studies examining dose escalation with Filgrastim are in progress and there is a report of improved survival in patients with small-cell lung cancer that requires confirmation. 

Two other early pilot studies of Filgrastim confirmed the rapid neutrophil recovery after chemotherapy in solid tumors, the safety of subcutaneous administration, and the good tolerability profile. In a study of patients with postchemotherapy mucositis examining semiquantitatively the number of neutrophils using a mouthrinse assay, there was evidence that neutrophils produced by Filgrastim were able to leave the circulation and to enter tissues. The availability of neutrophils to the tissues may explain the reduction in mucositis reported in early studies.

**Studies in Hematologic Malignancies**

Trials in hematologic malignancies have yielded similar results to those in solid tumors, but more work has been performed in the setting of dose intensification in lymphoma because it is perceived to be one of the malignancies more responsive to chemotherapy.

The major areas of potential clinical application of the hematopoietic colony-stimulating factors in patients include their use in facilitating recovery from the myelosuppressive effects of induction and/or consolidation chemotherapy, for harvesting peripheral blood stem cells after intensive chemotherapy that may be used for autologous stem-cell support for bone marrow ablation, and for cycle activation/maturatation therapy.

**Use of Filgrastim in acute myeloid leukemia (AML).** Although rHuG-CSF had been shown to accelerate recovery from myelosuppressive chemotherapy, its use in the setting of AML remained controversial because of the in vitro observations that G-CSF could stimulate leukemic growth. Ohno et al conducted a prospective, randomized study to determine the efficacy and safety of Filgrastim after a standard course of intensive chemotherapy in 108 patients with relapsed or refractory acute leukemia. Treatment with Filgrastim at 200 μg/m²/d was begun 2 days after the last dose of induction chemotherapy and continued until an ANC greater than 1.5 × 10⁹/L. Treatment with Filgrastim was shown to accelerate the recovery of neutrophils, shortening it by 1 week compared with the group that did not receive Filgrastim (Fig 5). This was observed despite the mean chemotherapy dose being greater in the Filgrastim-treated group. Although the incidence of febrile neutropenia was the same between the two groups, the incidence of documented infections was reduced in the Filgrastim-treated patients. No dif-
ference was observed in remission rates between the two groups.

Ohno et al 82 next explored whether the administration of Filgrastim before the start of chemotherapy and continuing after chemotherapy would result in enhanced cell kill because of growth factor-mediated recruitment of quiescent leukemic cells, improved remission rates, accelerated recovery of neutrophils, and reduced febrile neutropenia and infectious complications associated with leukemic therapy. Filgrastim was administered from 2 days before the initiation of chemotherapy and continued until the ANC reached 1.5 × 10^9/L. This study again showed faster recoveries for the neutrophil counts in the Filgrastim-treated patients; however, the incidence of febrile neutropenia was the same between the treatment groups. Although the incidence of documented infection was also similar between the groups, the incidence of documented infections after the third week showed a trend to be lower in the Filgrastim-treated group, but this did not reach statistical significance. Fifty percent of the Filgrastim-treated patients achieved a complete remission compared with 37% of the placebo patients; this was not statistically significant. There was no difference between the groups in event-free survival or in disease-free survival in the patients who did achieve a complete remission.

Estey et al 83 investigated whether Filgrastim administered before, during, and after fludarabine plus cytosine arabinoside chemotherapy affected the complete response rate, infection, blood count recovery, or survival in patients with newly diagnosed AML or MDS. A total of 112 patients received Filgrastim at 400 mg/m^2/d 1 day before and/or during chemotherapy. Filgrastim was continued until a complete remission was achieved. Results were compared with those in 85 newly diagnosed patients previously treated with the same chemotherapy regimen but without Filgrastim. Overall, Filgrastim administered in this manner had no effect on complete remission or infection rates in this population in which most of the patients were elderly with poor prognostic factors.

Age is a poor prognostic factor in AML, and a trial was designed to test the hypothesis that Filgrastim, used as supportive care, could improve the outcome of elderly patients with this disease. 84 Two hundred thirty-four patients with de novo or secondary AML were randomized to Filgrastim or placebo started 3 days after the last dose of chemotherapy when the marrow contained less than 5% blasts. The Filgrastim-treated group showed significant reductions in days to neutrophil recovery, days with fever, and days on intravenous antibiotics compared with the placebo group. Complete remission rates, overall survival, risk of fatal infection, numbers of documented infections, and duration of hospitalization were not significantly different between the two treatment groups. The initial report of this study included patients with de novo and secondary AML. Filgrastim was also delayed for several days, and this may have potentially confounded the interpretation of the results.

A recently presented European, randomized, double-blind, placebo-controlled, phase 3 study of Filgrastim in more than 500 patients receiving remission induction and early consolidation therapy for de novo AML 85 showed no difference in overall survival or complete response rate between the two treatment groups (complete response rate was 74% in patients younger than 50 years of age and 64% in patients older than 50), but the rate of neutrophil recovery was 5 days faster in the Filgrastim-treated group. This was accompanied by significant reductions in the duration of fever, parenteral antibiotic use, and hospitalization. These endpoints were also reduced by Filgrastim for consolidation chemotherapy courses. It appeared likely that, by shortening the neutropenic period, the use of Filgrastim led to fewer life-threatening infections (fungal infections in particular) without increasing the risk for leukemic death that had been a concern due to the presence of G-CSF receptors in many AML blasts. 86

Patients with AML who relapse after allogeneic transplantation have a poor prognosis. Few respond to chemotherapy and almost none survive long-term. For this reason, Giral et al 87 evaluated the ability of Filgrastim to reincrease remission by stimulating residual normal donor marrow cells. Seven women who relapsed within 360 days after allogeneic transplantation received Filgrastim. Three of the seven had a complete hematologic and cytogenetic remission, with the re-establishment of hematopoiesis of donor origin. In two of the patients, fluorescence in situ hybridization showed preferential stimulation of normal donor cells without differentiation of the leukemic clone. These investigators concluded that Filgrastim might be effective in selected patients with early relapse after allogeneic transplantation. One problem with the interpretation of the results was that cyclosporin and steroids were discontinued 7 and 14 days, respectively, after the start of Filgrastim treatment, allowing for the effect of graft-versus-leukemia effect to be exerted. What role this might have played in the results observed remains to be determined and would require further investigation.

A recently published report using lenograstim (CHO cell-derived rHuG-CSF) studied its effect on infection-related mortality during chemotherapy-induced neutropenia in elderly patients (ie, 65 years of age or older) receiving induction chemotherapy. 88 Although there was no difference in the overall survival, treated patients had a greater rate of complete remission than those who received placebo.

Use of Filgrastim in lymphoma. Eighty patients with non-Hodgkin lymphoma (NHL) were entered into a randomized trial 89 receiving the weekly chemotherapy regimen VA-PAC-E (vincristine, adriamycin, prednisone, etoposide, cytotoxan, and bleomycin) with and without Filgrastim administered subcutaneously at the conventional dose. The protocol allowed both groups identical dose-modification criteria and intravenous antibiotic policies. Severe neutropenia occurred in 37% of the Filgrastim-treated patients and 85% of control patients. Fever with neutropenia occurred in 22% of the Filgrastim group as opposed to 44% of the control group of patients. There were significantly fewer treatment delays with shorter durations of delay in the Filgrastim-treated patients (P = .01). Overall, patients treated with Filgrastim received a greater dose intensity, and, although the protocol did not allow maximum intensification of doses, this level of dose intensity did not result in any significant
differences in survival at the time of analysis. The inability to deliver dose intensity may, in part, explain the poorer outcome of chemotherapy in elderly lymphoma patients.54,55 Several studies suggest Filgrastim can reduce the need for dose reductions and delays with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy.56 This seems important because, when CHOP is administered at full doses to the elderly, a very high rate of febrile neutropenia (47%) and consequent hospitalization results.57 Recently, in a small, nonrandomized study of P-VEBEC (epirubicin, cyclophosphamide, etoposide, vinblastine, bleomycin, and prednisone) chemotherapy, it was suggested that elderly patients receiving Filgrastim had reduced mortality and a trend towards improved event-free survival. Clearly, additional studies are required to determine what effects Filgrastim has on survival. However, its use may be important, particularly in elderly patients with otherwise incurable lymphomas.

Use of Filgrastim in acute lymphoblastic leukemia (ALL). A German Acute Lymphoblastic Leukemia Pediatric Cooperative Group (ALL-BFM) has conducted a randomized phase 3 study of the efficacy of Filgrastim in children with high-risk ALL, randomized to receive nine alternating cycles of chemotherapy either alone or followed by Filgrastim administered prophylactically at conventional doses. In both groups, the planned interval between chemotherapy courses was a minimum of 21 days. Of the 34 patients analyzed, the incidence of febrile neutropenia, the number of culture-confirmed infections, and the total duration of intravenous antibiotic use was significantly reduced in the Filgrastim-treated group, allowing for tighter adherence to the treatment schedule.58 However, with a median follow-up of 3.3 years, no difference was observed with regards to the estimated event-free survival of 4 years (41% in both groups).

A recently published prospective, multicenter trial studied the simultaneous administration of Filgrastim and induction chemotherapy for ALL in adults.59 Patients receiving Filgrastim had a significant reduction in median duration of neutropenia (ANC <1.0 X 10^9/L) compared with patients who did not receive growth factor (8 days v 12.5 days; P < .002). There were trends for reduced overall infections (43% v 56%; P = .25) and nonviral infections (16 infections v 32 infections). There was a trend towards more rapid completion of chemotherapy, with delays of 2 weeks or greater occurring in only 24% of the Filgrastim-treated patients versus 46% of the control patients (P = .01). The studies to date in patients with lymphoma and leukemia have been small. A survival advantage in patients receiving Filgrastim for lymphoma or leukemia has not as yet been shown, unlike the recent results reported by Bezwooda et al50 for patients with breast cancer receiving high-dose therapy supported with Filgrastim-mobilized PBPCs (see below). The reason for the latter may be due to small numbers of patients in each study or inherent resistance of a subpopulation of tumor cells capable of regrowth.

Use of Filgrastim With Radiotherapy

Filgrastim has been used to treat the neutropenia associated with radiotherapy; however, only small studies have been reported and firm conclusions cannot be made. Eleven patients with isolated leukopenia while receiving fractionated radiotherapy received rhU-G-CSF at occurrence of leukopenia and continued treatment for the duration of the radiotherapy.61 The treatment was well tolerated, with mild bone pain in 1 patient and transient increases in serum alkaline phosphatase values in 4 patients reported. The investigators concluded that rhU-G-CSF could be used clinically to abrogate neutropenia caused by continuous fractionated radiotherapy.

Four consecutive patients receiving craniospinal irradiation, but no previous or concurrent chemotherapy, for intracranial tumors received intermittent subcutaneous injections of Filgrastins.62 All 4 patients exhibited a rapid return of their neutrophil counts to normal values, thus avoiding treatment delays because of neutropenia. In a later report, the same investigators administered Filgrastim to patients receiving extended-field radiotherapy.63 A group of 8 patients receiving craniospinal irradiation received Filgrastim when an ANC of 1.5 X 10^9/L was approached. The neutropenia was promptly corrected, avoiding unscheduled treatment interruptions. An additional 4 patients receiving mantle-type radiotherapy for NHL or Hodgkin disease were also treated with Filgrastim; all 4 of these patients had received previous courses of cytotoxic chemotherapy. Again, there was an increase in ANC on the next day. The investigators suggested that these results indicate that Filgrastim is a reliable and effective treatment for radiotherapy-induced neutropenia even when administered on an intermittent dosage schedule. The prophylactic use of Filgrastim to allow uninterrupted administration of radiotherapy was deemed valuable in another study.64

Rescue From Febrile Neutropenia

Filgrastim has a role as an adjunct to the use of antibiotics in the treatment of febrile neutropenia. Maher et al65 did a multicenter, randomized, placebo-controlled, double-blind study to determine if neutrophil and fever recovery were accelerated when Filgrastim was started after the onset of chemotherapy-induced febrile neutropenia. Patients received Filgrastim or placebo within 12 hours of a standard course of piperacillin and tobramycin, and treatment continued until an ANC greater than 0.5 X 10^9/L and 4 afebrile days (temperature < 37.5°C) had elapsed. The results of the study in the intent-to-treatment analysis showed that Filgrastim reduced the days of neutropenia (3.3 days v 4.3 days with ANC <0.5 X 10^9/L; P = .005), fever (4.1 v 5.1 days; P = .12), and febrile neutropenia (4.8 days v 6.3 days; P = .001). The mean number of days of hospitalization on study was reduced also from 10.0 days to 8.7 days. Filgrastim seemed more beneficial for the subset of patients with culture-confirmed or clinically documented infections than for patients with possible or unlikely infections. The relative risk of prolonged hospitalization for the Filgrastim group was half that of the placebo group.

In a recently reported double-blind study of the use of Filgrastim versus placebo in 186 pediatric patients for the treatment of febrile neutropenia, study drug was initiated within 24 hours of starting antibiotics and was discontinued.
upon withdrawal of antibiotics or when an ANC of 1.0 × 10^9/L was reached. Patients treated with Filgrastim had a more rapid neutrophil recovery to an ANC greater than 0.5 × 10^9/L (3 days vs 5 days; \( P < .05 \)), a higher median ANC at discharge (1.65 × 10^9/L vs 0.73 × 10^9/L; \( P < .005 \)), fewer full days of antibiotic therapy (5 days vs 6 days; \( P < .05 \)), and shorter hospital stays (5 days vs 7 days; \( P < .05 \)). There was no significant difference in duration of fever or of neutrophil recovery to 0.2 × 10^9/L between the two groups.

Dose-Intensified Chemotherapy

Importance of Dose Intensification

Clearly the major obstacle in the therapy of small-cell lung cancer and most other chemosensitive malignancies remains the development of cytotoxic drug resistance. Chemotherapy fails either because chemosensitive tumor cells remain after inadequate treatment or because resistant malignant clones predominate despite therapy. Adequate management of drug-related toxicity should ensure that chemotherapy regimens are administered at an effective dose, but avoiding or defeating drug resistance is proving a more difficult proposition.

Ongoing prospective studies are in progress to assess the impact of these high-dose therapies up-front as consolidation of first remission or good initial response in poor prognosis NHL, in germ-cell cancers refractory to cisplatin (or even up-front in poor-prognosis groups), and in breast cancer, as adjunct to primary therapy in patients with more than three positive axillary lymph nodes at diagnosis. Recently, the first report of a randomized clinical trial comparing the results of high-dose chemotherapy with standard-dose chemotherapy administered to women with measurable metastatic breast cancer. In this study, 90 women were randomized to receive either two courses of high-dose chemotherapy with stem-cell support or six to eight courses of standard-dose chemotherapy alone. The stem-cell support was either marrow (9 women) or double transplantation with PBPC (36 women), with the latter mobilized by chemotherapy and Filgrastim. The 45 patients treated with high-dose chemotherapy fared better than those treated with standard-dose chemotherapy, with complete response rates of 51% and 4%, respectively. The overall response rates were 96% and 53%, respectively. Patients who received high-dose therapy remained in remission longer and survived longer (90 weeks vs 45 weeks for median survival). Although this study was not large, it does suggest that high-dose chemotherapy is superior to standard-dose chemotherapy. Longer follow-up will be needed to quantify the effect that is suggested.

The use of Filgrastim to increase the intensity of doxorubicin treatment in patients with metastatic breast cancer by increasing the dose and reducing the interval between cycles resulted in a high response rate, but a disappointing duration of remission. However, Filgrastim-driven accelerated chemotherapies are being explored in other clinical settings.

Use of Filgrastim as Support of New Chemotherapeutic Agents

Various studies indicate that myeloid growth factors allowed intensified dosing or scheduling of promising new chemotherapeutic agents, such as paclitaxel (TAXOL), Topotecan, gallium, and Vinorelbine (Navelbine), and that using these agents at intensified doses may be of benefit. In an ECOG study, paclitaxel at 250 mg/m² (24-hour continuous infusion) was administered with Filgrastim on days 3 through 12 to 26 patients with transitional cell carcinoma of the urothelium, achieving a response rate of 42%. Similar results were seen in a study by Hutter et al and also in a study of 3-hour infusion of paclitaxel to patients with advanced, untreated malignancies. Link et al showed that paclitaxel dosing could be intensified with a flexible schedule of Filgrastim dosing. In this study, rather than reducing the dose of paclitaxel after a cycle complicated by febrile neutropenia, the dose of Filgrastim was increased. Only if a second episode of febrile neutropenia occurred was the dose of paclitaxel reduced. With the flexible dosing schedule, 81% of the cohorts treated in this manner were able to maintain chemotherapy at the targeted level. By increasing the dose of Filgrastim when indicated, patients at high risk did not develop recurring episodes of febrile neutropenia.

Another study in patients with urothelial cancer included a report with gallium nitrate and iofosfamide in which there was a 68% response rate. Filgrastim was able to support this highly myelosuppressive regimen. Irinotecan, a topoisomerase 1 inhibitor (ie, it causes single-strand breaks in the DNA of tumor cells), has been studied in patients with advanced lung cancer. Filgrastim was administered as support of the chemotherapy that also included etoposide, because both irinotecan and etoposide have known neutrophil toxicity. The combination of drugs appeared to be active against lung cancers, especially small-cell lung cancer. Diarrhea and leukopenia were the dose-limiting toxicities.

Some other studies using Filgrastim to support new, intense chemotherapeutic cytotoxic regimens are given in Table 2.

Bone Marrow Transplantation

Myeloablative chemotherapy requires cellular reconstitution and colony-stimulating factors alone are not sufficient to produce recovery. However, because prolonged neutropenia occurs after bone marrow transplantation, this was one of the first settings in which Filgrastim was studied.

An early study was performed by Taylor et al using Filgrastim administered by a 30-minute bolus infusion at a dose of 60 µg/kg/d beginning 24 hours after marrow infusion in 18 patients with Hodgkin’s disease. Recovery to ANCs of 0.1, 0.5, and 1.0 × 10^9/L occurred 4, 9, and 14 days faster, respectively, than in control patients.

Sheridan et al administered Filgrastim as a continuous subcutaneous infusion starting at 20 µg/kg/d 24 hours after autologous marrow infusion administered in support of chemotherapy to 15 patients with relapsed Hodgkin’s disease and NHL, germ cell tumors, or ALL. The median time to an ANC greater than 0.5 × 10^9/L was 11 days, compared with 20 days for historical control patients. There were significantly fewer days of parenteral antibiotic therapy (11 days vs 18 days) and less isolation in reverse-barrier nursing (10 days vs 18 days). There was no effect on platelet recovery, but there was a trend for fewer platelet transfusions in the
patients treated with Filgrastim. No significant toxicity was seen except for localized erythema at 2 of 88 infusion sites, and no significant difference was seen in the incidence of veno-occlusive disease of the liver or interstitial pneumonia.

In a prospective, randomized trial of high-dose chemotherapy and autologous transplantation, Filgrastim at 10 or 20 μg/kg/d reduced the median time to neutrophil recovery (10 days vs 18 days) compared with a control group not receiving Filgrastim. The median number of days with fever (1 day vs 4 days) and with neutropenic fever were also significantly reduced. Days on intravenous antibiotics and the duration of hospitalization were also shorter in the Filgrastim-treated group, although the two endpoints did not reach statistical significance.

Mobilization of PBPCs

High-dose chemotherapy has been an important addition to cancer treatment, but it causes severe pancytopenia and myelosuppression, increasing the risk of morbidity and/or mortality. Marrow transplantation is required after myeloblastic regimens to prevent permanent bone marrow hypoplasia, but even with myeloid growth factors, normalization of bone marrow function can be prolonged. Multilineage engraftment with PBPCs is generally more rapid than engraftment after marrow transplants, and PBPCs are easily collected by apheresis. In some situations, it is desirable to mobilize PBPCs by either chemotherapy plus colony-stimulating factors, or, preferably, by colony-stimulating factors alone, such as Filgrastim. Peripheral blood cell harvests may contain fewer occult tumor cells and may confer an advantage over marrow. In addition, it has been reported that a greater number of natural killer cells found in the blood may increase the functionality of T cells in PBPC grafts.

Mobilization in Normal Individuals

The kinetics of mobilization with Filgrastim in normal individuals shows that peak blood levels of clonogenic progenitor cells and CD34+ cells on days 5 or 6 of Filgrastim administration. There is interindividual variation in PBPC mobilization response to Filgrastim that may be partially explained by the age of the donors. In one study, there was an inverse correlation of peak CD34+ values with age seen in 10 volunteers aged 18 to 67 years, and a decline in mobilization response with age was also reported by Chatta et al. In this latter study, peripheral blood levels of GM-CFC were approximately 60% less in the elderly group compared with the young group (P = .03). Dose-response studies with normal volunteers have been limited and studies are not easily compared because of differences in leukapheresis techniques.

Mobilization in Cancer Patients

Dührsen et al were among the first to report the increases in various clonogenic hematopoietic progenitor cells on day 5 of Filgrastim treatment in patients with cancer (Fig 6). These results have been confirmed in subsequent studies, including those by Sheridan et al and de Luca et al, and follows a different kinetic profile compared with that of the neutrophilia induced by Filgrastim in normal volunteers. There is substantial interpatient variation in the peak levels of progenitor cells after Filgrastim treatment that has been confirmed to be related to the extent of previous chemotherapy or radiation therapy whose agents damage marrow function and reduce the number of stem cells (Table 3).

Use of Mobilized Progenitor Cells

Sheridan et al examined the ability of Filgrastim-mobilized PBPCs to reconstitute hematopoiesis in 17 patients with poor-prognosis, nonmyeloid malignancies. There were two historical control groups (one receiving marrow without cytokine support and the other receiving marrow with Filgrastim after transplantation) of patients with the same eligibility criteria treated in two previous studies; these control patients did not receive Filgrastim-mobilized PBPCs. After an initial apheresis performed without a mobilizing stimulus, Filgrastim at 12 μg/kg was administered as a continuous subcutaneous infusion for 6 days. Leukapheresis was performed again on days 5, 6, and 7 and at least 7 L was processed each time. The apheresis product was cryopre-
served, as was the marrow. Fourteen of the 17 patients received high-dose chemotherapy, and the cryopreserved materials were infused on day 0, followed by Filgrastim at 24 \( \mu g/kg/d \) starting on day 1. The Filgrastim therapy was tapered off over 6 days once the ANC was greater than 1.0 \( \times 10^9/L \).

Platelet recovery was significantly faster in Filgrastim-mobilized PBPC-treated patients than in historical-control patients, with the plateau count reaching 50 \( \times 10^9/L \) a median of 15 days after infusion of PBPCs, compared with 39 days in the control group \((P = .0006)\). The median number of platelet transfusions was 24 (range, 6 to 144) in the Filgrastim-treated group, 71 (12 to 314) for the first historical control group, and 85 (24 to 162) in the second historical control group. The accelerated platelet recovery decreased the transfusion requirements in patients receiving Filgrastim-mobilized PBPCs.

Bensinger et al\(^{[66]}\) also studied the ability of Filgrastim to mobilize PBPCs that could be used to repopulate the marrow of patients who had received myeloablative therapy. Twelve patients with various malignancies received Filgrastim at 16 \( \mu g/kg/d \) subcutaneously for 5 to 7 days, and cells were collected for 2 to 5 days beginning on day 4 after start of Filgrastim. One patient received Filgrastim at 5 \( \mu g/kg/d \) on days 0 through 13 after PBPC transfusion, but none of the other 11 patients received cytokines after infusion. The mean days to the recovery of ANCs to 0.1, 0.5, and 1.0 \( \times 10^9/L \) were 11.4 (range, 9 to 13), 12.7 (range, 10 to 15), and 13.6 (range, 11 to 16), respectively. The mean day to platelet transfusion independence was 13.3 (range, 7 to 49). The time to ANC of 0.5 or 1.0 \( \times 10^9/L \) and to a plateau count of 20 \( \times 10^9/L \) was more rapid than in historical control patients also treated with busulfan and cyclophosphamide who received marrow alone or followed by posttransplantation administration of cytokines (rHuG-CSF or rHuGM-CSF).

Chao et al\(^{[69]}\) studied engraftment rates using two different methods of PBPC mobilization and three different supportive care methods. Eighty-five patients with relapsed Hodgkin’s disease received autologous transplants. All patients received PBPCs with or without additional marrow infusions. Patients were assigned sequentially to group 1 (no growth factor during PBPC collection or infusion), group 2 (rHuGM-CSF after infusion), group 3 (Filgrastim after infusion), or group 4 (Filgrastim to mobilize and after infusion). Aphereses were performed daily until a target of 10\(^6\) mononuclear cells/kg was collected. Patients in group 4 received Filgrastim at 10 \( \mu g/kg/d \) intravenously or subcutaneously 4 days before apheresis commenced and continued until the collection was completed.

The median number of aphereses required to obtain the target number of cells was nine, eight, eight, and four in group 1 (no growth factor), group 2 (rHuGM-CSF posttransplant), group 3 (Filgrastim posttransplant), and group 4 (Filgrastim for mobilization and posttransplant), respectively. Time to myeloid engraftment was statistically significantly faster in patients in group 4 compared with groups 2 and 3 (10 days vs 12 days and 12 days; \( P < .01 \)), and patients in group 4 also engrafted within a narrower span of time. There was a statistically significant difference \((P < .001)\) in time to a stable platelet recovery defined as a platelet count greater than 20 \( \times 10^9/L \) without the need for further platelet transfusions. Platelet recovery in group 4 was a median of 13 days compared with medians of 18 to 33 days in the other groups.

A recently published European study showed the benefits of PBPCs mobilized with Filgrastim compared with autologous marrow after high-dose chemotherapy.\(^{[48]}\) Fifty-eight heavily pretreated patients with advanced Hodgkin’s disease or high-grade NHL were randomized to receive either mobilized PBPCs \((n = 27)\) or autologous marrow \((n = 31)\). All patients received Filgrastim after stem cell support. The patients who received Filgrastim-mobilized PBPCs had fewer days with platelet transfusions (6 days vs 10 days), and the time to platelet recovery \((=20 \times 10^9/L)\) was also less (16 days vs 23 days, \( P = .02 \); Fig 7). The time to an ANC greater than 0.5 \( \times 10^9/L \) was also reduced (11 days vs 14 days, \( P = .005 \)), as was time in the hospital.

### Role of Filgrastim After Infusion of Filgrastim-Mobilized PBPCs

Once it was established that PBPCs were capable of restoring hematopoiesis after myeloablative chemotherapy, studies were performed to evaluate the clinical benefit of the use of growth factors after infusion of growth-factor–mobilized PBPCs. In one study, 37 patients received both Filgrastim and rHuG-CSF for mobilization.\(^{[69]}\) One day after infusion of PBPCs and marrow, patients were randomized to receive no growth factor or a combination of Filgrastim and rHuG-CSF until neutrophil recovery. Patients receiving posttransplant growth factor reached an ANC of 0.5 \( \times 10^9/L \) in 10 days, compared with 16 days for patients who did not receive factors \((P = .0004)\). In addition, the duration of hospitalization was shorter in patients receiving growth factors (19 days vs 21 days; \( P = .0112 \)). The use of growth factors post-PBPC transplantation seemed to have a modest clinical benefit in this study.

In a slightly different study design, 41 patients received Filgrastim-mobilized PBPCs with or without marrow and were then randomized to receive Filgrastim posttransplantation or not.\(^{[90]}\) The median time to an ANC of 0.5 \( \times 10^9/L \) was faster in the Filgrastim-treated patients (10.5 days vs 16 days; \( P = .0001 \)), and Filgrastim was associated with a statistically significant reduction in neutrophil engraftment among patients who received PBPCs without marrow (11 days vs 17 days; \( P = .0003 \)) as well as in those receiving marrow also (10 days vs 14 days; \( P = .02 \)). There were statistically significant decreases in days of hospitalization and days of intravenous antibiotic therapy.

Recently, a report was published discussing the use of Filgrastim after PBPC transplantation.\(^{[90]}\) A total of 692 patients receiving high-dose chemotherapy were analyzed for engraftment kinetics as related to CD34 content. Patients had PBPCs collected after cyclophosphamide-based mobilization chemotherapy with or without Filgrastim, until \(=2.5 \times 10^6\) CD34\(^+\) cells were collected. After high-dose chemotherapy, the PBPCs were infused. Patients enrolled later in the study received posttransfusion Filgrastim, and this was associated with a faster neutrophil recovery \((P = .0001; \text{Table 4})\).
Future Direction for Cell Therapy

Brugger et al. have explored the use of autologous PBPCs generated ex vivo to restore hematopoiesis in patients who have had high-dose chemotherapy for the treatment of cancer. The CD34+ cells collected from Filgrastim-treated patients were grown in a medium containing autologous plasma and several recombinant human hematopoietic growth factors. No toxic effects were observed with the infusion of the ex vivo-expanded cells, and they promoted a rapid and sustained hematopoietic recovery. In this study, the starting number of CD34+ cells was $1 \times 10^6$, less than 10% of the usual starting population for cell growth.

Bensinger et al. studied the feasibility of using Filgrastim to mobilize granulocytes in normal donors. Eight normal marrow-donor adults volunteered to be granulocyte donors after administration of Filgrastim. Immediately after marrow donation, the seven volunteers received Filgrastim at 3.5 to 6 µg/kg/d for a mean of 11.5 days (range, 9 to 14 days), and granulocytes were collected by leukapheresis. This study showed that Filgrastim was safe to administer to normal adults, that it significantly improved the quantity of granulocytes collected, and that these granulocyte transfusions resulted in significant circulating levels of granulocytes in the neutropenic recipients.

MDS and AA

MDS are hematologic disorders characterized by dysplastic changes in at least two hematopoietic lineages and chronic cytopenia. Patients may have a relatively indolent course characterized by recurrent infections, requirement for red blood cell and/or platelet transfusions, and an increased risk of transformation to AML. Early in the development of rHuG-CSF, Negrin et al. and Kobayashi et al. conducted studies in small numbers of patients with predominantly advanced MDS (refractory anemia with an excess of blasts [RAEB] and refractory anemia with excess of blasts in transformation [RAEB-t]) to determine if rHuG-CSF could improve neutropenia and the complications related to this particular cytopenia. In these initial clinical trials, all patients experienced an improvement in their ANCs. In one study, 5 of 13 patients had a twofold increase in the reticulocyte counts, and in 2 of 9 patients who were red blood cell transfusion-dependent, administration of rHuG-CSF decreased red blood cell transfusion requirements.
A phase 3, international, multicenter, randomized clinical trial was then conducted to evaluate the long-term effects of Filgrastim on the natural history of advanced MDS in patients classified as RAEB or RAEB-t. Patients were randomized to receive either Filgrastim or the best supportive care until progression of their disease. A primary goal of this study was to determine if maturation induction with Filgrastim could decrease the self-renewal of the malignant clone and, thereby, reduce the incidence of conversion of MDS to AML. One hundred two patients were randomized and a neutrophil response was observed in all Filgrastim-treated patients within 2 to 3 weeks. The incidence of progression to AML for RAEB and RAEB-t patients was 14% and 60%, respectively, in the Filgrastim-treated group, and 19% and 60% in the observation group. The median time to progress to AML was 16 months in the observation group and had not been reached at the time of the initial report in the Filgrastim-treated group. For high-risk RAEB patients, the median survival was shorter in the Filgrastim-treated group compared with the observation group, with an increase in nonleukemic, disease-related deaths. Survival for RAEB-t patients was similar in both treatment groups. The reason for this difference in survival in the RAEB patients remains unexplained and is being studied further. The clinical benefit of Filgrastim has not been proven in the treatment of patients with advanced MDS. Whether Filgrastim might be useful in the setting of an acute episode of febrile neutropenia in patients with MDS has not yet been studied.
AA is a progressive hematologic disease that can cause neutropenia. Use of hematopoietic growth factors has been shown to increase neutrophil counts in some patients with moderate AA, but, in general, patients with very severe hypoplasia do not respond well to any growth factor. Patients with AA are a heterogeneous group, confounding interpretation of results of a clinical trial.

In a Japanese study with 20 children with severe or intermediate AA, Filgrastim at 400 μg/m²/d increased the neutrophil counts of 12 patients. Increasing the doses to as much as 1,200 μg/m²/d in five patients who did not respond to the initial dose produced an increase in neutrophils in three of them. In a later study, this group of investigators administered high doses of Filgrastim (400 to 2,000 μg/m²/d) to 10 children with severe AA and ANC less than 0.05 x 10⁹/L. In 6 of the 10 children, ANC were increased 10- to 60-fold.

SCN

SCN includes patients diagnosed with neutropenia at birth or shortly thereafter, which is generally accompanied by frequent and severe infections (for reviews see Welte and Dale). The estimated frequency of these conditions is approximately 1 to 2 cases per million population. In cyclic neutropenia, patients typically have 21-day oscillations of blood neutrophil counts, with the levels fluctuating between the lower limit of normal and 0. During the periods of severe neutropenia, the patients are prone to severe infections, but otherwise are relatively well. The category of idiopathic neutropenia includes patients without evidence for congenital, neoplastic, or immunologic causes for neutropenia. The clinical problems of patients with idiopathic neutropenia tend to vary considerably, but, in general, the patients with the more severe neutropenia have the greater frequency of fever episodes and infections.

**Therapy**

SCN is often recognized when patients present with infections and are found to have a low blood neutrophil count. Antibiotic therapy for these infections follows the same principles as for other patients, ie, broad-spectrum antibiotics for severe infections until the pathogen is identified, targeted therapy for specific pathogens, and administration of antibiotics until the signs and symptoms of infection have cleared. The use of prophylactic antibiotics (eg, trimethoprim-sulfamethoxazole) for these patients is common. Isolation of patients in their homes, away from crowds, or in the hospital in general has not proven to be of much value. However, the most promising therapy is continuous daily treatment with Filgrastim.

Seventy-five patients with the confirmed diagnosis of SCN (congenital, cyclic, or idiopathic neutropenia) were originally enrolled in phase 1 to 2 trials with Filgrastim.

Initially, severe congenital neutropenic patients started Filgrastim at 3 μg/kg/d and the dosage was adjusted accordingly to achieve a complete response, except as otherwise indicated. In greater than 95% of the patients with severe congenital neutropenia, all patients with cyclic neutropenia, and all patients with idiopathic neutropenia, Filgrastim in-
duced an increase of the median ANC to greater than $1.0 \times 10^9/L$. The subcutaneous dose of Filgrastim necessary to reach and maintain this ANC varied from patient to patient and ranged between 0.8 and $70 \\mu g/kg/d$. In the majority of patients with severe congenital neutropenia who were complete responders, the bacterial infections and the requirement for intravenous antibiotic treatment were significantly less in the maintenance treatment period compared with the pretreatment or dose-finding period.

One hundred twenty-three patients (60 congenital, 21 cyclic, and 42 idiopathic neutropenia) with recurrent infections and severe chronic neutropenia ($ANC < 0.5 \times 10^9/L$) were enrolled in a multicenter phase 3 trial. Patients were randomized to either immediately beginning Filgrastim (3.45 to 11.5 $\mu g/kg/d$) subcutaneously or entering a 4-month observation period followed by Filgrastim administration. Blood neutrophil counts, marrow-cell histology, and incidence and duration of infection-related events were monitored. Of the 123 patients enrolled, 120 received Filgrastim. On therapy, 108 patients had a median ANC $> 1.5 \times 10^9/L$. Examination of marrow aspirates showed increased proportions of maturing neutrophils. Infection-related events were significantly decreased ($P < .05$) with approximately 50% reduction in the incidence and duration of infection-related events and almost 70% reduction in duration of antibiotic use. This study also indicated that treatment of patients with SCN with Filgrastim results in a stimulation of marrow production and maturation of neutrophils, an increase in circulating neutrophils, and a reduction in infection-related events.

The patients in the phase 1/2/3 studies were entered into a long-term maintenance treatment phase once a response to Filgrastim was documented. In these patients treated for as long as 8 years, the median Filgrastim dose (in micrograms per kilogram per day) used to maintain an ANC greater than $1.0 \times 10^9/L$ varied by diagnosis: congenital, 12 $\mu g/kg/d$ (range, 0.8 to 72.0 $\mu g/kg/d$); cyclic, 3.6 $\mu g/kg/d$ (range, 0.06 to 12.0 $\mu g/kg/d$); and idiopathic, 1.5 $\mu g/kg/d$ (range, 0.1 to 28.0 $\mu g/kg/d$). The general improvement noted in these trials of Filgrastim for SCN was sustained with long-term therapy (Fig 8).

Microbiologically documented infections were less frequent during this long-term treatment. Using intravenous antibiotic therapy as a proxy for suspected serious infections, these types of infections diminished significantly with Filgrastim therapy. During maintenance treatment, most patients did not require intravenous antibiotic treatment again. Long-term treatment improved dramatically the quality of life of chronic neutropenia patients as judged by significantly less hospitalization, more normal daily life activities, and normal growth and development. This long-term treatment trial has also shown that Filgrastim, administered daily for years (up to 8 years), does not lead to an exhaustion of myelopoiesis and does not lead to generation of anti-G-CSF or antineutrophil antibodies (Fig 8).

### Leukemic Transformations in Patients With SCN

Worldwide phase 1/2/3 clinical trials with Filgrastim and the Severe Chronic Neutropenia International Registry have provided information on 420 patients. Before the era of growth factors, reports describe patients with congenital neutropenia who developed AML, suggesting that congenital neutropenia may be a premalignant condition. Within the 420 patients, only in the cohort of 220 patients with congenital neutropenia treated with Filgrastim for as many as 8 years have 16 cases of AML or MDS been reported. None of the patients with cyclic or idiopathic neutropenia developed AML/MDS, and no relationship was seen with dose or duration of dosing with Filgrastim. Intriguingly, all patients tested with severe congenital neutropenia associated with progression towards MDS and AML ($n = 6$) show G-CSF receptor mutations. These receptor mutations in patients with AML and MDS, present in cells of the myeloid lineage only, were nonsense mutations leading to truncation of the C-terminal cytoplasmic region crucial for maturation signaling. In these patients with MDS or AML secondary to severe congenital neutropenia, both the mutated and the normal alleles of the G-CSF receptor were expressed. Additionally,

### Table 4. Use of Filgrastim After PBPC Infusion

<table>
<thead>
<tr>
<th>n</th>
<th>Method of Mobilization</th>
<th>Filgrastim Postinfusion</th>
<th>Median Days to ANC $&lt; 0.5 \times 10^9/L$ (range)</th>
<th>Median Days to ANC $&gt; 20 \times 10^9/L$ (range)</th>
<th>Median Days in Hospital (range)</th>
<th>Median Days on IV Antibiotics (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>405</td>
<td>Chemotherapy Filgrastim</td>
<td>Yes</td>
<td>10 (5-51)</td>
<td>9 (4-46+)</td>
<td>12 (0-34)</td>
<td>9 (0-31)</td>
</tr>
<tr>
<td>27</td>
<td>Chemotherapy</td>
<td>Yes</td>
<td>10 (7-23+)</td>
<td>10 (0-50+)</td>
<td>12 (8-53)</td>
<td>9 (1-31)</td>
</tr>
<tr>
<td>18</td>
<td>Chemotherapy</td>
<td>No</td>
<td>13 (9-19)</td>
<td>9 (6-16)</td>
<td>12 (10-28)</td>
<td>8 (3-20)</td>
</tr>
</tbody>
</table>

Abbreviations: Plts, platelets; IV, intravenous.
40 patients with severe congenital neutropenia were tested and found to have no G-CSF receptor mutations. None of these 40 patients without G-CSF receptor mutation showed cytogenetic or clinical signs of progression to MDS or AML. These findings support the notion that mutations in the G-CSF receptor gene, resulting in the truncation of the C-terminal maturation domain, are associated with progression from SCN to MDS/AML.\(^9\),\(^9\),\(^1\)\(^0\),\(^1\)\(^1\) Mechanisms other than G-CSF receptor mutations have also been associated with leukemic transformation with congenital neutropenia. Kalra et al\(^1\)\(^1\)\(^1\) reported 13 cases with leukemic transformation. Ten of these showed monosomy 7; five patients had activating Ras mutations, four of whom also had monosomy 7. In contrast to the G-CSF receptor mutations, monosomy 7 and Ras mutations were detected at the stage of leukemic transformation, but not during the neutropenic phase of the disease.

Use of Filgrastim in Acquired Immune Deficiency Syndrome (AIDS)

Leukopenia with neutropenia and lymphocytopenia is a major problem in patients infected with the human immunodeficiency virus (HIV) and is caused not only by the virus itself, but also by the various cytotoxic agents used to treat AIDS and associated complications such as opportunistic infections. AIDS is a difficult disease to study. The HIV regulates its own expression and has immense capacity for replication and mutation. The course of the infection is determined by the specific immune response of the host, including cellular activation, cytokine secretion, and immunopathogenicity.

Neutrophils are important effector cells in the host defense against pyrogenic bacteria in patients with HIV infection. A study was performed to examine the expression of neutrophil surface markers associated with neutrophil function, activation, and/or differentiation in patients with AIDS.\(^3\)\(^1\)\(^2\) These patients showed a significant increase in the expression of CD66, a marker of neutrophil activation, as well as a minor increase in the expression of CD67, another marker of neutrophil activation. There were decreases in expression of CD16. These changes may be associated with abnormalities of neutrophil function resulting in an increased risk of bacterial infections observed in HIV infection.

Neutrophil superoxide production was evaluated in 71 patients with HIV infection at different stages of the disease.\(^1\)\(^3\) Antiviral therapy with zidovudine (AZT) was associated with impaired superoxide production, but may have been also associated with the longer duration of disease for treated patients. AZT had no direct inhibitory effect on superoxide production in neutrophils in vitro. Impaired neutrophil oxidative metabolism may contribute to the increased risk of serious bacterial infections, certain opportunistic infections, and perhaps to the pathogenesis of HIV infection itself.

Antiviral therapy with zidovudine (AZT) was associated with impaired superoxide production, but may have been also associated with the longer duration of disease for treated patients. AZT had no direct inhibitory effect on superoxide production in neutrophils in vitro. Impaired neutrophil oxidative metabolism may contribute to the increased risk of serious bacterial infections, certain opportunistic infections, and perhaps to the pathogenesis of HIV infection itself.

A nonrandomized study of Filgrastim combined with recombinant human erythropoietin (EPOGEN; rHuEPO) showed that, even at low doses, it can ameliorate the neutropenia associated with AZT\(^1\)\(^4\) (Fig 9). For most patients, doses of 0.3 to 1.2 \(\mu g/kg\) were required to maintain a target ANC. Filgrastim has been shown to improve the tolerance to ganciclovir (used to treat cytomegalovirus infection), allowing delivery of full doses, important in treating cytomegalovirus infections.\(^1\)\(^5\)

An open-label, noncomparative study examined the efficacy and safety of Filgrastim for treating neutropenia in patients with AIDS.\(^1\)\(^6\) Twenty patients with neutropenia of any cause (except chemotherapy) were treated with adjusting doses of Filgrastim to achieve and maintain ANCs of 2 to 5 \(\times 10^3/mL\). The median time to reversal of neutropenia was 3 days (range, 2 to 9) at a median dose of 1 \(\mu g/kg/d\) (range, 0.8 to 3 \(\mu g/kg/d\)). The Filgrastim was well tolerated and showed no effect on virologic markers.

In another small study, 10 consecutive patients at risk of developing severe neutropenia because of the use of antiviral drugs received 5 \(\mu g/kg/week\) in two divided doses for 6 months.\(^1\)\(^7\) Even at low intermittent doses, Filgrastim was able to prevent severe neutropenia and neutropenic fever. Patients receiving cytotoxic chemotherapy as palliative therapy for HIV-related NHL were treated with Filgrastim to determine the maximum tolerated dose of chemotherapy (cyclophosphamide, epirubicin, vincristine, and prednisolone).\(^1\)\(^8\) Filgrastim was able to support intensification of cyclophosphamide and epirubicin in this regimen with acceptable hematologic toxicities.

Some other studies with rHuG-CSF in patients with AIDS are given in Table 5.

Use of Filgrastim in Non-Neutropenic Settings

Filgrastim has potential in the setting of non-neutropenic diseases, including liver transplant\(^1\)\(^9\) or fungal infections.\(^1\)\(^2\),\(^1\)\(^2\)

In a single-blind pilot study, 11 volunteers were treated with Filgrastim or saline, and blood samples were collected at different time points to assess cytokine release.\(^1\)\(^2\) Twenty hours after administration, Filgrastim had reduced lipopoly-saccharide-inducible tumor necrosis-\(\alpha\) production by 40%. In a second, double-blind, randomized, controlled study with 21 volunteers,\(^1\)\(^2\) patients received Filgrastim and/or placebo for 3 days and blood samples were taken. Filgrastim treat-
ment resulted in a significant increase in neutrophil counts, as would be expected. Blood sampled 24 hours after administration was incubated with various stimuli, including muramyldipeptide, lipoteichoic acid, steptolysin O, phorbol ester, and phytohemagglutinin. All these stimuli diminished the release of free tumor necrosis factor-α (TNF-α) into the blood. It was suggested that the counterregulation by Filgrastim against overactivation of the host defense to a widespread spectrum of bacteria may indicate that Filgrastim has potential in the prevention of infection in patients in whom sepsis is anticipated.

Sepsis and septic shock are serious diseases and are considered to be the leading causes of death in intensive care units, with mortality at 35% to 50%. A number of factors contribute to the increased incidence of sepsis and septic shock: more aggressive and comprehensive use of immunosuppressive therapies, invasive devices needed to deliver these agents, an aging population, and a worldwide increase in the frequency of antibiotic-resistant organisms. In 1991, the American College of Chest Physicians agreed to define sepsis as a subset of a systemic inflammatory response syndrome brought about by a variety of insults and mediated by a number of cytokines. The definition includes some of the classic definitions of sepsis: increased body temperature, rapid heartbeat, accelerated respiratory rate, and elevated neutrophil count.

Controversy exists concerning the role of neutrophils as an agent of injury in adult respiratory distress syndrome (ARDS). Studies have shown that neutrophils collected from the peripheral blood of patients with ARDS show evidence of preexisting activation. The cells have enhanced chemotaxis and can generate abnormally high levels of oxygen metabolites after in vitro stimulation. If patients with mild lung injury also have diseases that reduce the blood neutrophil count, lung impairment frequently worsens if circulating neutrophil counts return to normal values. However, a recent study reported in abstract form suggests that neutrophils produced in response to Filgrastim may in fact reduce the incidence of ARDS. Seven hundred fifty-six patients were enrolled in a multicenter, double-blind, placebo-controlled trial of Filgrastim administered to hospitalized patients with community-acquired pneumonia (Nelson et al, manuscript in preparation). The median age of patients was 68 years in both groups; a respiratory pathogen was isolated from 55% of the Filgrastim-treated and 60% of the placebo-treated patients. Patients with pneumonia are at risk for developing serious and life-threatening complications such as septic shock, empyema, and end-organ failures (eg, ARDS or disseminated intravascular coagulation [DIC]). Compared with placebo, patients treated with Filgrastim had a lower incidence of these conditions. They also had a higher incidence rate of resolved infiltrates on chest radiograph by day 28 (P = .005) and a shorter time to resolution of the infiltrate (P = .001).

Sepsis remains a leading cause of morbidity and mortality in the neonate despite major advances in neonatal care. Filgrastim has been shown to stimulate the proliferation and differentiation of neonatal neutrophil progenitor cells into mature neutrophils. In preliminary clinical trials with neonates, Filgrastim significantly increased circulating neutrophil counts. Forty-two babies (<72 hours old and weighing >800 g) with a diagnosis of presumed sepsis were randomized to receive placebo; 1, 5, or 15 µg/kg/d Filgrastim; or 5 or 10 µg/kg Filgrastim every 12 hours. Filgrastim increased the ANC in the circulation and in the bone marrow, and at a dose of 10 µg/kg every 24 hours, C3bi expression was increased. Similar results were obtained from another study of 12 neonates. Neutrophils and monocytes increased after treatment with Filgrastim at 5 µg/kg/d (median, 4 days; range, 1 to 8 days [P < .01 for both]). The platelet count decreased in all neonates, but thrombocytopenia occurs in approximately 50% of neonates and was not unexpected.

Recently, the use of Filgrastim has been reported to have favorable effects on sepsis and rejection in patients receiving liver allografts. The effects of Filgrastim administration on the number of circulating neutrophils, the incidence of septic complications, the incidence and severity of rejection complications, and graft and recipient survival were investigated in 37 liver allograft patients. Filgrastim was administered for the first 7 to 10 days after transplantation, could be discontinued if the ANC reached 20 × 10^9/L, and could be restarted if the count was less than 10 × 10^9/L. The results of this study were compared with results obtained from 49 previous liver allograft patients who were not treated with Filgrastim. The Filgrastim-treated patients had significant increases in their granulocyte counts and a decreased number of sepsis episodes per patient. They also had a lower percent-

### Table 5. Literature Indicating Benefits of rHuG-CSF in Patients With AIDS

<table>
<thead>
<tr>
<th>Condition Treated</th>
<th>No. of Patients</th>
<th>Key Clinical Outcomes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZT-induced neutropenia</td>
<td>19*</td>
<td>Increase in WBC and ANC</td>
<td>154</td>
</tr>
<tr>
<td>Neutropenia/anemia</td>
<td>22</td>
<td>Increase in BFU-E before EPO administered</td>
<td>155</td>
</tr>
<tr>
<td>AZT-induced neutropenia</td>
<td>11</td>
<td>Increased ANC</td>
<td>156</td>
</tr>
<tr>
<td>AZT-induced neutropenia</td>
<td>11</td>
<td>Increased ANC</td>
<td>157</td>
</tr>
<tr>
<td>Granulocytopenia</td>
<td>16†</td>
<td>Increased ANC in all 16, decrease, then increase in platelets</td>
<td>158</td>
</tr>
<tr>
<td>Granulocytopenia</td>
<td>14</td>
<td>Increased ANC immediately in all but 1 patient</td>
<td>159</td>
</tr>
<tr>
<td>KS/Pulmonary involvement</td>
<td>18</td>
<td>Some PR/CR; survival increased</td>
<td>160</td>
</tr>
</tbody>
</table>

Abbreviations: BFU-E, burst-forming unit-erythroid; CR, complete response; KS, Kaposi's sarcoma; PR, partial response; WBC, white blood cell count.

* Includes pediatric patients.
† Sixteen patients randomized from 32 charts.
age of sepsis-related deaths (8% ± 22%), and the incidence of acute rejection was also decreased (22% ± 57%). The decreased rate of organ rejection may be due to some antirejection mechanism of Filgrastim, but a multicenter, randomized trial would be needed to confirm if Filgrastim is of benefit in this setting.

Other Neutropenic Conditions

Many drugs predictably cause neutropenia, including antineoplastic agents, but other drugs can cause neutropenia with unpredictable frequency (eg, phenothiazines, aminopyrine, and semisynthetic penicillins). Filgrastim has been used extensively to hasten neutrophil recovery in these settings. Table 6 lists some publications with rHuG-CSF in the drug-induced agranulocytosis setting. Because of the nature of the condition, most reports are of individual cases, and controlled clinical studies in this indication have not been published (see review by Sprinkelman et al).

Neutropenia is sometimes associated with rheumatoid arthritis as part of the Felty syndrome and systemic lupus erythematosus. The neutropenia of Felty syndrome has been attributed to several mechanisms, including serum inhibitory factor against myeloid precursor cells, antigranulocyte antibodies, and circulating immune complexes. The role of Filgrastim in Felty syndrome is not completely defined. Some patients have neutrophil responses, although in some patients, there is exacerbation of arthritis during neutrophil recovery. Table 6 also lists some studies of rHuG-CSF in patients with Felty syndrome or lupus.

SAFETY OF FILGRASTIM

In all studies, the safety profile and patient tolerance of Filgrastim have been very good, with mediulary bone pain (seen in approximately 10% to 20% of the patients treated by the conventional subcutaneous route) as the only clinically significant and relatively frequent side effect. Safety data are available from several clinical settings including standard-dose chemotherapy, high-dose therapy with or without marrow or PBPC support, and SCN.131,132

In all studies of Filgrastim, overall events attributed to Filgrastim were infrequent. Local reactions (pain, swelling, and redness at the injection site) were very rarely reported, and fever and chills were not observed. The laboratory changes noted included increases in alkaline phosphatase, leukocyte alkaline phosphatase, and uric acid and were sustained so long as treatment was continued. In addition, the label contains a precaution that safety and efficacy has not been stipulated when Filgrastim is administered simultaneously with antimetabolites (eg, 5-fluorouracil), nitosoureas, or mitomycin C, or with concomitant radiotherapy.

In the SCN patient population, spleen volume as measured by computed tomography/magnetic resonance imaging tended to increase early in Filgrastim treatment and then for most patients decreased toward baseline values.106

In normal donor studies, the side effects of Filgrastim reported have been limited to musculoskeletal pain. Normal volunteers given a short duration of rHuG-CSF 5 years ago have not shown any evidence of abnormal bone marrow findings.133

COST EFFECTIVENESS AND QUALITY OF LIFE

Current cost-effectiveness analyses, performed both in Europe and the United States, suggest that prophylactic therapy of patients with chemotherapy-induced febrile neutropenia with recombinant colony-stimulating factors may not be justified if the expected risk of neutropenic fever is less than 20%, but can be justified if it is greater than 40%.134 One of the problems is that these risks are often poorly recorded in the literature and are naturally difficult to assess for an individual patient. Moreover, some chemotherapy regimens commonly used for, eg, small-cell lung cancer, are associated with a sufficient risk of febrile neutropenia to warrant the use of prophylactic Filgrastim, whereas many other probably equally effective regimens in this disease are less toxic.135 Older patients with impaired marrow reserve (due to previous chemotherapy or radiotherapy or to heavy involvement by tumor) or patients with significant immunodepression (eg, AIDS patients) should also be considered as candidates for prophylactic Filgrastim use.130 Outside high-dose chemotherapy protocols, the routine prophylactic use of recombinant

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Table 6. Literature Indicating Benefits of rHuG-CSF in Patients With Drug-Induced Agranulocytosis and Idiopathic Neutropenia

<table>
<thead>
<tr>
<th>Cause of Neutropenia</th>
<th>No. of Patients</th>
<th>Key Clinical Outcomes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpromazine and antibiotics</td>
<td>10</td>
<td>Increase in ANC</td>
<td>161</td>
</tr>
<tr>
<td>Clomipramine</td>
<td>3</td>
<td>ANC recovery after 3 to 7 days</td>
<td>163</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>22</td>
<td>Increase in ANC</td>
<td>164</td>
</tr>
<tr>
<td>Methimazole</td>
<td>1</td>
<td>Increase in ANC</td>
<td>165</td>
</tr>
<tr>
<td>Clozapine</td>
<td>1</td>
<td>Increase in ANC</td>
<td>166</td>
</tr>
<tr>
<td>Ticlopidine</td>
<td>3</td>
<td>Increase in ANC</td>
<td>170</td>
</tr>
<tr>
<td>Felty syndrome</td>
<td>1</td>
<td>Increase in ANC; improvement in maturational arrest in bone marrow</td>
<td>171</td>
</tr>
<tr>
<td>Felty syndrome/chronic</td>
<td>1</td>
<td>Prolonged use reduced infectious episodes healing of wound and increase in ANC</td>
<td>172</td>
</tr>
<tr>
<td>Systemic lupus erythematousus</td>
<td>1</td>
<td>Increase in WBC and production of hypercellular marrow</td>
<td>174</td>
</tr>
</tbody>
</table>

Abbreviation: WBC, white blood cell.
Filgrastim has specific and selective actions due to the colony-stimulating factors. It stimulates the proliferation, restricted expression of G-CSF receptors on both myeloid precursor and mature cells. It stimulates the proliferation, differentiation, and activation of cells of the neutrophil lineage and reduces neutrophil maturation time.

In the clinical setting, Filgrastim plus Filgrastim-mobilized PBPCs allow the benefits of chemotherapy dose intensity to be studied, and early randomized studies suggest that myeloablative therapy is of benefit to patients with acute leukemia, lymphoma, or metastatic breast cancer. The use of Filgrastim has been shown to reduce morbidity and provide cost offsets by a reduction in hospitalization in patients with other diseases. In the SCN setting, Filgrastim decreases morbidity due to infections; in patients with AIDS, it decreases the morbidity associated with both the disease and with antiviral drugs. Filgrastim has been shown to be safe in the infectious disease setting, and interesting phase 2 data are now being reported. Table 7 identifies the approved indications for Filgrastim in the United States and Europe. Filgrastim is a very promising agent with much more development and potential yet to be realized.

**ACKNOWLEDGMENT**

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10 YEARS OF FILGRASTIM (r-METHUG-CSF)


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Filgrastim (r-metHuG-CSF): the first 10 years
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