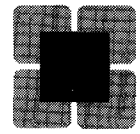

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The use of G-CSF and GM-CSF in bone marrow transplantation

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Bone marrow transplantation is accepted as potentially curative therapy for a variety of patients with hematologic malignancies and other disorders. The most important causes of morbidity are infections and bleeding secondary to prolonged cytopenias. Granulocyte colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) have been shown to potentially enhance bone marrow engraftment which has translated into reduced morbidity and mortality. Additionally, growth factors such as G-CSF and GM-CSF may increase numbers of circulating peripheral progenitor cells to serve as the source of "marrow" for transplantation. This review summarizes the current available data using G-CSF and GM-CSF in bone marrow transplantation and discusses potential areas of study with additional cytokines.

INDEX TERMS: GRANULOCYTE COLONY-STIMULATING FACTOR; GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR; BONE MARROW TRANSPLANTATION
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KEY TO ABBREVIATIONS: **BMT**= BONE MARROW TRANSPLANTATION; **CFU-GM**= GRANULOCYTE-MACROPHAGE COLONY FORMING UNITS; **G-CSF**= GRANULOCYTE COLONY-STIMULATING FACTOR; **GM-CSF** = GRANULOCYTE-MACROHPAGE COLONY-STIMULATING FACTOR; **HLA**= HUMAN LEUKOCYTE ANTIGEN; **IL**=INTERLEUKIN; **PBPC**= PERIPHERAL BLOOD PROGENITOR CELLS

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BONE MARROW transplantation (BMT) is widely accepted as the treatment of choice for selected patients with leukemia, lymphoma, and a variety of other oncologic and hematologic disorders. Many study groups are searching for ways to reduce treatment-related morbidity and mortality and boost cure rates. Growth factors used in conjunction with BMT have shown promise in a number of studies. This article reviews the most recent data on the use of growth factors in BMT.

RATIONALE FOR BMT

The use of BMT in antitumor therapy is based on three assumptions: (1) sufficient doses of chemotherapy, radiation therapy, or both can totally eradicate a given tumor; (2) the dose of antitumor agents is largely limited by their toxicity to the patient's normal bone marrow; (3) if normal bone marrow is available for transplantation, higher and potentially curative antitumor doses of chemotherapy, radiation therapy, or both can be administered, and the donor marrow can save the patient from iatrogenic

TABLE 1
CLINICAL TRIALS OF GM-CSF* IN AUTOLOGOUS BONE MARROW TRANSPLANTATION

Author (reference)	Number of patients	GM-CSF dose	Marrow dose (nucleated cells x 10^8/kg)	Median days to ANC† >500/μL (historical controls)	Deaths (historical controls)	LOS in days (historical controls)	Comment
Brandt (7)	19	2 to 32 μg/kg/day Continuous infusion for 14 days	0.54 x 10^8	14 (19)	11% (21%)		Most patients had fall in ANC when GM-CSF stopped
Devereaux (8)	12	100 to 400 μg/m^2/day Continuous infusion for 3 to 21 days	1.88 x10^8	16 (25)	17% (5%)	30 (30)	
Nemunaitis (9)	15	15 to 240 μg/m^2/day	—	14 (25)	7%	29 (41)	GM-CSF well tolerated at doses up to 240 μg/m^2
Blazer (10)	25	16 to 256 μg/m^2/day	0.2 to 0.99 x 10^8	23 (24)	16% (19%)		Responders had increased progenitors infused
Link (11)	9	500 μg/m^2/day Continuous infusion for 28 days	—	15 (23)	—		
Lazarus (12)	16	11 μg/kg/day	2.5 x 10^8	14 (20)‡	—	37	Remission and relapse rates unchanged with GM-CSF

*Granulocyte-macrophage colony-stimulating factor
†Absolute neutrophil count
‡$P = .0002$

death. Thus, the basic premise of BMT is that it allows for maximal dose-intensity of antitumor agents.

Typically, BMT has three major components: (1) treatment with high doses of chemotherapy or radiation therapy (the preparative regimen) or both, which, it is hoped, eradicates the patient's underlying disorder; (2) infusion of a matched (or partially matched) donor's marrow (allogeneic BMT) or infusion of the patient's previously harvested own hematopoietic cells (autologous BMT); and (3) intensive supportive care over the next several weeks while the patient is pancytopenic and at risk of infection and bleeding.

Historically, most of the early morbidity and mortality associated with BMT has occurred during the period of pancytopenia while awaiting marrow engraftment. Since bone marrow engraftment generally took approximately 3 weeks, serious septic events were not uncommon and induction mortality rates were generally 10% to 20%.[1-4]

Hematopoietic growth factors, such as granulocyte colony stimulating factor (G-CSF) and granulocyte-macrophage CSF (GM-CSF), stimulate a variety of bone marrow progenitor cells.[5,6] The risk of infection and subsequent morbidity and mortality in BMT patients is directly related to the duration of neutropenia prior to bone marrow engraftment. It has been hoped that G-CSF and GM-CSF might accelerate bone marrow engraftment, reduce infections, reduce mortality rates, and make the whole BMT process safer.

GROWTH FACTORS WITH AUTOLOGOUS BMT

Autologous BMT was a logical setting for clinical trials of growth factors. Since G-CSF and GM-CSF stimulate the differentiation, proliferation, and function of specific myeloid cells, investigators hoped that administering G-CSF or GM-CSF after BMT would accelerate bone marrow recovery. Autologous BMT does not involve the immunologic issues that are associated with allogeneic BMT (ie, graft-vs-host disease and graft rejection) and that could confound the basic question, "Will growth factors speed marrow engraftment?"

Tables 1 and *2* outline the results of trials using GM-CSF and G-CSF after autologous BMT. Most

of these trials shared a similar, simple design, as shown in *Figure 1*.[7–14] Patients received a standard preparative regimen followed by infusion of autologous bone marrow. Then G-CSF or GM-CSF was given intravenously on a daily basis, usually for 14 to 21 days. Most of these studies were phase II studies using historical controls for comparisons.

TABLE 2
CLINICAL TRIALS OF G-CSF* IN AUTOLOGOUS BONE MARROW TRANSPLANTATION

Author (reference)	Number of patients	G-CSF dose	Marrow dose (nucleated cells x 10^8/kg)	Median days to ANC† >500/μL (historical controls)	Deaths (historical controls)
Sheridan (13)	15	20 μg/kg/day *Continuous infusion*	—	11 (20)	20% (11%)
Taylor (14)	18	60 μg/kg/day IV push	>1 x 10^8	13 (22)	0%

*Granulocyte colony-stimulating factor
†Absolute neutrophil count

This clinical experience points to several general observations. The historical control data are fairly uniform. Autologous BMT without growth factors leads to an approximately 3-week period of neutropenia and is associated with an induction mortality rate of 5% to 22%. Adding either GM-CSF or G-CSF after autologous BMT shortens the duration of neutropenia to approximately 2 weeks. Rates of platelet recovery and erythrocyte recovery are generally not affected by this schedule of the use of GM-CSF or G-CSF. There was also a general lack of data to predict which subset of patients might benefit from growth factors. The speed of engraftment did not seem to correlate with the number of infused marrow cells or with the dose of bone marrow.

The study of GM-CSF by Brandt et al[7] used a 14-day dosing schedule. When the GM-CSF infusion was stopped, the majority of patients experienced a fall of leukocyte counts over 3 to 4 days. Brandt et al also looked at bone marrow morphology. Samples of bone marrow were collected at 5-day intervals for 20 days. Marrow cellularity was low for the first 10 days after transplantation, and the authors felt that marrow cellularity proved to be an insensitive indicator of earliest myeloid recovery. The toxicity of GM-CSF in this series consisted largely of myalgia, edema, and pleural effusions. Significant toxicity occurred with a dose of 32 μg/kg/day, and the onset was rapid and included erythroderma, weight gain, generalized edema, and hypotension.

Lazarus et al[12] described a phase II Eastern Cooperative Oncology Group trial using GM-CSF after autologous BMT for relapsed non-Hodgkin's lymphoma patients. Recovery to an absolute neutrophil count of 500/μL was statistically significantly faster than controls (median 14 days vs median 20 days, $P = .0002$). The time to platelet engraftment was not different in patients receiving GM-CSF compared with historical controls. Lazarus also noted that cessation of GM-CSF treatment was accompanied by a transient decrease in granulocyte counts in most patients.

Generally, GM-CSF was well tolerated. In addition to fever and myalgia, two patients experienced knee effusions. Eight of 16 BMT patients who received GM-CSF achieved a complete remission (range 4 to 21 months after BMT), which was similar to that seen in historical controls. Only 2 of 16 patients experienced documented infections.

The series by Blazer et al[10] is particularly noteworthy. They reported that when bone marrow was

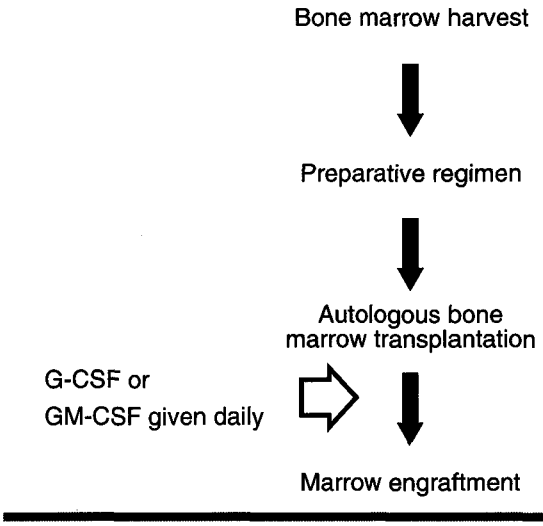

FIGURE 1. Schematic depiction of the use of growth factors after autologous bone marrow transplantation.

purged with the chemical 4-hydroperoxycyclophosphamide (4HC) enhanced marrow engraftment did *not* occur when patients received GM-CSF. This led to the conclusion that purging the marrow with 4HC kills progenitor cells that are the likely target of GM-CSF; a lack of adequate numbers of progenitor cells led to a lack of response to GM-CSF.

G-CSF also has been shown to decrease the duration of neutropenia. Sheridan et al[13] showed that the time required to reach an absolute neutrophil count of 500/μL was 11 days in G-CSF patients compared with 20 days in historical controls ($P < .0005$). They also noted a trend toward a shorter length of hospital stay (23 days vs 30 days). G-CSF was remarkably well tolerated, with no evidence of bone pain. In fact, the only adverse reaction to G-CSF was erythema at subcutaneous infusion sites.

Taylor et al[14] also demonstrated that patients treated with G-CSF and autologous BMT experience a statistically significant reduction in the time required to reach an absolute neutrophil count of 500/μL compared with historical controls (median 13 days vs 22 days, $P = .002$). Side effects were also minimal, with only occasional bone pain and myalgia noted.

Several randomized, double-blind, placebo-controlled trials used GM-CSF after autologous BMT for lymphoid cancer. Nemunaitis et al[15] enrolled 128 patients in a trial of either a daily 2-hour infusion of GM-CSF for 28 days or placebo. The patients given GM-CSF reached an absolute neutrophil count of 500/μL 7 days earlier than patients who received placebo (19 days vs 28 days; $P < .001$). The patients receiving GM-CSF also had fewer infections and required 6 fewer days of hospitalization. The authors appropriately concluded that GM-CSF significantly lessens morbidity after autologous BMT for lymphoid neoplasia.

These data have been confirmed by two additional trials. Gulati and Bennett[16] used autologous BMT to treat 24 patients with relapsed Hodgkin's disease. Twelve received GM-CSF after BMT, and 12 received placebo in a randomized, double-blind trial. Patients receiving GM-CSF reached an absolute neutrophil count of 1000/μL in 16 days, vs 27 days for placebo ($P = .02$). The median length of stay for patients receiving GM-CSF was also shorter (32 days vs 40.5 days, $P = .004$). An important aspect of this trial showed that the group treated with GM-CSF had lower total hospital charges after infusion of autologous marrow when compared with the placebo group (median in-hospital charges $39,800 vs $62,500, $P = .005$). The authors felt that the difference in hospital charges was the result of a lower use of antibiotics, laboratory tests, and ancillary services such as physical therapy.

Advani et al[17] also conducted a double-blind, placebo-controlled trial using GM-CSF after autologous BMT. Patients who received GM-CSF achieved an absolute neutrophil count of greater than 500/μL faster than those receiving placebo (median 12 days vs median 16 days, $P = .02$). In addition, the occurrence of bacterial infections was significantly reduced in the GM-CSF group ($P = .04$). The time to platelet independence was similar in both groups. Additionally, purging marrow with monoclonal antibodies ("relevant B or T cell monoclonal antibodies plus complement") seemed to have a deleterious affect on marrow engraftment.

Nemunaitis et al[18] recently reported the *long-term* follow-up of patients who received GM-CSF after autologous BMT. They demonstrated that, with a median follow-up of 774 days after autologous BMT, rates of tumor relapse in patients receiving GM-CSF were similar to those seen in previous BMT studies. This supports the theory that GM-CSF does not have an adverse effect on relapse rates in lymphoid neoplasia. In addition, bone marrow engraftment in patients receiving GM-CSF was durable.

In summary, the use of G-CSF or GM-CSF on a daily basis after autologous BMT enhances the rate of neutrophil recovery. This generally translates into a decreased incidence of infection and decreased mortality rates. Three randomized, placebo-controlled trials have documented that using GM-CSF after BMT results in a more rapid neutrophil engraftment. Purging bone marrow with either 4HC or monoclonal antibodies may negate some of the positive effects of growth factors after BMT. Additionally, some authors have documented a reduced length of stay and decreased hospital charges when growth factors are used.

GROWTH FACTORS AND PERIPHERAL BLOOD PROGENITOR CELLS

Bone marrow progenitor cells, or stem cells, circulate in the peripheral blood. These peripheral blood progenitor cells (PBPCs) may serve as a source of "marrow" for autologous BMT. Thus, if the patient's marrow is contaminated by metastatic cancer, then autologous BMT is still feasible, provided one can

harvest an adequate number of PBPCs for transplantation. Clinical trials have shown that the use of PBPCs as the source of hematopoietic cells leads to successful marrow engraftment and that survival rates after transplantation are comparable to results using autologous marrow.[19–22]

The technique to harvest PBPCs is straightforward. The patient undergoes several leukapheresis procedures, and peripheral nucleated cells undergo cryopreservation. The timing of leukapheresis is felt to be important in optimizing the yield of PBPC collection. Prior treatment with cyclophosphamide generally results in a surge of CD34-positive cells as bone marrow recovers from the cytotoxic effects of this chemotherapeutic agent.[23] While some centers do not routinely use cyclophosphamide "priming" to harvest PBPCs, many feel that such a technique enhances the quantity of PBPCs collected and reduces the number of pheresis procedures required for harvesting.

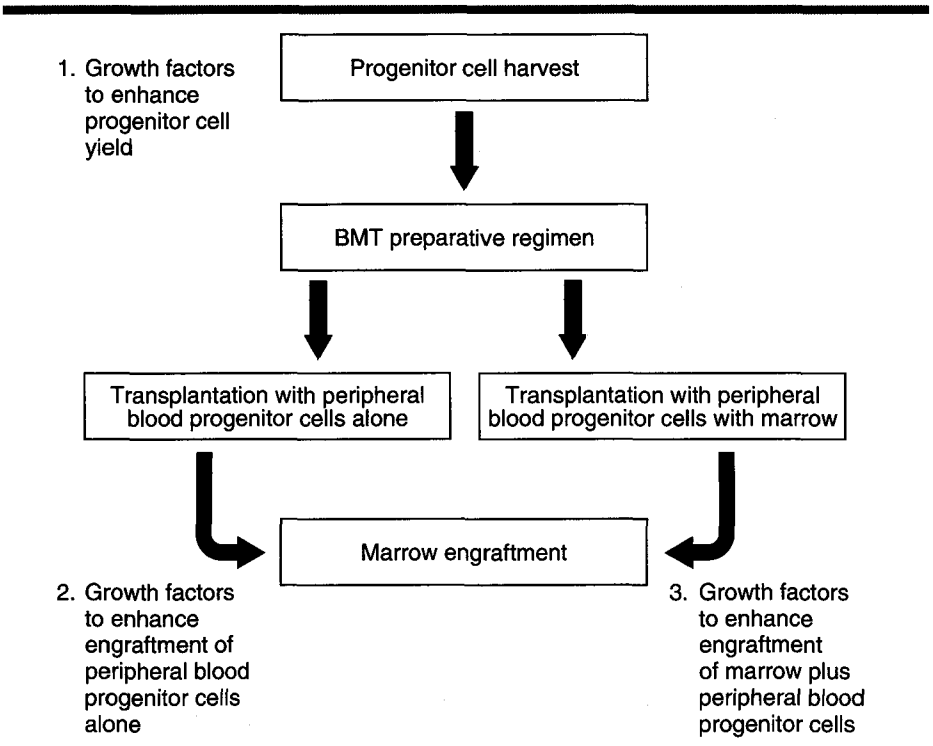

FIGURE 2. Schematic depiction of the use of growth factors with peripheral blood progenitor cells.

The use of PBPCs in BMT has led to a variety of novel trials using growth factors. These include (1) the use of growth factors to enhance the yield of PBPC harvesting prior to transplant; (2) the use of growth factors after PBPC transplantation; and (3) the use of growth factors with both autologous bone marrow and PBPCs, as shown in *Figure 2*.

Several authors have shown that GM-CSF expands the circulating hematopoietic progenitor cell compartment. Socinski et al[24] treated patients with GM-CSF, given alone or after cytotoxic chemotherapy. They found that GM-CSF produced an 18-fold increase in peripheral blood granulocyte-macrophage colony-forming units (CFU-GM). When GM-CSF was given following cytotoxic chemotherapy, the results were more dramatic: the peak number of CFU-GM increased to 62 times pretreatment counts, and was 5 times higher than the peak occurring after GM-CSF was given alone before chemotherapy. The authors concluded that GM-CSF significantly increased the number of PBPCs, and this phenomenon was especially true when it was given after cytotoxic chemotherapy.

Gianni et al[25] also showed that GM-CSF given after high-dose cyclophosphamide dramatically increased (up to 1000-fold) the number of peripheral blood CFU-GM. This use of GM-CSF with cyclophosphamide resulted in high yields of progenitor cells collected with relatively few leukapheresis procedures. These PBPCs were then used for autologous hematopoietic cell transplantation. After receiving a preparative regimen of total-body irradiation and melphalan, patients received PBPC and GM-CSF. Complete hematopoietic recovery occurred in a very short time: an absolute neutrophil count of 500/μL was achieved in a median of 10 days, and a platelet count of 50 000/μL was achieved in a median of 11 days. In addition, the mucositis previously noted with this particular preparative regimen was less severe. The authors concluded that the use of GM-CSF and PBPCs led

to a dramatic increase in circulating progenitor cells, and that these cells, when used at the time of BMT, led to very rapid marrow engraftment. Several additional trials using GM-CSF and PBPCs have demonstrated rapid marrow engraftment.[26–29]

These studies confirm that GM-CSF improves the quantity of PBPCs, and that these cells lead to rapid marrow engraftment. In addition, Siena et al[29] studied the in vitro growth characteristics of PBPCs primed with GM-CSF. They found qualitatively normal hematopoietic colony growth as compared with bone marrow progenitors. Thus, the quality of mobilized PBPCs is similar to that of bone marrow.

G-CSF has also been shown to increase the number of circulating PBPCs. Sheridan et al[30] have recently reported that numbers of granulocyte-macrophage progenitor cells in peripheral blood increased 58-fold over pretreatment values when patients were treated with G-CSF before progenitor cell collection. The authors then used these cells after high-dose chemotherapy in 14 patients given a preparative regimen of busulfan and cyclophosphamide. The median time to neutrophil recovery of 500/μL was 9 days when PBPCs and G-CSF were given after BMT. Extremely rapid platelet recovery was also seen, with a median time of 15 days for platelet counts to reach 50 000/μL. This engraftment of both neutrophils and platelets was faster than that seen in patients receiving the same high-dose chemotherapy with autologous BMT and G-CSF alone (ie, without G-CSF-mobilized PBPCs), as well as when compared with a control population receiving high-dose chemotherapy and autologous BMT without G-CSF. The differences were statistically significant.

Thus, growth factors increase the number of circulating PBPCs, and these cells lead to successful marrow engraftment. These data are noteworthy because both neutrophil engraftment (about 10 days after BMT) and platelet recovery (12 to 14 days after BMT) are very rapid.

These data mainly involve PBPCs with GM-CSF or G-CSF after BMT. Are the PBPCs responsible for this quick engraftment? As stated above, Sheridan et al[30] found that the use of G-CSF with PBPCs led to quicker engraftment than the use of G-CSF alone. Would PBPCs alone, without growth factors after BMT, lead to rapid engraftment? Elias et al[31] compared marrow engraftment in patients receiving high-dose chemotherapy for breast cancer using either PBPCs alone or PBPCs with post-transplant GM-CSF. They found that PBPCs used with GM-CSF resulted in quicker engraftment of neutrophils than PBPCs alone; both groups had rapid platelet engraftment. Thus, both PBPCs and growth factors seemed to play a role in this enhanced marrow engraftment.

Additional data exist on the combined use of growth factors, PBPCs, and autologous bone marrow. That is, at the time of transplant, *both* autologous bone marrow and PBPCs have been infused in an attempt to enhance engraftment. Peters et al[32] have reported that PBPCs primed with GM-CSF and coupled with autologous BMT leads to extremely quick neutrophil engraftment. Bone marrow was harvested and PBPCs primed with GM-CSF were collected prior to BMT. Patients were then given a chemotherapeutic preparative regimen, and both PBPCs and autologous marrow were reinfused. GM-CSF was then given daily. The median time during which the white blood cell count was under 100/μL was reduced to 2 days, and the mean time during which the absolute neutrophil count was under 100/μL was reduced to 7 days in patients treated with this regimen.

We recently reported the results of our Cleveland Clinic study using G-CSF and PBPCs together with autologous bone marrow.[33] We studied patients with lymphomas, either non-Hodgkin's lymphoma or Hodgkin's disease, using the CBV preparative regimen (cyclophosphamide, BCNU [carmustine], and VP-16).

Prior to transplantation, all patients underwent a standard bone marrow harvest. Twelve days before admission, patients received an 8-day course of G-CSF (5 μg/kg/day). PBPCs were harvested on days 5, 7, and 8. G-CSF treatment resulted in an increased peripheral white blood cell count in all patients (range 28 000 to 72 000/μL). After the preparative regimen, both autologous marrow and PBPCs were reinfused. G-CSF was restarted at a dose of 16 mg/kg/day on the day of PBPC infusion. Median time required to reach a neutrophil count of 500/μL was 9 days after transplantation (range 7 to 13 days). The median time to achieve a platelet count of 20 000/μL (independent of platelet transfusions) was 13 days after transplantation. This rapid engraftment led to a shortened length of hospital stay, with an average length of 27 days (vs historical controls of 36 days).

PBPCs collected without chemotherapeutic or growth-factor priming may not result in enhanced

TABLE 3
GM-CSF* IN GRAFT FAILURE

Author (reference)	GM-CSF dose	Patients	Outcome
Nemunaitis (42)	Dose escalation 60 to 250 μg/m^2/day for 4 days (1 to 3 courses)	15 allogeneic BMT†	9 of 15 had ANC‡ > 500/μL by day 14 8 of 9 maintained this ANC; no increased incidence of graft-vs-host disease
		21 autologous BMT	11 of 21 responded to GM-CSF 0 of 7 with 4HC or VP-16 purged marrow responded vs 12 of 15 with unpurged or monoclonal antibody purging (*P*=.0007)
Vose (43)	250 μg/m^2 day	12 autologous BMT 2 marrow only 1 both stem cell and marrow	Median ANC 90 to 704 /μL vs historical control 48 to 408/μL (*P*=.008) did not matter if patient received marrowmor stem cells; most patients had stabilization or rise of neutrophils after GM-CSF stopped
Brandwein (44)	5 to 10 μg/kg/day for 14 days	6 autologous BMT	3 of 6 responded with 7- to 14-fold increase of ANC: 2 of 3 responders had ANC fall to pre-treatment levels 4 to 7 weeks after GM-CSF stopped

*Granulocyte-macrophage colony-stimulating factor
†Bone marrow transplantation
‡Absolute neutrophil count

engraftment. Lobo et al[34] recently reported a study comparing engraftment of patients receiving either autologous bone marrow or autologous marrow with *unprimed* PBPCs. No difference in engraftment was seen between the two groups.

Thus, priming of PBPCs (with chemotherapy, growth factors, or both) seems to play a key role in improving engraftment. The mechanism of this enhanced engraftment with PBPCs is unknown. However, it is possible that marrow engraftment occurs in several phases. Jones et al[35] have shown that initial engraftment after BMT involves committed progenitor cells, whereas a second sustained engraftment phase depends upon the pluripotential stem cell. PBPCs with growth factors may enhance this first phase of engraftment while maintaining an adequate number of pluripotential stem cells to ensure sustained engraftment.

The Duke University BMT program has also conducted a financial analysis of the impact of CSF-primed PBPCs.[36] Four groups of patients were studied. The first two groups underwent autologous BMT without PBPCs but with either GM-CSF or G-CSF. The third group of patients underwent autologous BMT with PBPCs and GM-CSF. The fourth group underwent autologous BMT with PBPCs and G-CSF. Hospital charges were dramatically reduced in the fourth group. The median hospital charge in patients undergoing autologous BMT with PBPCs and G-CSF was $77 000, compared with median charges of $92 000 to $100 000 in the first three groups. This was a statistically significant difference. Additionally, 31% to 54% of patients in the first three groups had charges over $125 000, whereas no patient undergoing autologous BMT with PBPCs and G-CSF had charges over $125 000. These important data show that enhanced marrow engraftment seen with these techniques leads not only to reduced toxicity and length of hospital stay, but also to decreased resource use and decreased hospital charges.

In summary, the use of PBPCs in autologous BMT is extremely exciting. Use of PBPCs plus either G-CSF or GM-CSF seems to dramatically enhance rates of neutrophil engraftment with or without the use of autologous bone marrow. Use of PBPCs with growth factors also dramatically enhances the rate of platelet engraftment. This enhanced platelet engraftment seems to be distinct from that seen when using growth factors with autologous bone marrow alone. However, no prospective trials exist comparing autologous BMT with BMT plus PBPCs.

Such trials are needed to ultimately define the value of growth factors and PBPCs. Furthermore, the optimal timing and dosing schedule for growth factors and PBPCs, both before and after BMT, is not yet defined.

GROWTH FACTORS IN GRAFT FAILURE

Graft failure is not a common complication of autologous BMT. However, approximately 2% of allogeneic BMT patients suffer from immunologically mediated graft rejection. This incidence may rise with use of T-cell-depleted marrow transplants or unrelated bone marrow donors, or in patients with aplastic anemia.[1,2,37–40] Immunologic rejection is not an issue in autologous BMT; however, between 2% and 8% of patients who undergo autologous BMT have evidence of failure of bone marrow engraftment.[41] For such patients, therapy has generally been supportive, and the outcome is generally poor.

Three series have looked at the use of GM-CSF in graft failure after BMT.[42–44] These results are shown in *Table 3*. Nemunaitis et al[42] defined graft failure as (1) an absolute neutrophil count of less than 100/μL by day 28 after BMT, or (2) failure to achieve an absolute neutrophil count of 100/μL by day 21 and the presence of a documented life-threatening infection. They examined both allogeneic and autologous BMT patients. A response was defined as an increase in absolute neutrophil count to ≥500/μL within 14 days of starting a course of GM-CSF. Patients received one to three courses of GM-CSF; each course was a 14-day daily dose of GM-CSF. Most allogeneic BMT patients responded. Of those who did, no increased incidence of graft-vs-host disease was seen. The autologous BMT patients had variable responses. Interestingly, none of the seven patients who received bone marrow purged with either 4HC or VP-16 had responses. This result coincides with that found by Blazer et al[10] using GM-CSF in autologous BMT with GM-CSF and marrow purged with 4HC. That is, neither Blazer's series nor this series documented any response to GM-CSF when marrow was purged with 4HC.

Vose et al[43] and Brandwein et al[44] also observed responses to GM-CSF in patients who underwent autologous BMT and experienced delayed engraftment. Vose treated patients who failed to achieve an absolute neutrophil count of 150/μL by day 30 after BMT with GM-CSF 250 μg/m²/day. Nine of 12 patients received autologous transplants of peripheral stem cells only. These patients were reported in aggregate form; median absolute neutrophil count rose from 90/μL to 704/μL. This rise of neutrophils was higher than that seen in a group of historical controls; the difference was statistically significant. This study is noteworthy in demonstrating that delayed engraftment with peripheral stem cell transplants may respond to GM-CSF.

Brandwein[44] studied six patients with very delayed engraftment. All patients were at least 55 days after BMT and had absolute neutrophil counts under 500/μL. Three of the six patients responded to GM-CSF; two of the three responders had to be retreated with GM-CSF after discontinuation to maintain an acceptable neutrophil count.

In summary, while graft failure is generally not a common problem in BMT, GM-CSF has been shown to reverse graft failure and lead to adequate neutrophil engraftment in many patients. Patients with chemically purged bone marrow have not, to date, responded to GM-CSF.

ALLOGENEIC BMT AND GROWTH FACTORS

The use of G-CSF and GM-CSF in allogeneic BMT has not been well studied. This is probably because of concern about potential adverse effects on the severity of graft-vs-host disease. While neither G-CSF nor GM-CSF directly stimulate T-cell proliferation, and while certain T cells are thought to be responsible for graft-vs-host disease, cytokines have the potential to increase production of interleukin-1 (IL-1) and tumor necrosis factor, which might affect the severity of graft-vs-host disease.

Two recent studies used GM-CSF in patients undergoing allogeneic BMT. Nemunaitis et al[45] reported a dose-escalation study using GM-CSF in 47 patients undergoing allogeneic BMT using sibling donors with identical human leukocyte antigen (HLA) statuses. The preparative regimens were not uniform, although most patients received cyclophosphamide and total-body irradiation. Patients were divided into two groups: one group received methotrexate as part of prophylaxis for graft-vs-host disease (the dose of methotrexate was 15 mg/m² intravenously on day 1, and 10 mg/m² intravenously on days 3, 6, and 11); the other group did not receive methotrexate. The clinical results of this study are shown in *Table 4*. Neutrophil recovery was more

TABLE 4
GM-CSF* IN ALLOGENEIC BONE MARROW TRANSPLANTATION

Author (reference)	GM-CSF dose	Number of patients	Median Day ANC† > 1000/μL (historical controls)		Comment
Nemunaitis (45)	Dose escalation study: Maximum tolerated dose, 250 μg/m²/day	Group I 27 (no methotrexate)	14 (19) P=.0017		No increased incidence of graft-vs-host disease
		Group II 18	20 (24) P=.0052		
			Median day ANC 300/μL‡	Median day ANC 500/μL§	
DeWitte (46)	8 μg/kg/day Continuous infusion	Group I (+GM-CSF) 29	13.9	15.8	No difference in incidence of graft-vs-host disease
		Group II (placebo) 28	18.1	19.9	

*Granulocyte-macrophage colony-stimulating factor
†Absolute neutrophil count
‡P<.02
§P not significant

rapid in the group that did not receive methotrexate prophylaxis. However, when compared with a group of historical control patients, both groups experienced faster neutrophil engraftment when using GM-CSF. The incidence of graft-vs-host disease was 55% in group 1 and group 2. The incidence of severe acute graft-vs-host disease was 29% in group 1 and 29% in group 2. This was not different from the control population.

This trial included a dose escalation of GM-CSF. The maximal tolerated dose was 250 μg/m²/day. At doses of 500 μg/m²/day, 60% of people experienced severe bone pain or chest pain, or both. Nemunaitis concluded that GM-CSF was well tolerated at 250 μg/m²/day, that the severity and incidence of acute graft-vs-host disease was not affected by GM-CSF, and that GM-CSF may enhance neutrophil engraftment after allogeneic BMT.

DeWitte et al[46] recently reported a prospective randomized trial of GM-CSF in allogeneic BMT using T-cell-depleted bone marrow. Fifty-seven patients were randomized to receive either GM-CSF or placebo after allogeneic BMT. The donors were all HLA-identical siblings. The preparative regimen was cyclophosphamide and total-body irradiation. T-cell depletion was performed either by counter-flow centrifugation or by a "cocktail" of monoclonal antibodies. Patients receiving GM-CSF were treated with 8 μg/kg/day as a continuous infusion for 14 days, starting 3 hours after bone marrow infusion. *Table 4* shows the neutrophil engraftment data. Neutrophil engraftment was enhanced to an absolute neutrophil count of both 300/μL and 500/μL in patients receiving GM-CSF. This was a statistically significant difference compared with the placebo group when studying an absolute neutrophil count of 300/μL. The difference between the two groups did not reach statistical significance when analyzing the number of days needed to reach an absolute neutrophil count of 500/μL. The length of time to platelet engraftment was not affected by GM-CSF use.

Transplant-related mortality was not different in the two groups in DeWitte's study. The incidence of acute graft-vs-host disease was also not different between the two groups. There was a trend towards a lower relapse rate in patients receiving GM-CSF, and this translated into a trend towards better survival. The toxicity rate of GM-CSF was generally acceptable. However, 8 of 29 patients who received GM-CSF had unexplained fever, and 4 had myalgia. The authors concluded that GM-CSF enhanced neutrophil recovery in T-cell-depleted allogeneic BMT. This did not result in an increase in the severity of graft-vs-host disease or in leukemic relapse.

Masaoka et al[47] have reported the only investigation of G-CSF in allogeneic BMT. They treated 36 patients undergoing allogeneic BMT on a dose-escalation study of G-CSF (200 to 800 μg/m²/day × 14 days).[47] Patients were generally treated with total-body irradiation. Graft-vs-host disease prophylaxis consisted of cyclosporine with or without

methotrexate. Regardless of the dose of G-CSF used, the average time needed to achieve a granulocyte count of 500/μL was 15 days. Patients receiving methotrexate had a significantly slower rate of engraftment than those who did not receive methotrexate. G-CSF was well tolerated and did not influence the rate or severity of graft-vs-host disease. Relapse rates of underlying disease were similar to historical controls. The authors concluded that G-CSF was well tolerated and seemed to result in decreased neutropenia.

More data on the use of growth factors in allogeneic BMT are needed. It is encouraging to see that early results have not demonstrated an increase in graft-vs-host disease. It is also encouraging that rates of leukemic relapse have not changed. Unlike most data cited for autologous BMT, allogeneic BMT frequently involves the treatment of *myeloid* leukemia. Growth factors have the potential to stimulate both nonmalignant and malignant myeloid cells. Relapse rates in studies employing growth factors in patients undergoing allogeneic BMT have not changed; this certainly supports the design of additional prospective trials. Such trials are necessary to optimally define the role of G-CSF and GM-CSF in allogeneic BMT.

LIMITATIONS OF GROWTH FACTORS IN BMT

Bone marrow toxicity is the most common dose-limiting factor of available chemotherapeutic agents. The fundamental purpose of BMT is to overcome bone marrow toxicity as the dose-limiting factor and thereby allow further escalation of drug dosage. It is hoped that additional escalation of drug dosage will translate into increased cure rates. If so, the dose-limiting factor of chemotherapy would become toxicity to other organs, such as the lungs and the liver, rather than toxicity to hematopoietic cells.

Since the primary action of growth factors is on hematopoietic cells, it is unlikely that the use of growth factors will allow significant additional dose escalation in preparative regimens for BMT. However, by reducing the toxicity associated with BMT, trials may ascertain whether transplantation earlier in the course of malignancy will be of benefit; reducing toxicity also allows for the study of more than one transplantation procedure for high-risk patients.

The optimal schedule or combination of growth factors to use in BMT is not presently defined. This review has highlighted many different ways of using GM-CSF and G-CSF. Other growth factors that may stimulate additional forms of progenitor cells, such as IL-1, IL-6, and stem-cell factor, are currently in clinical trials and may have significant clinical uses in the future.

Finally, in a recent editorial, Vose et al[48] correctly stated that the benefit achieved by using growth factors in BMT should not necessarily be extrapolated to standard-dose chemotherapy in the outpatient setting. There is little evidence to suggest that G-CSF or GM-CSF should be used *routinely* for patients with a standard risk profile receiving outpatient chemotherapy.

SUMMARY

Use of growth factors has become a standard part of the care of patients undergoing autologous BMT. There is little question of the ability of colony-stimulating factors to enhance marrow growth. But the best way to achieve maximal marrow engraftment is not precisely defined. The next several years will see further investigations of cytokine combinations after BMT, combinations of growth factors to enhance collection of circulating progenitor cells, and the use of growth factors in allogeneic BMT. Some basic questions that will be asked include the following: Which combinations of cytokines provide optimal progenitor cell yield? If adequate numbers of progenitor cells are harvested, is a bone marrow harvest necessary to achieve durable engraftment? Will rates of cancer relapse be affected by broader-acting growth factors such as stem-cell factor? Are multiple BMT procedures feasible, and will multiple transplantation increase cure rates? What is the optimal quantity and quality of progenitor cells for autologous BMT? Is there a role for PBPCs in allogeneic BMT? These and other very important questions await answers that will be defined in clinical trials.

To date, it is clear that the use of hematopoietic growth factors has made BMT safer. Morbidity and mortality rates have decreased. Patients are discharged from the hospital sooner, and this has translated into less overall cost for patients, insurance companies, and the health-care system. The next decade promises to be an exciting era during which advanced technology not only helps answer basic questions of hematopoiesis, but also improves patient care, and possibly decreases cost for the health-care system as a whole.

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