We have studied the impact of cell dose on short- and long-term graft function and outcome in 905 patients undergoing an unmanipulated allogeneic bone marrow transplantation (BMT) from an HLA-identical sibling (n = 735), a one-antigen mismatched related donor (n = 35), or a matched unrelated donor (n = 135). Median number of nucleated cells infused was 3.4 × 10^8/kg (25th percentile 2.4 × 10^8/kg, 75th percentile 5 × 10^8/kg). Patients were stratified according to cells infused in 3 groups: ≤2.4 × 10^8/kg (n = 247; low dose); >2.4 × 10^8/kg and ≤5 × 10^8/kg (n = 452; intermediate dose); and >5 × 10^8/kg (n = 206; high dose). Patients receiving high cell dose had significantly higher platelet counts on days +20, +50, +100, +180, and +365 after BMT (P < .01) and higher white blood cell counts on days +50, +100, and +180 after BMT (P < .01) as compared with other patients. The actuarial 5-year transplant-related mortality (TRM) was 41% versus 36% versus 28% (P = .01); overall survival was 45% versus 51% versus 56% (P = .0008); and disease-free survival was 41% versus 42% versus 48%, respectively, (P = .04) in patients receiving low, intermediate, or high cell dose. The cell dose effect was more pronounced in patients older than 30 years of age, with progenitor cells in PB grafts leading to faster hematologic recovery, with little influence on transplant mortality.

In conclusion, transplantation of high marrow cell dose is associated with reduced transplant mortality and improved survival and results in improved graft function both short and long term. (Blood. 2002;100:3930-3934)

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Table 1. Clinical data

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of patients</th>
<th>Median age, y (range)</th>
<th>Donor-recipient sex match, no. of patients (%)</th>
<th>Female to male</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAAs, No. of patients (%)</td>
<td>905</td>
<td>30 (1-66)</td>
<td></td>
<td>249 (28)</td>
<td>656 (72)</td>
</tr>
<tr>
<td>CML, No. of patients (%)</td>
<td>55 (6)</td>
<td>31 (2-77)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AML, No. of patients (%)</td>
<td>905 (100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALL, No. of patients (%)</td>
<td>163 (18)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDS, No. of patients (%)</td>
<td>61 (7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPDs, No. of patients (%)</td>
<td>55 (6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Donor type, No. of patients (%)**
- HLA-identical sibling: 735 (81)
- Mismatched related donor: 35 (4)
- Matched unrelated donor: 135 (15)

**Donor age, Median (Range)**
- Median donor age: 31 (2-77)

**Disease, No. of patients (%)**
- SAA: 94 (10)
- CML: 20 (33)
- AML: 234 (26)
- ALL: 163 (18)
- MDS: 61 (7)
- LPDs: 55 (6)

**Median follow-up for surviving patients, d (range)**
- 2510 (88-9094)

**Median follow-up for deceased patients, d (range)**
- 124 (1-6533)

**TBI, No. of patients (%)**
- No patients (%)
- First CR/CP: 448 (55)
- TBI, No. of patients (%)
- Yes: 656 (72)
- No: 249 (28)

**Interval between diagnosis and BMT, d (range)**
- 326 (22-6240)

**Median follow-up for deceased patients, d (range)**
- 124 (1-6533)

**Median follow-up for surviving patients, d (range)**
- 2510 (88-9094)

SAA indicates severe aplastic anemia; CML, chronic myeloid leukemia; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplasia; LPDs, lymphoproliferative disorders; CR, complete remission; CP, chronic phase; TBI, total body irradiation; and BMT, bone marrow transplantation.

The disease state is evaluated only in leukemia patients (n = 811). and myelodysplasia (MDS, n = 61). Of the 811 leukemia patients, 448 patients (55%) received allogeneic BMT in first remission or in first chronic phase, and 363 (45%) were classified as advanced. Patient characteristics are summarized in Table 1.

Conditioning regimen and graft-versus-host disease prophylaxis

Eighty-five percent (10%) were conditioned with cyclophosphamide (CY) 200 mg/kg alone; 651 patients (72%) received CY (120 mg/kg) and total body irradiation (TBI: 9.9-12 Gy); and 169 patients (18%) were prepared with thiotepa/CY- or busulfan/CY-containing regimens. Acute graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporin A (CyA) with or without methotrexate (MTX). Intravenous CyA (1-5 mg/kg daily) was administered until day +28 and then switched to the oral route (12.5 mg/kg daily). Methotrexate was administered at doses of 10 to 15 mg/m² on day +1 after BMT and 8 to 10 mg/m² on days +3, +6, and +11 after BMT. Acute and chronic GVHD were graded according to standard criteria.

Stem cell harvest and infusion

Bone marrow (BM) was collected using small-volume (2 mL) aspirations, 20 to 25 mL/kg of recipient body weight, under general anesthesia; it was processed through small-gauge needles (20 G) to avoid losing cells in the infusion filters. In cases of major ABO incompatibility, the BM was not depleted of red cells, but patients’ isohemagglutinins were reduced to a titer of 1:16 or less in saline, with 1 or 2 plasma exchanges. BM was administered intravenously after completion of the conditioning regimen.

Graft function

Platelet and white blood cell (WBC) counts were used to evaluate graft function, as were hemoglobin levels.

Statistical analysis

Transplant-related mortality (TRM) is defined as death due to causes unrelated to the underlying disease: Patients relapsing are censored as surviving at the time of relapse. Disease-free survival is defined as the probability of being alive free of disease; events are death in remission, relapse, and death due to the underlying disease, whichever occurs first. The Student t and Mann-Whitney tests were used for continuous variables and the χ² test for 2 × 2 tables. Actuarial probabilities of transplant-related mortality and overall survival were calculated with the Kaplan-Meier method, and the log-rank test was used to evaluate the differences between curves. The following factors were studied in multivariate Cox analysis for potential effect on transplant-related mortality rates: donor type, donor/recipient age, type and disease phase, cell dose, and conditioning regimen. The number-cruncher software (NCSS, version 5.0; JL Hintze, Kaysville, UT) was used to perform the analysis.

Results

Clinical data of patients receiving a low, intermediate, or high cell dose

The median nucleated cells infused was 3.4 × 10⁸/kg (25th percentile 2.4 × 10⁸/kg, 75th percentile 5 × 10⁸/kg). Patients were stratified into 3 groups according to low, intermediate, or high cell dose: ≤ 2.4 × 10⁸/kg (n = 247); > 2.4 × 10⁸/kg and ≤ 5 × 10⁸/kg (n = 452), and > 5 × 10⁸/kg nucleated cells (n = 206).

Table 2 outlines the clinical characteristics of patients in the 3 groups. Patients in the high cell dose group were significantly older (P = .0005), had significantly older donors (P = .001), and comprised significantly more alternative donor transplants (P = .05) and fewer patients receiving TBI (P = .000 01). Notably, all of these factors have a negative influence on TRM in univariate χ² analysis: recipient age more than 30 years (TRM 38% vs 29%, P = .004), donor age 30 or more years (TRM 38% vs 28%, P = .003), alternative donor transplants (TRM 44% vs 30%, P = .0004), and use of non-TBI regimens (TRM 36% vs 32%, P = .2).

Hematopoietic recovery

Platelet recovery is outlined in Figure 1 in patients stratified according to the cell dose received (low, intermediate, or high):

Table 2. Clinical data according to the cell dose infused at transplantation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low dose: 2.4 × 10⁸/kg</th>
<th>Intermediate dose: &gt; 2.4 × 10⁸/kg and ≤ 5 × 10⁸/kg</th>
<th>High dose: &gt; 5 × 10⁸/kg</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient age more than 30 y</td>
<td>37 (83)</td>
<td>50 (226)</td>
<td>55 (124)</td>
<td>.0005</td>
</tr>
<tr>
<td>Donor age more than 31 y</td>
<td>39 (88)</td>
<td>49 (225)</td>
<td>55 (16)</td>
<td>.001</td>
</tr>
<tr>
<td>Advanced-phase disease*</td>
<td>29 (105)</td>
<td>47 (170)</td>
<td>24 (88)</td>
<td>.1</td>
</tr>
<tr>
<td>Interval between diagnosis and BMT more than 365 d</td>
<td>52 (112)</td>
<td>51 (224)</td>
<td>45 (98)</td>
<td>.3</td>
</tr>
<tr>
<td>Alternative donor</td>
<td>13 (30)</td>
<td>21 (94)</td>
<td>20 (46)</td>
<td>.05</td>
</tr>
<tr>
<td>CML diagnosis</td>
<td>28 (63)</td>
<td>35 (159)</td>
<td>33 (76)</td>
<td>.2</td>
</tr>
<tr>
<td>TBI regimen</td>
<td>83 (187)</td>
<td>74 (338)</td>
<td>58 (133)</td>
<td>.0001</td>
</tr>
</tbody>
</table>

BMT indicates bone marrow transplantation; CML, chronic myeloid leukemia; and TBI, total body irradiation.

*The disease phase is evaluated only in leukemia patients.
Patients in the latter group had higher platelet counts at all time points up to 1 year after transplantation, and the difference was significant at the $P < .01$ level. Similar results were obtained when looking at WBC counts (Figure 2) on days +20, +50, and +180 after BMT ($P < .01$). Hemoglobin levels were comparable at different time points after transplantation.

### Cell dose and transplant-related mortality

The actuarial 5-year transplant-related mortality (TRM) was 41% versus 36% versus 28%, respectively, ($P = .01$) in patients receiving low, intermediate, or high cell dose (Figure 3). Crude TRM within day +100 was 24% for patients receiving a low cell dose, 19% for intermediate, and 16% for a high cell dose ($P = .008$). Crude TRM for patients surviving 100 days was 23%, 17%, and 11%, respectively, in the 3 groups ($P = .01$). The cell dose effect on TRM was more pronounced in patients with advanced-phase disease (47% vs 33% vs 26%, $P < .008$) as compared with patients with early-phase disease (32% vs 31% vs 21%, $P = .1$). The same was true for older patients (age $\geq$ 30 years; 56% vs 35% vs 27%, $P < .0001$) as compared with younger patients (age $< 30$ years; 31% vs 32% vs 20%, $P = .1$). The cell dose effect on TRM was seen in patients grafted from an alternative donor (62% vs 44% vs 33%, $P = .004$) as well as in patients grafted from an HLA-identical sibling (37% vs 31% vs 22%, $P = .006$). When patients were stratified according to diagnosis, the cell dose effect on TRM was most significant in patients with CML (52%, 39%, 18%, respectively, for low, intermediate, high cell dose; $P = .001$), MDS (39%, 35%, 25%; $P = .006$), and chronic lymphoproliferative disorders (70%, 22%, 20%; $P = .01$) as compared with patients with acute leukemia (29%, 26%, 21%; $P = .3$) and marrow failure (52%, 42%, 32%; $P = .2$). Acute GVHD was scored as grade 0, I, II, III, and IV, respectively, in 92 (10%), 332 (37%), 342 (38%), 104 (11%), and 35 (4%) patients. Acute GVHD grade III to IV was 42% and 32%, $P = .2$. Chronic GVHD was scored as absent, limited, or extensive, respectively, in 672 (74%), 186 (21%), and 47 (5%) patients and was not different in the 3 groups ($P = .2$).

### Survival and causes of death

The actuarial 5-year overall survival was significantly different in patients receiving low, intermediate, or a high cell dose: 45% versus 51% versus 56% ($P = .0008$; Figure 4). Deaths due to
GVHD and infections were 28% versus 23% versus 16%, respectively, in the 3 groups, \( P = .009 \). There was also a difference in the risk of being infected on day +30 after transplantation: Bacterial infections were seen in 16%, 14%, and 10% in the 3 groups (\( P = .1 \)); fungal infections in 32%, 11%, and 9% (\( P = .001 \)); and viral infections in 13%, 5%, and 4% (\( P = .01 \)), respectively, in the low, intermediate, and high group. Causes of TRM other than GVHD and infections were comparable (\( P = .5 \)). The number of deaths due to leukemia was similar in the 3 groups (17%, 14%, 14%; \( P = .8 \)), and this was confirmed in the Kaplan-Meier analysis.

### Disease-free survival

The actuarial 5-year disease-free survival was significantly different in patients receiving low, intermediate, or high cell dose: 41% versus 42% versus 48% (\( P = .02 \)).

### Leukemia relapse

The actuarial risk of relapse at 5 years in patients who received low, intermediate, and high cell dose was, respectively, 30% versus 33% versus 35% (\( P = 3 \)); for patients in first remission the actuarial risk is 19%, 23%, and 26% (\( P = .4 \)).

### Multivariate analysis

The cell dose was analyzed in multivariate Cox analysis for potential effect on TRM together with 6 other clinical factors: donor type, donor age, recipient age, type and disease phase, conditioning regimen, and year of transplantation. In multivariate Cox analysis on TRM, cell dose was a significant predictor (\( P = .002 \); relative risk 0.6) together with donor type (\( P = .0001 \)), year of transplantation (\( P = .0001 \)), conditioning regimen (\( P = .02 \)), and recipient age (\( P = .02 \); Table 3).

### Discussion

We have shown in the present study that graft function is improved both in the short and long term after transplantation of a high dose of allogeneic bone marrow cells: This results in significantly higher white blood cell counts up to 6 months after engraftment and higher platelet counts at all time intervals up to 1 year after transplantation. Although this would sound expected, we could not find this information in studies comparing different cell doses or different sources of stem cells.6-16 Many reports concentrate on duration of neutropenia or thrombocytopenia and disregard peripheral blood counts beyond day +20. In the present study, instead, we could show that graft function was particularly improved beyond day +20 and even beyond day +100, suggesting a long-lasting effect of a high marrow cell dose: One-year postengraftment median platelet counts for patients receiving low, intermediate, or high marrow cell dose were \( 130 \times 10^9/L, 167 \times 10^9/L, \) and \( 191 \times 10^9/L \), respectively.

The second finding is the strong correlation between cell dose and transplant mortality, in keeping with other studies.20-22: In multivariate analysis patients receiving a high cell dose (> \( 5 \times 10^9/kg \)) had a relative risk of dying of transplant-related complications of 0.6 when compared with patients receiving a low cell dose (< \( 1.7 \times 10^9/kg \)). This was almost entirely due to a reduction of lethal infections and GVHD: The risk of death caused by infection/GVHD (often related) was 28%, 23%, and 16%, respectively, in patients receiving a low, intermediate, or high cell dose (\( P = .009 \)). Other studies have shown a positive effect of cell dose on graft-versus-host disease,20,21,26 and the fact that we find reduced GVHD with improved graft function in patients receiving a high cell dose is in keeping with the recent demonstration of a strong correlation between severity of acute GVHD and platelet counts.27 In the present study the actuarial 5-year TRM in patients receiving low, intermediate, or high cell dose was, respectively, 41%, 36%, and 28% (\( P = .009 \)). This could not be explained by a selection of patients with good prognosis, because the high cell dose group contained more patients with risk factors such as older age and alternative donor transplants, and this is confirmed in the multivariate Cox analysis.

The reduction of transplant-related mortality was so strong that it produced improved overall survival. We did not find a significant difference in the leukemia relapse between the 3 groups of patients, notwithstanding a recent report on a possible favorable effect of a high cell dose.25 However, if GVHD and TRM are reduced, it is unlikely that leukemia relapse would also be reduced. In keeping with these observations, we find that the disease-free survival was significantly reduced in patients grafted with low cell dose in comparison with intermediate and high dose (41% vs 42% vs 48%; \( P = .02 \)).

One question that comes up concerns the cell component important in reducing transplant-related mortality. Is it stem cells, hemopoietic progenitors, lymphocytes, or other cells such as stromal or mesenchymal cells?3-19 Stem cell numbers would seem important, as shown by experimental data on radioprotection 30 and clinical data in human beings that cause reduction of hematologic reconstitution.5-31 The question is how other cells influence stem cell function. The high lymphocyte content of

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**Table 3. Results of multivariate Cox analysis for potential effect on TRM**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline value</th>
<th>Compared value</th>
<th>RR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell dose</td>
<td>( \leq 2.4 \times 10^9/kg )</td>
<td>&gt; ( 2.4 \times 10^9/kg ) and ( \leq 5 \times 10^9/kg )</td>
<td>0.76 (0.58-0.99)</td>
<td>.002</td>
</tr>
<tr>
<td></td>
<td>&gt; ( &gt; 5 \times 10^9/kg )</td>
<td>0.6 (0.41-0.88)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor type</td>
<td>HLA-identical sibling</td>
<td>Alternative</td>
<td>2.19 (1.61-2.97)</td>
<td>.0001</td>
</tr>
<tr>
<td>Year of transplantation</td>
<td>1987 or before</td>
<td>1997 or before</td>
<td>0.44 (0.33-0.6)</td>
<td>.0001</td>
</tr>
<tr>
<td></td>
<td>After 1997</td>
<td>0.32 (0.21-0.5)</td>
<td></td>
<td>.0001</td>
</tr>
<tr>
<td>Conditioning regimen</td>
<td>TBI: yes</td>
<td>TBI: no</td>
<td>1.36 (1.04-1.77)</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td>( \leq 30 ) y</td>
<td>( &gt; 30 ) y</td>
<td>1.02 (1.00-1.03)</td>
<td>.02</td>
</tr>
<tr>
<td>Recipient age</td>
<td>( \leq 31 ) y</td>
<td>( &gt; 31 ) y</td>
<td>1.01 (1.00-1.02)</td>
<td>.06</td>
</tr>
<tr>
<td>Donor age</td>
<td>Early</td>
<td>Advanced</td>
<td>1.25 (0.99-1.59)</td>
<td>.06</td>
</tr>
</tbody>
</table>

TRM indicates transplant-related mortality; RR, relative risk; CI, confidence interval; and TBI, total body irradiation.
Peripheral blood grafts, producing significant acute and chronic GVHD, may counterbalance the positive effect of cell dose and produce an overall negative effect, mostly when the total cell count exceeds $9 \times 10^9$/kg. This was also seen in a recent study on CD34$^+$ selected allografts, with a cutoff for increased TRM of $3 \times 10^9$/kg CD34$^+$ peripheral blood cells; thus, CD34$^+$ cells per se or their progeny, including antigen-presenting cells, could have a promoting role on graft-versus-host disease and thus impair graft function. Other accessory cells, such as mesenchymal stem cells (MSCs), instead, could play a positive role on graft function, by virtue either of a suppressive effect on GVHD or of a direct promoting effect on stem cells. These MSCs are found in bone marrow but not in peripheral blood harvest.

In conclusion, this study indicates a very strong cell dose effect on graft function when using bone marrow as a stem cell source, resulting in lower transplant mortality. Data from the literature and our own institution (A.B., unpublished data, 2000) would suggest that this is not the case for peripheral blood grafts. Therefore, graft function and low transplant toxicity is not solely the result of a large CD34$^+$ cell dose and is probably influenced by other cell subpopulations. Some of these, such as mesenchymal stem cells, are being studied as candidates for the cell dose effect. At present we would recommend using a high marrow cell dose for best graft function and low transplant mortality: How to manipulate peripheral blood grafts to mimic this effect remains to be determined.

Acknowledgment

The great work of our nursing staff is gratefully acknowledged.

References