

# Circulating Granulocyte Colony-Stimulating Factor (G-CSF) Levels After Allogeneic and Autologous Bone Marrow Transplantation: Endogenous G-CSF Production Correlates With Myeloid Engraftment

By Mitchell S. Cairo, Yu Suen, Leonard Sender, Eileen R. Gillan, Winston Ho, J. Michael Plunkett, and Carmella van de Ven

**Myeloid engraftment after bone marrow transplantation (BMT) is influenced by a number of variables, including cytoreductive chemoradiotherapy, genetic disparity, number of reinfused committed myeloid progenitor cells, healthy microenvironment, and the presence of hematopoietic growth factors. Granulocyte colony-stimulating factor (G-CSF) stimulates proliferation of myeloid progenitor cells and enhances myeloid engraftment after BMT. We investigated the temporal relationship between endogenous G-CSF production and myeloid engraftment in both children and adults after allogeneic (ALLO) and autologous (AUTO) BMT. Circulating endogenous G-CSF levels ranged between 0 and 2552 pg/mL. The correlation coefficient between circulating serum G-CSF levels and the peripheral absolute neutrophil count (ANC) was  $r = -.875$  ( $P < .001$ ). The endogenous serum G-CSF level was**

**highest during the first week after BMT, when the ANC was  $\leq 200/\mu\text{L}$  ( $699 \pm 82.3$  pg/mL) ( $P < .001$ ). Both children and adults demonstrated a similar inverse relationship between circulating G-CSF level and degree of neutropenia. One patient failed to engraft after AUTO BMT and also failed to generate any endogenous G-CSF production. Lastly, once the serum G-CSF level decreased to less than 200 pg/mL, a mean of  $6.1 \pm 0.9$  days elapsed before the ANC was  $\geq 500/\mu\text{L}$  for 2 consecutive days. This study demonstrates that endogenous G-CSF production is associated with myeloid engraftment in both children and adults after AUTO and ALLO BMT and that the rate of increase and decrease in endogenous G-CSF may be predictive of either failure to engraft or duration of neutropenia.**

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**B**ONE MARROW transplantation (BMT) has become a successful treatment method for a variety of conditions, including leukemias and solid tumors. Myeloid engraftment ( $\geq 500$  PMN/polymorphonuclear leukocytes per microliter) usually occurs anywhere between 2 to 8 weeks after cytoreductive therapy and marrow reinfusion. The length and degree of neutropenia after BMT is significantly associated with a high incidence of infectious morbidity. Prolonged neutropenia may be associated with a number of variables after BMT, including chemoradiotherapy, quantity of infused committed myeloid progenitors, previous cytotoxic therapy and reduced marrow reserve, concurrent myelosuppressive cytotoxic therapy, graft rejection, graft versus host disease (GVHD), defective microenvironment, toxicity to myeloid progenitor cells after BM purging, and decreased production of hematopoietic growth factors.

Myelopoiesis was recently demonstrated to be in part dependent on the continuous or intermittent supply of highly specific hematopoietic growth factors. Granulocyte colony-stimulating factor (G-CSF), first purified to homogeneity from the human bladder carcinoma cell line 5637, significantly modulates myelopoiesis and mature PMN functional activation.<sup>1-5</sup> Purified G-CSF supports proliferation and maturation of myeloid progenitor cells, induces egress of mature neutrophil storage pools from BM reserves, and results in functional activation of mature effector neutrophils. Recently, exogenous administration of recombinant human G-CSF (rhG-CSF) enhanced myeloid engraftment and reduced infectious morbidity associated with neutropenia after both allogeneic (ALLO) and autologous (AUTO) BMT.<sup>6-8</sup> In the current study, we investigated the temporal relationship between endogenous circulating G-CSF levels and myeloid engraftment in both children and adults after ALLO and AUTO BMT and correlated the increase and decrease in circulating G-CSF levels with degree of neutropenia.

## PATIENTS AND METHODS

**Patients.** Twelve patients, eight females and four males, aged 3 to 56 years, were studied before cytoreductive therapy and after

BMT until their absolute neutrophil count (ANC) was  $\geq 1,000/\mu\text{L}$  for 3 consecutive days. Seven AUTO and five ALLO BMTs were performed for a variety of leukemias, lymphomas, and solid tumors. Their pretransplant characteristics are shown in Table 1. Patients with acute nonlymphoblastic leukemia (ANLL) received either busulfan (4 mg/kg/day for 4 days) and cyclophosphamide (50 mg/kg/day for 4 days) (BuCy), or 1,400 total body irradiation (TBI) and Cy (60 mg/kg/day for 2 days); patients with brain tumors received VP-16 (1,500 mg/m<sup>2</sup> for 3 days) and thiotepa (900 mg/m<sup>2</sup> for 3 days); patients with non-Hodgkin's lymphoma (NHL) received either Cy (1.8 g/m<sup>2</sup>/day for 4 days), VP-16 (400 mg/m<sup>2</sup>/every 12 hours for 3 days), BCNU (600 mg/m<sup>2</sup>/day for 1 day), BuCy, or FxTBI (1,200 rad), VP-16 + CTX; patients with acute lymphoblastic leukemia (ALL) had their BM purged with vincristine (1.5  $\mu\text{g}/\text{mL}$ ) and VP-16 (50  $\mu\text{g}/\text{mL}$ ) and were treated with 1,200 TBI, VP-16 (1,800 mg/m<sup>2</sup> once) and Cy (60 mg/kg  $\times$  2 days); and patients with breast cancer received thiotepa (100 mg/m<sup>2</sup>  $\times$  3 days), mitoxantrone (12 mg/m<sup>2</sup>  $\times$  3 days), and Cy (60 mg/kg for 2 days). Patients receiving GVHD prophylaxis received either methotrexate (MTX) on days 1, 3, 6, and 11 after BMT in combination

*From the Division of Hematology/Oncology, Children's Hospital of Orange County; Division of Research Immunology and Bone Marrow Transplantation, Children's Hospital of Los Angeles, Los Angeles; and Division of Bone Marrow Transplantation, St Joseph Hospital, Orange, CA.*

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*Address reprint requests to Mitchell S. Cairo, MD, Director, Hematology/Oncology Research and Bone Marrow Transplantation, Children's Hospital of Orange County, 455 S Main St, Orange, CA 92668.*

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Table 1. Pretransplant Characteristics

Patient/Age/Sex	Diagnosis	Preparative Therapy	BMT	GVHD Prophylaxis
1/4/F	NHL Second CR	CY, VP-16, BCNU	6/6 ALLO	MTX, CSP
2/3/F	ANLL First CR	Bu, CY	6/6 ALLO	MTX
3/3/M	ANLL First Rel	TBI, CY	4/6 ALLO	MTX, CSP, ATG
4/5/F	ANLL First CR	Bu, CY	6/6 ALLO	MTX
5/13/M	ALL Third CR	VP-16, CY, TBI	AUTO	—
6/4/F	GBM First PR	VP-16, TT	AUTO	—
7/9/F	BSG First PR	VP-16, TT	AUTO	—
8/48/F	AML Second CR	Bu, CY	AUTO	—
9/21/M	NHL Second CR	Bu, CY	AUTO	—
10/56/F	Breast CA First PR	Mito, TT, CY	AUTO	—
11/46/F	NHL Second CR	TBI, CY, VP-16	6/6 ALLO	CSP
12/24/M	NHL First CR	TBI, CY, VP-16	AUTO	—

Abbreviations: NHL, non-Hodgkin's lymphoma; ANLL, acute nonlymphocytic leukemia; GBM, glioblastoma multiformi; ALL, acute lymphoblastic leukemia; BSG, brain stem glioma; CY, cyclophosphamide, VP-16, etoposide; BCNU, bischloronitrosourea; BU, Busulfan; TBI, total body irradiation; TT, thiotepa; Mito, mitoxantrone; MTX, methotrexate; CSP, cyclosporine; ATG, antithymocyte globulin; GVHD, graft v host disease; ALLO, allogeneic; AUTO, autologous; PR, partial remission; CR, complete remission.

with cyclosporine (CSP), a long course of MTX, or MTX + CSP + 6 doses of antithymocyte globulin (ATG, 10 mg/kg/day for 6 days).<sup>9</sup>

**Patient samples.** Samples were obtained from each patient before administration of preconditioning therapy, on day 0, and every 2 days thereafter until the ANC was  $\geq 1000/\mu\text{L}$  for 3 consecutive days. Serum samples were also obtained from control subjects not undergoing BMT, including six cord samples, six children, and six adults. Positive control G-CSF samples were also obtained from standard G-CSF curves obtained by coincidence of rhG-CSF with normal donor serum samples and from supernatants obtained from stimulated peripheral mononuclear cells by phytohemagglutinin/phorbol myristate acetate (PHA/PMA).

**Enzyme-linked immunosorbent assay (ELISA) for detecting hG-CSF.** G-CSF levels from serum were measured by a sandwich ELISA assay. Microtiter plates were coated with polyclonal rabbit anti-rhG-CSF Ig, rhG-CSF standards, and positive controls. Test samples were added and incubated at 37°C overnight. Horseradish peroxidase-conjugated mouse anti-G-CSF monoclonal antibody (MoAb) was added and incubated for 2 hours at 37°C. Plates were washed, and tetramethylbenzidine was added as the substrate. The reaction was stopped after 30 minutes by addition of sulfuric acid (0.5N). Optical density of the samples was measured at 450 nm with a BioRad (Richmond, CA) enzyme immunoassay (EIA) reader. Sensitivity of the assay ranged from 50 to 5,000 pg/mL of G-CSF. Various concentrations of rhG-CSF (0 to 5,000 pg/mL) were used for the standard curve. Supernatant of a human glioblastoma (astrocytoma grade III cell line U-87 MG) and stimulated (PHA 2  $\mu\text{g}/\text{mL}$  and PMA 20 ng/mL) MNC culture supernatant samples were used as positive controls (U-87 MG, 10,000 pg/mL; activated supernatant from adult MNC, 1,400 pg/mL). All samples were run in duplicate, and data are the mean  $\pm$  SEM. G-CSF serum levels by ELISA were correlated with an NFS-60 bioassay (performed by Amgen, Boulder, CO).

**Statistical analysis.** All results are mean values plus or minus SEM of blood serum samples. The probability of existence of significant differences when two groups were compared was determined by unpaired Student's *t* test, and the probability of significant differences existing when multiple groups were examined was determined by analysis of variance (ANOVA) followed by Student-Newman-Keuls multiple-range tests to define the unique subsets within the study. Correlation statistics were determined by Pearson's correlation coefficient. This and all other statistical analyses were performed with the Biostat I statistical program (Sigma Soft,

Placentia, CA) for the IBM personal computer.  $P < .05$  was considered significant.

## RESULTS

**Control subjects.** Serum obtained from newborns (cords), children, and adults with normal circulating ANC had undetectable circulating G-CSF levels ( $< 50$  pg/mL).

**BMT patients.** The G-CSF levels ranged from 0 to 2,552 pg/mL. The correlation coefficient between circulating serum G-CSF levels and the ANC was  $r = -.875$  ( $P < .001$ ) (Fig 1). The circulating G-CSF level increased significantly during the early post-BMT period and correlated significantly with the degree of peripheral neutropenia. The circulating serum G-CSF level was significantly higher when ANC was  $\leq 200/\mu\text{L}$  ( $699 \pm 82.3$  pg/mL) as compared with ANC between 200 and 500 ( $71 \pm 14$  pg/mL) ( $P < .001$ ) and when ANC was  $> 500/\mu\text{L}$  ( $4.9 \pm 5.0$  pg/mL) ( $P < .001$ ). In addition, the inverse relationship between the circulating serum G-CSF level and the degree of neutropenia was similar in both pediatric patients and adult patients (for pediatric patients, ANC  $< 200/\mu\text{L}$

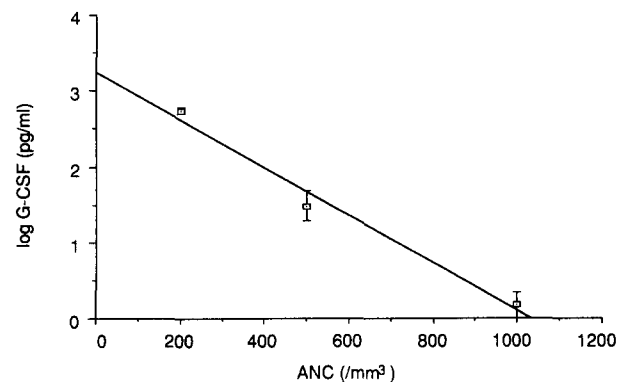


Fig 1. Post-BMT myeloid engraftment: Correlation analysis of endogenous serum G-CSF v ANC. Serum G-CSF levels (mean  $\pm$  SEM) of 12 post-BMT patients were determined by ELISA. Correlation coefficient between the G-CSF and ANC was computed by Pearson's correlation-coefficient analysis:  $r = -.875$ ,  $P = < .001$ .

923.5 ± 109.3 pg/mL, ANC 200 to 500/ $\mu$ L 77.3 ± 18.1 pg/mL, and ANC >500/ $\mu$ L 8.4 ± 8.0 pg/mL; for adult patients, ANC <200/ $\mu$ L 333.6 ± 43.7 pg/mL, ANC 200 to 500/ $\mu$ L 59.9 ± 21.0 pg/mL, and ANC >500/ $\mu$ L ≤ 20 pg/mL). There was a significant increase in endogenous G-CSF production in children as compared with adults after AUTO BMT when the ANC was <200/ $\mu$ L (585 ± 71 v 338 ± 46 pg/mL) ( $P$  < .01). Last, there was also a significant difference in endogenous G-CSF production between AUTO and ALLO transplants when the ANC was ≤200/ $\mu$ L (ALLO 988.8 ± 131.6 v AUTO 408.9 ± 45.4 pg/mL) ( $P$  < .001).

The inverse relationship between circulating endogenous serum G-CSF levels and the ANC is best shown in Fig 2, which is an example of the inverse relationship between circulating ANC and endogenous serum G-CSF level in both a pediatric and an adult patient undergoing BMT. On day 0, when the ANC was within normal limits, the circulating endogenous G-CSF level was nondetectable. However, as the ANC began to decrease, the circulating endogenous G-CSF level began to increase, reaching a peak between day 5 and day 10. As the ANC began to increase after myeloid engraftment, the endogenous G-CSF level began to disappear and again became nondetectable.

One patient in our series failed to engraft after an AUTO BMT with 4-HC purging (100  $\mu$ g/mL). He was then retransplanted using a two-antigen-mismatched related sibling after preparative therapy with TBI and Cy. His course is shown in Figure 3. During his first BMT, when he failed to demonstrate any evidence of myeloid engraftment, he also failed to generate any endogenous G-CSF production. After his relapse on day 30, he was retransplanted. During the second transplant, however, he demonstrated a significant increase in endogenous G-CSF production, resulting in myeloid engraftment (Fig 3).

The mean number of days before myeloid engraftment (PMN >500/ $\mu$ L for 2 days) in the entire series of patients

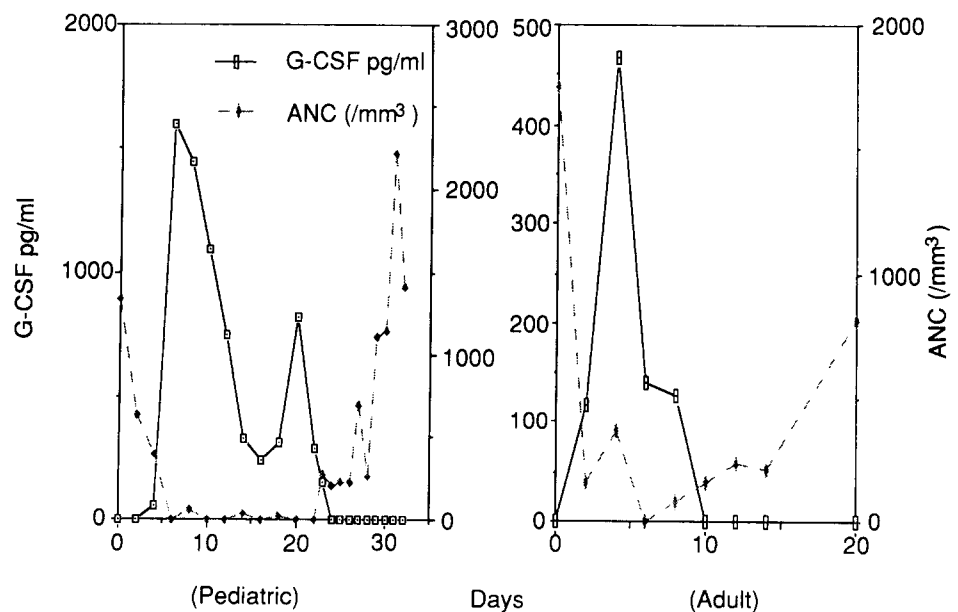
was 16.8 ± 1.8, not significantly different from the mean number in 40 ALLO and AUTO BMTs we previously performed. Once the G-CSF level decreased to 200 pg/mL, an average of 3.6 ± 0.7 days elapsed before the G-CSF level was nondetectable (<50 pg/mL). In addition, once the serum G-CSF level decreased to 200 pg/mL, an average of 6.1 ± 0.9 days elapsed before the ANC was >500/ $\mu$ L for 2 consecutive days. Thus, a decrease in the endogenous serum G-CSF level to less than 200 pg/mL was predictive of complete myeloid engraftment in an average of 6 more days.

## DISCUSSION

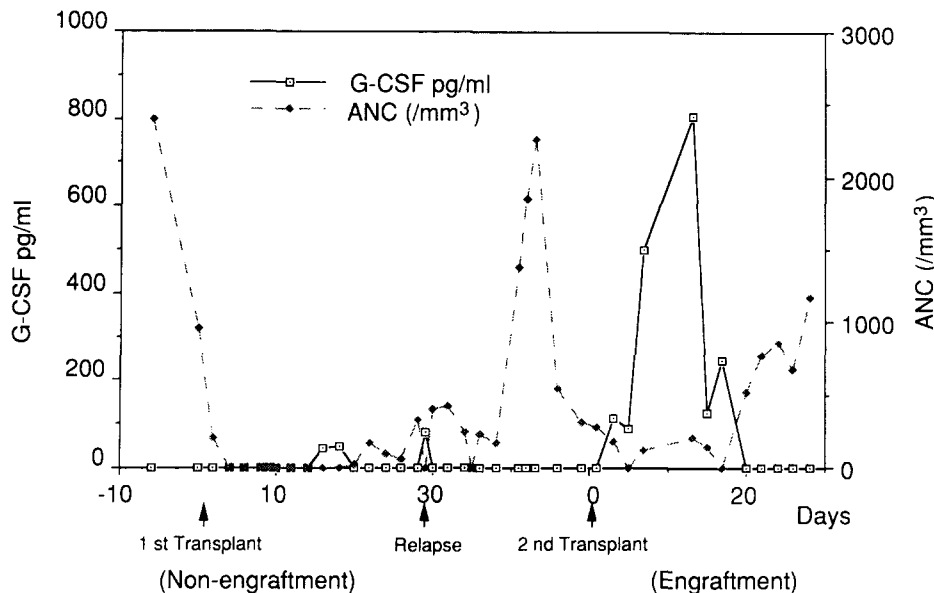
In normal individuals and under steady-state conditions without evidence of neutropenia, the circulating serum G-CSF level is nondetectable (<30 to 50 pg/mL).<sup>10</sup> However, in patients with aplastic anemia, cyclic neutropenia, and congenital forms of neutropenia such as Kostmann's syndrome, the circulating endogenous G-CSF level increases significantly from previous nondetectable levels.<sup>10,11</sup> Previous studies demonstrated hematopoietic colony growth-promoting activities in the plasma of BMT recipients, including growth-promoting activities for granulocyte-macrophage colony production.<sup>12</sup>

The present study demonstrated a significant correlation between the degree of neutropenia post-BMT and endogenous serum G-CSF production. Both children and adults demonstrated a similar inverse relationship between serum G-CSF level and circulating ANC. In addition, there was no significant difference between patients undergoing ALLO or AUTO BMT with regard to the inverse relationship between circulating ANC and serum endogenous G-CSF levels. Neither was there any particular chemoradiotherapy preparative regimen that could be identified as an independent factor in inducing endogenous G-CSF production.

Only one patient in our study failed to demonstrate



**Fig 2.** ANC v circulating endogenous G-CSF levels. Example of a pediatric patient (patient 2) and an adult patient (patient 9) profile of serum G-CSF levels, ANC level v number of days after BMT. G-CSF levels were determined by ELISA.



**Fig 3. Patient profile (patient 3) post-BMT (first) nonengraftment v (second) engraftment: Endogenous serum G-CSF v ANC. Serum G-CSF levels and ANC v number of days after first transplant (nonengraftment) and after second transplant (engraftment). Serum G-CSF levels were determined by ELISA.**

myeloid engraftment after an AUTO BMT. This patient showed no endogenous G-CSF production in the first 20 days after BMT. However, during his second transplant, he developed a significant increase in his endogenous G-CSF level and subsequent recovery of his ANC level. The failure to generate a significant increase in serum G-CSF level during the first transplant also may have been secondary to residual leukemia and inhibition of G-CSF production. Although only one of our patients initially failed to demonstrate myeloid engraftment, this significant early increase in serum endogenous G-CSF level after BMT may be an important predictor of myeloid engraftment.

Our results also suggest that a decrease in endogenous serum G-CSF level to 200 pg/mL is an important prognostic factor in predicting time to final myeloid engraftment. Once the endogenous G-CSF level decreased to 200 pg/mL, only  $6.1 \pm 0.9$  days elapsed before the circulating ANC was  $>500/\mu\text{L}$  for 2 consecutive days. Therefore, the increase and decrease in the endogenous G-CSF level may be an important predictive factor in determining how many days are still required before myeloid engraftment is complete.

Exogenous administration of pharmacologic doses of rhG-CSF have previously been used after both ALLO and AUTO BMT. Sheridan et al<sup>8</sup> administered rhG-CSF after BMT to 15 patients with nonmyeloid malignancies. Neutrophil recovery was demonstrated to be accelerated in G-CSF-treated patients as compared with 18 historical controls. Kitayama et al<sup>6</sup> also administered rhG-CSF to patients after ALLO BMT. Taylor et al<sup>7</sup> administered rhG-CSF for a maximum of 28 days after AUTO BMT in patients with refractory or relapsed Hodgkin's disease. Recovery of ANC  $>500/\mu\text{L}$  was significantly faster in rhG-CSF-treated patients in all three of these BMT studies.

Pharmacologic administration of rhG-CSF results in a very significant increase in circulating G-CSF levels. Sheridan et al<sup>8</sup> demonstrated that patients receiving 20  $\mu\text{g}/\text{kg}/\text{day}$  of rhG-CSF after AUTO BMT, had mean circulating

G-CSF levels of 40,000 pg/mL. This mean level of 40,000 pg/mL was almost 40-fold higher than the peak endogenous G-CSF level demonstrated in our patients when their ANC was  $<200/\mu\text{L}$  for a similar time. Normal endogenous production of G-CSF is derived from monocytes, macrophages, endothelial cells, and fibroblasts. Although significant neutropenia usually occurs after BMT, endogenous production of G-CSF after BMT probably develops from residual endothelial cells, tissue macrophages, and fibroblasts.

Kawakami et al<sup>13</sup> examined the endogenous levels of G-CSF in patients before, during, and after acute infections. The G-CSF level was  $25 \pm 20$  pg/mL in the preinfectious period, but during the acute stage of infection, the endogenous G-CSF level increased to  $731 \pm 895$  pg/mL (range 30 to 3,200 pg/mL). The level returned to normal after the acute stage of infection. Therefore, the endogenous G-CSF levels demonstrated during acute infections<sup>13</sup> are similar to the endogenous levels we demonstrated after BMT.

Recently, circulating serum levels of erythropoietin (Epo) were measured after ALLO and AUTO BMT.<sup>14-16</sup> All three studies also demonstrated an inverse relationship between serum Epo levels and circulating hemoglobin levels. In addition, although the Epo levels were normal during the conditioning and early posttransplantation periods, they were inappropriately low for the degree of anemia during the later stages of BMT, especially in patients with nephrotoxicity.<sup>11,12</sup> However, once complete engraftment occurred, circulating Epo levels again returned to appropriate levels.

The duration and severity of neutropenia in patients undergoing chemotherapy significantly correlates with the percentage of serious bacterial infections.<sup>17</sup> Exogenous rhG-CSF was recently shown to reduce the duration and degree of neutropenia in a number of other clinical conditions (including congenital agranulocytosis, aplastic anemia, cyclic neutropenia) as well as after administration of

myelosuppressive chemotherapy.<sup>6-8,18-22</sup> As demonstrated in this study, endogenous G-CSF production also appears to hasten granulocyte recovery and is correlated with myeloid engraftment after both AUTO and ALLO BMT in both children and adults. Failure to produce endogenous G-CSF may be predictive of a failure to engraft. In addition, an acute increase in endogenous G-CSF production and subsequent decrease in circulating endogenous G-CSF levels may be predictive of the number of days before full myeloid engraftment. Further studies are underway to expand this

pilot study and to correlate the dose-response of exogenous rhG-CSF studies with our endogenous G-CSF levels to determine the most effective dose of rhG-CSF to promote BM myeloid engraftment.

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