A PROSPECTIVE STUDY OF G-CSF PRIMED BONE MARROW AS A STEM CELL
SOURCE FOR ALLOGENEIC BONE MARROW TRANSPLANT IN CHILDREN: A
PEDIATRIC BLOOD AND MARROW TRANSPLANT CONSORTIUM (PBMTC)
STUDY

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Abstract

A prospective multi-center trial was conducted to evaluate the safety and feasibility of granulocyte-colony stimulating factor primed bone marrow (G-BM) in children receiving allogeneic bone marrow transplantation (BMT). Forty two children with a median age of 9.8 years (range 0.8-17) were enrolled. Donors with median age of 9.2 y (range 1.1-22) received 5 mcg/kg/day of subcutaneous G-CSF for 5 consecutive days. Bone marrow was harvested on the fifth day. No donor experienced complications related to G-CSF administration or marrow harvest. Median nucleated (NC) and CD34 cells infused was $6.7 \times 10^8$/kg (range 2.4-18.5) and $7.4 \times 10^6$/kg (range 2-27.6) respectively. Neutrophil and platelet engraftment was at a median of 19 days (13-28), and 20 days (9-44) respectively. Thirteen patients (32%) developed grade II graft-versus-host disease (GVHD) and 5 of 40 evaluable patients (13%) developed chronic GVHD (3 limited and 2 extensive). Higher cell dose was not associated with increased risk of acute or chronic GVHD. Overall survival and event free survival at 2 years were 81% and 69% respectively. Collection of G-BM from pediatric donors is safe, and can result in high NC and CD34 cell doses that facilitate engraftment after myeloablative BMT without a discernable increase in the risk of GVHD.
Introduction:

Bone marrow transplantation is an important therapeutic intervention in children and adults with malignant and non-malignant disorders. Although for many years the most commonly used hematopoietic stem cell source has been bone marrow, in recent years peripheral blood stem cells (PBSC) mobilized with granulocyte-colony stimulating growth factor (G-CSF) have become an alternative source of hematopoietic stem cells. In initial clinical trials, PBSC have been shown to offer the advantage of higher stem cell dose and faster engraftment than unmodified bone marrow \(^1\). PBSC have become the preferred stem cell source for most adults receiving HLA-identical sibling donor transplantation, but are not as widely accepted as a standard stem cell source in pediatric patients. In a recent analysis by the International Bone Marrow Transplant Registry (IBMTR), the use of PBSC in pediatric patients was associated with higher risk of chronic GVHD and transplant-related mortality \(^2\). In addition, apheresis of young children is often challenging and may result in blood product exposure and central line placement for the donor \(^3\).

Several studies have shown that higher bone marrow cell dose is associated with more rapid engraftment, less risk of fatal GVHD and improved survival in both related and unrelated marrow transplants \(^4,5\). Treatment of the bone marrow donor with G-CSF prior to the harvest results in a significant increase of total nucleated cell count and CD34+ progenitor cells. Studies in adults utilizing G-CSF primed bone marrow harvest (G-BM) have shown improved engraftment kinetics compared to conventional marrow, similar to
PBSC, without increased incidence of chronic GVHD $^{6-8}$. Thus, G-BM may offer some or all of the benefits of PBSC compared to conventional bone marrow (BM), with fewer of the risks of PBSC. We conducted a prospective multi-center pilot study to evaluate the safety and feasibility of G-BM in pediatric patients receiving HLA-identical sibling donor marrow transplant.

**Patients and Methods:**

**Patient accrual and characteristics**

Between February 2003 and November 2005, pediatric patients undergoing allogeneic transplantation from an HLA-identical sibling were asked to participate in a pilot study of G-BM. The study was approved by the Pediatric Blood and Marrow Transplant Consortium (PBMTC) scientific committee and the institutional review board of each participating institution, and monitored by the PBMTC Data Safety Monitoring Committee. An informed consent was obtained from all patients, donors or their legal guardians.

**Bone marrow collections**

All donors received 5 mcg/kg/day of G-CSF (Filgrastim, Neupogen®, Amgen, Thousand Oaks, CA) as a single subcutaneous injection for 5 consecutive days. G-BM harvest was performed on the fifth day with a target volume of 15-20 mL/kg of patient’s weight, not to exceed 20 ml/kg of donor’s weight. The bone marrow was infused the same day it was...
collected. Plasma or red cell depletion was performed if there was any ABO incompatibility, following institutional guidelines.

Evaluations and definitions

Bone marrow product and donor peripheral blood were analyzed for CD34+ subsets and T-cell subsets by flow cytometry using previously published methods. Growth factor use was not permitted unless the absolute neutrophil count (ANC) was less than 500 cells/mm³ on day 21 post transplant. Neutrophil engraftment was defined as the first of 3 days with an ANC greater than 500 cells/mm³ after the post transplant nadir. Platelet engraftment was defined as the first of 7 consecutive days with a platelet count greater than 20,000 cells/mm³ without platelet transfusions. Patients receiving a transplant for hematologic malignancies beyond first complete remission were considered as “high risk”, and all others as “standard risk”. Acute and chronic GVHD were graded by Seattle criteria. Patients who died while in relapse after transplantation were categorized as having died of relapse. Patients who died without disease recurrence were categorized as experiencing non-relapse mortality.

Donors were evaluated during the days of G-CSF administration, following bone marrow collection, and 30 days later for any adverse events attributable to G-CSF.

Statistics:

The primary aim of this study was to evaluate safety and feasibility of G-CSF administration to pediatric donors. Additional study outcomes include overall and event-free survival, time to neutrophil and platelet engraftment, incidence of acute and chronic
GVHD, disease risk classification (high vs. standard risk), total nucleated (TNC), CD34 and CD3 cell counts. In addition, each patient was classified as 'standard risk' or 'high risk' according to the definition above.

The associations between risk classification and overall and event-free survival were evaluated by Kaplan-Meier estimates of survival and log-rank tests \(^\text{12}\). Associations between transplant factors and survival were evaluated using Cox proportional hazards regression, and associations with continuous outcomes (e.g., time to engraftment and CD34) were evaluated using the nonparametric Spearman correlation coefficient \(^\text{13,14}\). All tests were judged significant at the 0.05 level.

**Results**

**Study population**

Characteristics of the 42 patients are shown in Table 1. There was a predominance of patients with hematologic malignancies and 17 patients had high-risk disease. Five of those patients had received one (N=4) or 2 prior transplants (N=1). Median follow-up time of surviving patients was 886 days (range 510-1391 days). Median donor age was 9.2 years (range 1.1-22 years). All donors received the prescribed doses of G-CSF without any adverse events except for grade 1 skeletal pain reported in 2 donors that resolved with acetaminophen. None of the donors required hospitalization related to the GCSF administration.
Bone marrow product

The median TNC and CD34+ cell counts were 6.7 x 10^6/kg (range 2.4-18.6) and 7.4 x10^6/kg (range 2.0-27.6) of recipient weight, respectively. The median number of CD3+ cells infused was analyzed in 34 patients and was 26.9x10^6/kg (range 1.5-74). To assess for potential contamination of the collected bone marrow by peripheral blood, the absolute number of CD34+ cells was measured in the blood and bone marrow on the day of the collection in 30 donors. The absolute CD34+ cell count was significantly higher in bone marrow compared to peripheral blood, with a median of 513 cells/µL (116-1005) vs 50 cells/µL (8-247) respectively (p <0.0001).

Engraftment

All patients achieved neutrophil engraftment at a median time of 19 days (range, 13-28 days). Median time to platelet recovery was 20 days (range, 9-44 days). Two patients were excluded from the analysis; one patient relapsed early and went to receive salvage therapy and another died of complications prior to reaching platelet recovery. One patient with severe aplastic anemia experienced secondary graft failure one year post transplant and received a second transplant from the same donor and is alive with complete donor engraftment. There is significant association between total nucleated cell count and both neutrophil and platelet engraftment. Doubling of the total nucleated cell count was associated with a 2.5 day reduction in time to neutrophil engraftment (p=0.01) and a 5 day reduction in time to platelet engraftment (p=0.005). Neither platelet nor neutrophil engraftment was associated with CD34+ cell count.
Acute and chronic graft-versus-host disease

Thirteen of 41 patients developed grade II acute GVHD with a cumulative incidence of 32% (95% CI: 18-46). Grade III-IV acute GVHD was not observed. While higher total nucleated cell count was not associated with acute GVHD (p=0.26), a lower CD34+ cell count was associated with increased risk of grade II acute GVHD (p=0.02). Median CD34 count for patients with or without acute GVHD was $6.0 \times 10^6$/kg versus $10.5 \times 10^6$/kg. There was no association between CD3 count and acute GVHD (p=0.13). Five of 40 evaluable patients developed chronic GVHD with a cumulative incidence of 13% (95% CI: 2-23). Two patients developed extensive and three limited chronic GVHD. There was no association between higher total nucleated cell count, CD34+ cell count and chronic GVHD (p=0.15 and p=0.6, respectively). There was no association between CD3+ cell count and chronic GVHD (p=0.96).

Relapse and survival

Eleven patients relapsed, 7 with high-risk disease and 4 with standard risk disease. Eight of those patients died secondary to disease relapse and 3 patients are alive without disease after salvage chemotherapy. Two patients died of transplant related complications; one from sepsis in the setting of acute GVHD and another from multi-system organ failure. The estimated event free survival (EFS) and overall survival at 2 years was 69% (95% CI: 56-84) and 81% (95% CI: 70-94), respectively. The EFS was significantly better among standard risk patients compared to high-risk patients, 83% (95% CI: 69-100) versus 50% (95% CI: 32-79) (p=0.01). Similarly, overall survival was significantly better
among standard risk compared to high risk patients, 96% (95% CI: 88-100) versus 61% (95% CI: 42-88) (p=0.005). (Figure 1).

**Discussion:**

A number of groups have recently reported on the outcome of patients who received G-CSF mobilized PBSC during HLA matched allogeneic transplantation. These results have consistently shown that use of PBSC as a stem cell source led to more rapid engraftment of both neutrophil and platelets compared to BM. In a randomized trial, the Seattle group reported that allogeneic PBSC resulted in significantly faster neutrophil and platelet engraftment and improved survival without increase in the incidence of acute or chronic GVHD with a relatively short follow-up of one year. This trial included only few pediatric patients since only donors >40kg were included. Other groups have reported significantly higher risk of chronic GVHD in recipients of PBSC transplants. Despite the lack of prospective randomized trials of PBSC in children, a recent study from the PBMT group revealed that 23% of all allogeneic matched sibling transplants in children utilized PBSC as the source for transplant. Results from 3 separate studies in children using PBSC as stem cell source in matched related donor transplants have shown a chronic GVHD disease rate of 63-75%, twice of what is expected in pediatric patients receiving unstimulated bone marrow.

Recently, a retrospective analysis was performed by the IBMTR of 143 PBSC and 630 BM transplants from HLA identical sibling donors in children aged 8 to 20 years with acute leukemias. This analysis revealed significantly higher rates of cGVHD (p=0.001)
and transplant related mortality (p=0.001) in recipients of PBSC, with recipients of BM having significantly better survival than PBSC recipients (p=0.01) \(^2\).

There have been several small single arm and randomized studies of G-BM in adult recipients. Isola et al reported on 10 patients who received G-CSF stimulated allogeneic bone marrow and compared their results to historic bone marrow recipient. The G-BM group attained neutrophil engraftment nine days earlier and platelet engraftment six days earlier than historical controls receiving unstimulated HLA-identical sibling BM \(^7\). Couban reported on 29 allogeneic transplants using G-BM and showed that platelet and neutrophil engraftment was faster than historic controls with unstimulated marrow but both groups had similar length of hospital stay, febrile days, and days on antibiotics \(^6\). A prospective randomized trial comparing G-BM versus G-PBSC found no significant difference in neutrophil or platelet engraftment, but a significantly increased risk of acute (17 vs. 46%) and chronic (27 vs. 77%) GVHD in recipients of PBSC \(^8\). Similar results were reported by Serody et al \(^23\).

We report the first multi-center experience using G-CSF primed bone marrow from pediatric donors as a source of stem cells for transplantation in children. Our study demonstrates the safety of the use of G-CSF in pediatric donors. The youngest donor in our study was 1 year old and only one donor was >18 years of age. Donors tolerated G-CSF administration without reported symptoms except for mild, reversible skeletal pain. Previous studies of G-CSF in pediatric donors have reported a rate of skeletal pain ranging from 11-17.5% \(^3,21\). This higher incidence of pain is probably related to the higher dose of G-CSF (10 mcg/kg) used for PBSC mobilization in these trials. In a recent
study by the PBMTC 97% of donors <6 years and 67% of donors 7-12 years of age required central line placement for PBSC collection. The risk of central line placement in otherwise healthy pediatric donor should be balanced against the risk of bone marrow harvest since both have the risk of general anesthesia.

This trial demonstrates that priming with a lower dose of G-CSF (5 mcg/kg) results in nucleated and CD34+ cells yields that are comparable to PBSC collections and higher than that achieved in BM collections, while avoiding the high CD3+ cell collections typical for PBSC. The number of CD3+ cells collected was similar to unstimulated BM and 10 fold lower than that observed with PBSC. Additionally the high number of nucleated and CD34 positive cell count concentration observed might allow the collection and successful transplant of smaller bone marrow volume, and thus decreasing the morbidity in small pediatric donors. This approach might also be helpful in increasing the number of cells infused when pediatric donors are significantly younger or smaller than the recipients. Time to neutrophil engraftment in our study was longer than that previously reported with G-BM, but our patients did not receive post transplant growth factor support, while other studies utilized post transplant growth factor. Platelet engraftment for our patients was faster than that reported in other pediatric trials.

Our study further demonstrates that infusing higher number of nucleated cells and CD34+ cells is not associated with an increased risk of acute or chronic GVHD. Of note, none of the patients in our study developed grade III or IV acute GVHD. Compared to other pediatric studies, the incidence of chronic GVHD in our study is much lower than that observed using PBSC in children.
The use of higher nucleated cell dose in the bone marrow has been associated with improved survival \(^4\). G-BM can provide a potentially safer alternative for higher cell dose without increasing the risk of GVHD and transplant related mortality observed in children receiving PBSC.

Our study has provided sufficient data to conclude that G-BM is a safe and feasible source of stem cells from related pediatric donors. These promising results have lead to the development of ASCT0631, a Children’s Oncology Group phase III randomized trial comparing G-BM to unstimulated marrow, which will open in late 2007.

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Author contribution: Study design (HF, JL, EN, AW and SG). Data analysis (HF, DB). All authors participated in study conduct, interpretation of data and approval of final manuscript.

The authors report no relevant conflict of interest.

All participating institutions in the Pediatric Blood and Marrow Transplant Consortium (PBMTC) study are listed in the affiliations note on the title page of this article.
References:

Table 1. Patient and Transplant Characteristics

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age in years (range)</td>
<td>9.8 (0.8-17)</td>
</tr>
<tr>
<td>Gender, male/female</td>
<td>22/20</td>
</tr>
</tbody>
</table>

**Diagnosis**

- **High Risk**
  - ALL ≥ CR 2*: 5
  - AML ≥CR 2/refractory*: 6
  - Advanced MDS/JMML: 4
  - NHL CR 2: 2

- **Standard Risk**
  - AML CR 1: 9
  - ALL CR 1: 2
  - CML CP 1: 2
  - MDS-RA: 3
  - Severe Aplastic Anemia: 6
  - Sickle Cell Disease: 1
  - Red Cell Aplasia: 1
  - Metabolic Disorder: 1

**Preparative regimen**

- TBI/Cy ± VP-16: 12
- BU/CY ± other: 24
- Cy/ATG: 6

**GVHD prophylaxis**

- CSP/MTX: 26
- FK/MTX: 7
- CSP or MTX: 4
- CSP/Prednisone: 1
- FK/MMF: 3

*5 of those patients had prior allogeneic transplants; $One patient received a syngeneic transplant and had no GVHD prophylaxis. Abbreviations: BU: Busulfan; CY: Cyclophosphamide; TBI: Total body irradiation 1200-1320 cGy; CSP: Cyclosporine; FK: Tacrolimus; MTX: Methotrexate; ATG: Antithymocyte Globulin
Figure 1. Kaplan-Meier estimates of the percentage of event free (A) and overall survival (B) at 24 months

A.

B.
A prospective study of G-CSF primed bone marrow as a stem cell course for allogeneic bone marrow transplant in children: a Pediatric Blood and Marrow Transplant Consortium (PBMTC) study

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