CD34⁺ Cell Dose Predicts Survival, Posttransplant Morbidity, and Rate of Hematologic Recovery After Allogeneic Marrow Transplants for Hematologic Malignancies

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After autologous or allogeneic transplants of peripheral blood stem cells (PBSC), an adequate dose of CD34⁺ cells is necessary to ensure early and sustained hematopoietic engraftment and favorable clinical outcome. There are no comparable data on the relationship between CD34⁺ cell dose and recovery after allogeneic bone marrow transplants (BMT). Twenty-eight patients with hematologic malignancies received a BMT from an HLA-identical sibling, using T-cell depletion and cyclosporin for graft-versus-host disease prophylaxis and delayed donor lymphocyte transfusions in an attempt to prevent leukemia relapse. The treatment-related mortality (TRM), primarily due to infections and cytopenias, was significantly higher for 13 patients receiving less than 1 x 10⁶ CD34⁺ cells/kg (64.9% ± 12.8% vs 6.9% ± 6.4%, P = .003). Survival at a median follow-up of 1 year was also lower in the group receiving less than 1 x 10⁶ CD34⁺ cells/kg (30.8% ± 12.8 vs 74.3% ± 13.7%, P = .005). The CD34⁺ cell dose was the only variable significantly associated with TRM. The dose of CD34⁺ cells also correlated with speed of hematopoietic recovery. Patients receiving more than 2 x 10⁶ CD34⁺ cells/kg showed significantly earlier recovery of monocytes and a trend for earlier recovery of lymphocytes. They achieved platelet and red blood cell transfusion independence earlier, required less granulocyte colony-stimulating factor support during ganciclovir treatment, and spent fewer days in the hospital after transplantation. These results suggest that, for allogeneic T-cell-depleted BMT, the higher CD34⁺ cell doses may improve outcome in engrafting patients.

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MATERIALS AND METHODS

Patients. Of 31 consecutive patients undergoing allogeneic BMT between September 1993 and September 1995, 28 patients who received an HLA-matched sibling transplant and had CD34⁺ cell quantitation available on their grafts were studied. Ages ranged from 12 to 58 years. Twenty had chronic myelogenous leukemia (CML), with 14 in chronic phase, 3 in accelerated phase, and 3 in blast transformation. Three had myelodysplastic syndrome (MDS) in transformation to acute myeloid leukemia. Three had multiple myeloma (MM), and two had acute myeloblastic leukemia (AML), with one in second remission and one in resistant relapse at the time of BMT. Eleven patients transplanted with progressing disease, CML in blast crisis (3), transformed MDS (3), patients with marrow fibrosis in accelerated phase of CML (3), AML in relapse (1), and myeloma relapsing after autologous BMT (1), were categorized as high risk for early transplant mortality. The remaining 17 (CML CP [14], MM responsive to chemotherapy [2], and AML in second remission [1]) were considered to be at standard risk. Patients gave written informed consent to be treated under one of three local institutional review board approved protocols (93-H-212, 94-H-0092, and 94-H-0182).

BMT. Twenty-seven patients were conditioned with 120 mg/kg cyclophosphamide and 1.360 cGy total body irradiation (TBI) in 8 fractions. Busulfan at 16 mg/kg replaced TBI in one patient with MM who had received previous TBI and autologous BMT. To accomplish T-cell depletion, BM was processed by methods previously described, including an initial automated concentration procedure followed by centrifugal counterflow elutriation of the resulting cell concentrate using the Beckman JE-5.0 rotor and chamber (Beckman, Palo Alto, CA). Elutriation fractions were collected at flow rates of 110 ml/min and 140 ml/min at 3,000 rpm. Cells remaining in the chamber were collected by turning the rotor off (RO fraction) and flushing out the chamber. The marrow graft consisted of the entire RO elutriation fraction, sometimes with a portion of the 140 or 110 fraction added to achieve the target T-cell dose of 2 x 10⁶/kg recip-

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ent body weight. For 5 cases in which the CD34+ cell dose was less than 0.5 × 10^6/kg in the RO fraction, all fractions were combined and administered to the patient as a T-replete graft. All patients received cyclosporin (3 mg/kg intravenously [IV]) from day -4, which was converted to an oral dose of 10 mg/kg when tolerated and was continued for a minimum of 3 months after BMT. To confer graft-versus-leukemia reactivity after T-depleted BMT, donor T-cell transfusions of 2 × 10^6/kg and 5 × 10^6/kg were administered on days +30 and +45, respectively, to patients who did not develop grade II or greater graft-versus-host disease (GVHD).19 Patients who had less than 0.2 × 10^9/L neutrophils on day +21 received granulocyte colony-stimulating factor (G-CSF) at 5 μg/kg/d until the count exceeded 5.0 × 10^9/L for 2 consecutive days. G-CSF was also administered during ganciclovir treatment, if needed, to maintain a neutrophil count greater than 1 × 10^9/L. Red blood cell (RBC) and platelet transfusions were administered to maintain a hemoglobin level greater than 9 g/dL and a platelet count greater than 10 × 10^9/L.

Hospital discharge and readmission criteria. Patients were discharged from the hospital after transplantation when they were afebrile, had no active GVHD, had a neutrophil count greater than 0.5 × 10^9/L, had a platelet transfusion requirement of less than 2 transfusions per week, and were in positive weight balance without a requirement for parenteral feeding. During the first 100 days after transplantation, patients were readmitted to the hospital for fever, new onset GVHD grade II or more, cytomegalovirus (CMV) reactivation, and intractable nausea, vomiting, diarrhea, or weight loss.

CMV surveillance, prophylaxis, and treatment. Patients and donors were screened for antibodies to CMV before BMT. All patients received CMV-negative blood products. For antiviral prophylaxis, all CMV-seropositive patients and CMV-seronegative patients with seropositive donors received acyclovir at 500 mg/m² IV every 8 hours from day -5 to day +30 and then 800 mg orally every 6 hours from day +31 to day +100. CMV reactivation, defined as detection of pp65 antigen in blood or culture of CMV from blood or broncho-alveolar lavage, was treated with ganciclovir at 5 mg/kg IV twice daily for 10 to 14 days, followed by 5 mg/kg three times weekly until day +100. Ganciclovir-induced neutropenia was treated with G-CSF at 5 μg/kg/d IV three times a week. All patients received IV Ig at 500 mg/kg weekly and the dose was increased to 1000 mg/kg weekly upon CMV reactivation.

CMV interstitial pneumonia (IP) was treated with ganciclovir at 5 mg/kg twice daily and IV Ig at 500 mg/kg daily. Foscarnet was added if there was no response. Lymphocyte transfusions from CMV-seropositive donors were administered as immunotherapy for CMV IP in 3 patients.

Flow cytometry. Samples of each elutriation fraction and of the final narrow graft were adjusted to a cell concentration of 5 to 10 × 10^6/mL. One hundred microliters of each cell suspension was incubated with monoclonal antibodies for 20 minutes at 4°C. After incubation, RBCs were lysed with NH₄Cl and washed with phosphate-buffered saline. Flow cytometry was performed using the FACScan (Becton Dickinson, Mountain View, CA). For both the CD34+ cell and the CD3+ T-cell quantifications, two different gating methods were used during acquisition, depending on the differential counts of the elutrated fractions.14 For samples with a mononuclear cell (MNC) count of less than 10%, a region (Rr) was drawn to include all MNCs, and live gate acquisition was performed only on cellular events that fell into that region. In general, 10,000 events were acquired in the live gate, with a minimum of 50,000 total events scanned. For samples with a MNC count of greater than 10%, open gate acquisition was performed for a minimum of 10,000 events in the entire cell population. If a CD34+ cell cluster was not clearly visible, up to 100,000 events were acquired. The absolute numbers for CD3+ T cells and CD34+ hematopoietic progenitor cells were calculated by multiplying the percentage of positive cells (minus isotype control values) by the percentage live gate (if used) and by the total nucleated cell content of the fraction, as determined by conventional automated cell counting (Coulter S-plus V; Coulter, Hialeah, FL).

Statistics. Kaplan-Meier analysis was performed to determine actuarial probabilities of transplant-related mortality (TRM) and survival.15 Differences between survival were calculated using the log rank test. To compare hematopoietic recovery, the highest count achieved in each 7-day period in the first 10 weeks after BMT was used to calculate the mean cell count for three CD34+ cell dose groups (<1, 1 to 2, and >2 × 10^6 CD34+ cells/kg). Differences between the groups in blood counts, transfusion and growth factor requirements, and inpatient stay were compared using unpaired t-tests for specific time points.

RESULTS

Transplant outcome and CD34+ cell dose. Sixteen patients survive between days 180 and 850 post-BMT. Twenty-four of 26 patients at risk because either the recipient or donor were seropositive for CMV reactivated CMV and were treated with ganciclovir. Twelve developed acute GVHD grade II or more and required high-dose steroid treatment. All patients engrafted.

Twelve patients died: 9 of transplant-related complications and 3 from disease relapse. Transplant-related deaths occurred between 28 and 147 (median, 38) days after transplantation. Relapse deaths occurred on days 64, 113, and 504. Transplant-related deaths were from infection in 7 patients (CMV IP [5], adenovirus [1], and aspergillosis [1]) or associated with cytopenias (intracranial hemorrhage on day 27 [1] and platelet transfusion reaction for prolonged thrombocytopenia after ganciclovir treatment on day 147 [1]).

Table 1 shows results ranked according to the dose of CD34+ cells received. Five patients received CD34+ cell doses of 2.0 to 3.8 × 10^6/kg, 10 between 1.06 and 1.6 × 10^6, and 13 less than 1.0 × 10^6. Eight of the transplant-related deaths occurred in the group of patients receiving less than 1.0 × 10^6/kg CD34+ cells, 1 death in the group receiving 1 to 2 × 10^6/kg, and no deaths in the group receiving more than 2 × 10^6 CD34+ cells/kg.

Factors affecting TRM and survival. Figure 1A shows the probability of TRM (ie, excluding relapse as a cause of death) according to the CD34+ cell dose received. The group receiving less than 1 × 10^6 CD34+ cells/kg had a significantly higher risk of transplant-related death (64.9% ± 12.8% vs 6.9% ± 6.4%, P = .003). Survival was also significantly greater in the group receiving more than 1.0 × 10^6 CD34+ cells/kg (74.3% ± 13.7% vs 30.8% ± 12.8%, P = .005; Fig 1B).

Table 2 shows the actuarial probability of TRM for variables known to be associated with transplant outcome. CD34+ cell dose was the only factor significantly associated with TRM. Poor prognosis categories of age greater than 30 years, high-risk disease (defined in the Materials and Methods), and grade II or more acute GVHD did not significantly affect TRM. Lymphocyte doses administered ranged from 0.12 × 10^9/kg to 49.8 × 10^9/kg. Eleven patients received less than 2 × 10^9 CD3+ cells/kg and 17 received 2 × 10^9 or more, including 5 patients who received T-cell-replete grafts. Within this range there was no significant effect of lymphocyte dose on TRM. To determine whether
CD34+ dose was independent from lymphocyte dose and disease risk, the probability of TRM was compared between high and low CD34+ cell doses and high versus low lymphocyte doses (including or excluding T-replete BMT) and high-risk versus standard-risk malignancies. The only determinant significantly affecting outcome in these subgroups was CD34+ cell dose and hematologic recovery.

CD34+ cell dose and hematologic recovery. Recovery of neutrophils, monocytes, and lymphocytes was compared for the CD34+ cell dose ranges less than 1, 1 to 2, and greater than 2 × 10⁶/kg (Fig 2A, B, and C). Monocyte recovery occurred significantly earlier in the group of patients receiving greater than 2 × 10⁶ CD34+ cells. There was a trend for earlier recovery of lymphocytes in the same group. There was no difference in recovery rates of neutrophils. Quality of hematologic recovery, measured as weeks from BMT to independence from platelet or RBC transfusions and G-CSF dose received, was compared for the three CD34+ cell dose ranges. Figure 3 shows that patients receiving greater than 2 × 10⁶ CD34+ cells/kg required significantly less G-CSF to maintain neutrophil counts during ganciclovir treatment for 2 days versus 17.5 days (1 × 10⁶ CD34+ cells/kg, P = .003) and 27 days (≤1 × 10⁶ CD34+ cells/kg, P = .0015). There was also a trend for patients receiving greater than 2 × 10⁶/kg CD34+ doses to achieve platelet and RBC transfusion independence 4 weeks and 3 weeks sooner, respectively, than patients receiving lower CD34+ cell doses. In addition, patients receiving greater than 2 × 10⁶/kg CD34 cells experienced fewer inpatient days during the first 100 days after transplantation, than the other patients receiving 1 to 2 or less than 1.0 × 10⁶/kg CD34+ cells (39 ± 52 and 62, respectively, P = .05; Fig 4).

**DISCUSSION**

Various parameters have been used as surrogates to estimate the stem cell dose in studies of autologous and allogeneic transplantation. In the past, most investigators have relied on the total nucleated cell count of the marrow to predict adequacy of engraftment cell dose. Standard harvesting techniques that provide in the range of high and low CD34+ cell doses (Fig 2A, B, and C). Monocyte recovery occurred significantly earlier in the group of patients receiving greater than 2 × 10⁶ CD34+ cells. There was a trend for earlier recovery of lymphocytes in the same group. There was no difference in recovery rates of neutrophils. Quality of hematologic recovery, measured as weeks from BMT to independence from platelet or RBC transfusions and G-CSF dose received, was compared for the three CD34+ cell dose ranges. Figure 3 shows that patients receiving greater than 2 × 10⁶ CD34+ cells/kg required significantly less G-CSF to maintain neutrophil counts during ganciclovir treatment for 2 days versus 17.5 days (1 × 10⁶ CD34+ cells/kg, P = .003) and 27 days (≤1 × 10⁶ CD34+ cells/kg, P = .0015). There was also a trend for patients receiving greater than 2 × 10⁶/kg CD34+ doses to achieve platelet and RBC transfusion independence 4 weeks and 3 weeks sooner, respectively, than patients receiving lower CD34+ cell doses. In addition, patients receiving greater than 2 × 10⁶/kg CD34 cells experienced fewer inpatient days during the first 100 days after transplantation, than the other patients receiving 1 to 2 or less than 1.0 × 10⁶/kg CD34+ cells (39 ± 52 and 62, respectively, P = .05; Fig 4).
rate of hematologic recovery has been established, and prediction of transplant outcome breaks down in mismatched transplants and when the marrow is manipulated. As a possibly more relevant measure of stem cell function, CFU-GM assays have been used. These assays take 12 to 14 days to determine of cell collection and correlates with colony forming capacity of recipient body weight. For BM allografts, there is little information on the relationship of CD34+ cell dose with survival.1,2 For PB allografts, the lowest CD34+ cell dose resulting in complete and sustained engraftment has been reported to be 2.5 × 10^6/kg of recipient body weight.11 For BM allografts, there is little information on the relationship of CD34+ cell dose with outcome. Furthermore, in comparing CD34+ cell numbers with engraftment, a number of factors with a profound modifying influence on engraftment must be considered. These are the degree of histocompatibility between donor and recipient, the nature of the preparative regimen, T-lymphocyte depletion or other manipulations, and the source of the stem cells, ie, whether from marrow, PB, or umbilical cord blood.20

In our study, we considered the CD34+ cell content of the graft to be especially relevant, because the conditions for engraftment were limiting, first because the marrow was T-cell--depleted and second because the elutriation procedure was associated with considerable stem cell loss. We found the CD34+ cell dose to be the only factor significantly predicting TRM. None of the other variables (age, disease risk, lymphocyte dose of the graft, and grade II or more acute GVHD) independently affected TRM. Our results may have been influenced by the small number of patients studied and therefore should be considered suggestive rather than definitive. In previous studies, T-cell depletion is reported to be associated with delayed immunologic reconstitution14 and active CMV infection early after transplantation.22 This may have been the reason for the frequent and early reactivation of CMV in this series. Our data suggest that, in T-depleted BMT, increasing the CD34+ cell dose may favorably affect transplant outcome by reducing death from infection of all types. Of the 8 transplant-related deaths in recipients of CD34+ cell doses less than 1 × 10^6/kg, 6 were from infection (CMV IP [4], adenovirus [1], and aspergillus [1]). Only 1 patient receiving more than 1 × 10^6 CD34+ cells/kg died (from CMV IP). It is not clear why a low CD34+ cell dose should correlate with an increased risk of fatal viral infections. It is possible that the earlier recovery of lymphocytes and monocytes we observed with higher CD34+ cell doses could reduce mortality from infection by improving monocyte-related antigen presentation of infectious agents to T cells.

The CD34+ cell dose appeared to affect hematologic reconstitution in several aspects. Patients receiving higher CD34+ cell doses required significantly less G-CSF support during ganciclovir administration and showed a trend for

![Fig 1. Actuarial probability of TRM (A) and overall survival (B) for patients receiving less than 1 or more than 1 × 10^6 CD34+ cells/kg.](http://www.bloodjournal.org/content/102/11/3226/F1)

Table 2. Risk Factors for TRM

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>% TRM (± SD)</th>
<th>P</th>
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<tbody>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
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<tr>
<td>&lt;30</td>
<td>12</td>
<td>26 (12.5)</td>
<td>.3</td>
</tr>
<tr>
<td>&gt;30</td>
<td>16</td>
<td>44.5 (12.5)</td>
<td>.35</td>
</tr>
<tr>
<td>Disease risk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>17</td>
<td>30 (11)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>11</td>
<td>48 (15.5)</td>
<td></td>
</tr>
<tr>
<td>Acute GVHD grade</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>O-I</td>
<td>16</td>
<td>32.2 (11.6)</td>
<td>.8</td>
</tr>
<tr>
<td>II-IV</td>
<td>12</td>
<td>41.7 (14)</td>
<td></td>
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<tr>
<td>Lymphocyte dose (&gt;10^5/kg)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt;2</td>
<td>11</td>
<td>28.6 (13.4)</td>
<td>.6</td>
</tr>
<tr>
<td>≥2</td>
<td>17</td>
<td>41.5 (12)</td>
<td>.4</td>
</tr>
<tr>
<td>T depletion</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>T-</td>
<td>23</td>
<td>40.5 (10)</td>
<td></td>
</tr>
<tr>
<td>T+</td>
<td>5</td>
<td>20 (18)</td>
<td></td>
</tr>
<tr>
<td>CD34 dose (&gt;10^6/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>13</td>
<td>64.9 (12.8)</td>
<td>.003</td>
</tr>
<tr>
<td>&gt;1</td>
<td>15</td>
<td>6.9 (6.4)</td>
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earlier platelet and RBC transfusion independence. The absence of any effect of CD34+ cell dose on neutrophil recovery may be explained by the routine use of G-CSF for patients with neutropenia. The small group of patients receiving greater than 2 × 10^6 CD34+ cells/kg also had fewer inpatient days. There was no effect of CD34+ cell dose on disease relapse. Predictably, there was a higher mortality in the high-risk patient group. However, Table 3 shows that the CD34+ cell dose appeared to influence TRM in both high- and standard-risk patient groups. Similarly, the dose of lymphocytes in this largely T-depleted transplant group did not affect transplant outcome. Given the small sample size and the retrospective nature of the study, these findings should be interpreted with caution; however, a large analysis of CD34+ cell dose as an independent variable for transplant outcome in T-cell-depleted BMT is warranted.

### Table 3. Actuarial Probability of TRM for High and Low CD34 Dose Groups According to Lymphocyte Dose

<table>
<thead>
<tr>
<th>Lymphocyte Dose</th>
<th>% TRM (±SD) Including T-Replete</th>
<th>% TRM (±SD) Excluding T-Replete</th>
<th>Disease Risk</th>
<th>% TRM (±SD)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>High CD34 (&gt;1.0 × 10^6/kg)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>High risk</td>
<td>0 (0)</td>
<td>NS</td>
</tr>
<tr>
<td>&lt;2 × 10^6/kg</td>
<td>11 (10.5)</td>
<td>14 (0)</td>
<td>Low risk</td>
<td>10 (8.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Low CD34 (&lt;1.0 × 10^6/kg)</td>
<td>72.2 (16)</td>
<td>85.7 (13)</td>
<td>High risk</td>
<td>75 (20)</td>
<td>NS</td>
</tr>
<tr>
<td>&lt;2 × 10^5/kg</td>
<td>90 (25)</td>
<td>50 (25)</td>
<td>Low risk</td>
<td>97 (19)</td>
<td>NS</td>
</tr>
</tbody>
</table>

For disease risk, see the Materials and Methods. Abbreviation: NS, not significant (P > 2).

Fig 2. Hematopoietic recovery after transplantation according to the CD34+ cell dose received. (A) Lymphocytes, (B) monocytes, and (C) neutrophils. (—) Greater than 2 × 10^6 CD34+ cells/kg; (—) 1 to 2 × 10^6/kg; and (—) less than 1 × 10^6/kg. The mean counts for each of the three CD34+ cell dose groups and P values at different timepoints are shown.
These results suggest that, even in engrafting patients, the CD34+ cell dose may affect early mortality after BMT. Furthermore, a higher CD34+ cell dose may reduce the cost of marrow and blood stem cell transplants by minimizing transfusion and growth factor requirements and time spent in hospital. It is likely that CD34+ cell doses in unmanipulated transplants of blood and marrow are usually well in excess of $10^6$/kg. However, stem cell loss from physical methods used to deplete T cells or select CD34+ cells can reduce the stem cell dose infused to suboptimal levels. Our findings suggest that further studies evaluating CD34+ cell dose in a large patient cohort are needed. In designing new T-cell–depleted BMT protocols it may be prudent to ensure that CD34+ cell doses much greater than $1 \times 10^6$/kg are administered in HLA-matched allogeneic BMT. Increased CD34+ cell collections can now be readily achieved with G-CSF–stimulated PB progenitor cell collections from normal donors24 or by combining allogeneic BM and CD34+ blood cells.25 These approaches could compensate for CD34+ cell loss due to T-cell depletion or other manipulations and allow the administration of sufficient T-cell–depleted stem cells for optimum results.

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