Identification of Patients Who May Benefit From Prophylactic Immunotherapy After Bone Marrow Transplantation for Acute Myeloid Leukemia on the Basis of Lymphocyte Recovery Early After Transplantation

By Ray Powles, Seema Singhal, J Jennifer Treleaven, Samar Kulkarni, Clive Horton, and Jayesh Mehta

Two hundred and one patients (median age, 29 years) with acute myeloid leukemia (AML) underwent bone marrow transplantation (BMT) from HLA-identical sibling donors after conditioning with melphalan-total-body irradiation (TBI) (57%), cyclophosphamide-TBI (35%), or chemotherapy alone (8%). Graft-versus-host disease (GVHD) prophylaxis included cyclosporine alone (68%), cyclosporine-methotrexate (26%), or T-cell depletion (6%). The probability of relapse was calculated as a function of the absolute lymphocyte count (10^9/L) on days 27 to 30 posttransplant (<0.1 v 0.1, <0.2 v 0.2, and <0.3 v 0.3). In each of these 12 comparisons, the probability of relapse was higher for the group with the lower lymphocyte count. Because the difference was most significant (P = .004) for an absolute lymphocyte count of <0.2 on day 29 (3-year relapse probability, 42%) versus 0.2 (16%), this variable was included in a Cox model to determine factors independently affecting relapse. Multivariate analysis showed that conditioning regimens other than melphalan-TBI, a low lymphocyte count on day 29, French-American-British (FAB) subtypes M4-7, and a nucleated cell dose of 2.42 x 10^9/kg was associated with a higher risk of relapse. We conclude that slow lymphocyte recovery after allogeneic BMT, to < 0.2 x 10^9/L 29 days in this analysis, appears to be associated with a higher risk of relapse in patients with AML. This group of patients may benefit from posttransplant immune manipulations such as abbreviated GVHD prophylaxis, or donor cell or cytokine administration to enhance graft-versus-leukemia reactions to reduce relapse.

MATERIALS AND METHODS

The database of the Leukaemia Unit contains prospectively-collected information on over 1,600 patients treated in the Royal Marsden Hospital since 1978.24,25 This was searched to identify a group of 201 AML patients who underwent allogeneic BMT in the first remission (CR) from HLA-identical sibling donors between 1981 and 1995. Patients receiving blood-derived stem cells or blinded marrow/blood cells26 were excluded. Patient characteristics are shown in Table 1.

Conditioning regimens. These consisted of 110 mg/m^2 of melphalan or 120 mg/kg cyclophosphamide with 950 to 1,150 cGy single-fraction total-body irradiation (TBI)11,12 or chemotherapy alone (240 mg/m^2 melphalan alone27 or 16 mg/kg busulfan with 120 mg/kg cyclophosphamide28) and have been described in detail elsewhere.

GVHD prophylaxis. Before the intravenous formulation of cyclosporine became available, the drug was administered intramuscularly at a dose of 25 mg/kg from day −1 for 5 days and then orally at a dose of 12.5 mg/kg. After the intravenous formulation of cyclosporine became available, the drug was administered intravenously at a dose of 3 mg/kg from day −1 until oral intake was resumed. The drug was then given orally at a dose of 12.5 mg/kg. Patients receiving a short course of methotrexate in addition to cyclosporine received 15 mg/m^2 of the drug on day 1 and 10 mg/m^2 on days 3 and 6, or on days 3, 6, and 11.

The murine anti-CD52 monoclonal antibodies Campath-1G or Campath-1M (kindly provided by Drs G. Hale and H. Waldmann, Cambridge, UK) were used to deplete the donor marrow of T cells in a minority of patients.29

Acute GVHD was usually diagnosed and graded on the basis of clinical findings and was treated with conventional (2 mg/kg) or high (10 to 20 mg/kg) dose corticosteroids.

Donor marrow. Marrow was harvested from the iliac crests of HLA-identical sibling donors under general anesthesia on the day of transplantation. The target cell number for the harvest was 2 x 10^8 nucleated cells per kg recipient body weight. The marrow was usually not depleted of T cells and was infused into the patient essentially unmanipulated except as required for donor-recipient ABO blood group incompatibility.30

Supportive care. All patients were treated in protective isolation in rooms with positive-pressure ventilation. Blood products transfused...
Patients were censored at the time of nonrelapse death (death in remission) or the last follow-up in continuous remission. The log-rank test was used to compare the differences.

The following factors were also analyzed in univariate and multivariate fashion to determine their effect on the probability of relapse: age (<20 v ≥20 years), conditioning regimen (melphalan-TBI v cyclophosphamide-TBI v chemotherapy only), donor-recipient ABO blood group compatibility (match v mismatch), French-American-British (FAB) subtype (M1-3 v M4-7 v unknown), GVHD prophylaxis (cyclosporine alone v cyclosporine-methotrexate v T-cell depletion), leukocyte count at presentation (<5.1 × 10^9/L v ≥5.1), nucleated cell dose (≥2.42 v >2.42 × 10^9/kg), occurrence of acute or chronic GVHD (yes v no), remission-transplant interval (<4 months v ≥4 months), and the absolute lymphocyte count on day 29 after BMT (≥0.2 × 10^9/L v >0.2). The effect of the karyotype could not be analyzed because results were not available for more than half the patients (who were referred for BMT in remission after prior therapy elsewhere).

Among the different variables, a very strong correlation was seen between GVHD prophylaxis and conditioning regimen (r = 0.35; P < .0001) with 11 of 12 T-cell–depleted grafts being conditioned with cyclophosphamide-TBI and 47 of 53 cyclosporine-methotrexate grafts being conditioned with melphalan-TBI.

### RESULTS

Twenty-five patients relapsed at 3 to 46 months (median, 10); 23 died of relapsed disease and 2 are alive after cell- and cytokine-mediated immunotherapy. Seventy-two patients died of transplant-related causes at 0.5 to 123 months (median, 3). Eighty-four patients are alive in continuous remission at 12 to 191 months (median, 91). Four of 12 patients receiving T-cell–depleted marrow relapsed, as did 14 of 136 patients receiving cyclosporine, and 7 of 53 patients receiving cyclosporine-methotrexate.

As Table 2 shows, for each of the lymphocyte levels compared on the 4 posttransplant days studied, patients with the lower lymphocyte counts had higher relapse rates. This difference was significant at the P < .01 level for 3 of the 12 comparisons. The significance was the highest (P = .004) for day 29 and lymphocytes <0.2 versus ≥0.2 × 10^9/L (Fig 1).

Table 3 shows that the mode of GVHD prophylaxis including the administration of methotrexate did not affect the lymphocyte count significantly.

Table 4 shows the results of the Cox analysis. Patients conditioned with melphalan-TBI had a significantly lower probability of relapse (Fig 2), and this factor dominated the risk of relapse. An absolute lymphocyte count of <0.2 × 10^9/L on day 29 was also significantly associated with a higher risk of relapse, as were FAB subtypes other than M1-3 and a higher
infused cell dose. Because of the strong correlation between the conditioning regimen and GVHD prophylaxis, the effect of the latter, if any, was not discernible.

The higher lymphocyte count on day 29 was also associated with a significant survival benefit (Fig 3). This effect was partly also due to a lower toxic death rate among patients with the higher lymphocyte count (59 of 153 vs 22 of 35; 1-year probabilities 35% vs 65.5%; P = .003).

Finally, the beneficial effect of the higher lymphocyte count on relapse was independent of GVHD because, as Fig 4 shows, the lower lymphocyte count was associated with a slightly higher probability of GVHD.

**DISCUSSION**

Our data show that slower lymphocyte recovery in the fourth week after allogeneic BMT for AML in first remission, as reflected by an absolute lymphocyte count of <0.2 × 10^9/L on day 29, is associated with an increased risk of disease recurrence independent of GVHD. This observation has practical significance because this identifies a group of patients who may be suitable candidates for early intervention to enhance graft-versus-leukemia.

**Table 4. Multivariate Analysis of Factors Affecting Relapse**

<table>
<thead>
<tr>
<th>Favorable Variable</th>
<th>Unfavorable Variable</th>
<th>Risk Ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melphalan-TBI</td>
<td>Other</td>
<td>3.2 (1.8-5.6)</td>
<td>.0001</td>
<td></td>
</tr>
<tr>
<td>Day 29 lymphocyte</td>
<td>Day 29 lymphocyte</td>
<td>2.9 (0.9-9.1)</td>
<td>.02</td>
<td></td>
</tr>
<tr>
<td>count &lt;=0.2 × 10^9/L</td>
<td>count &gt;0.2 × 10^9/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAB subtype M1-3</td>
<td>Other</td>
<td>1.6 (1.0-2.7)</td>
<td>.03</td>
<td></td>
</tr>
<tr>
<td>Nucleated cell dose</td>
<td>Nucleated cell dose</td>
<td>1.7 (1.1-1.8)</td>
<td>.05</td>
<td></td>
</tr>
<tr>
<td>&lt;=2.4 × 10^9/kg</td>
<td>&gt;2.4 × 10^9/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Can factors operating so early in the course of an allograft really make a difference? In the early days or weeks after the allograft, the disease burden is at its lowest. At this time, the chances of eliminating it completely through immunological mechanisms should logically be the highest. There is evidence to show that factors operating very early in the course of the transplant can affect relapse rates.

A study from the International Bone Marrow Transplant Registry showed that the use of methotrexate for GVHD prophylaxis in patients with acute lymphoblastic leukemia decreased relapse independently of its effect on GVHD, possibly due to the direct antileukemic action of methotrexate. Blaise et al showed that the addition of an antibody against the interleukin-2 receptor (33B3.1) to standard GVHD prophylaxis early after transplantation delayed acute GVHD, but did not decrease its incidence. This delay was associated with an increased relapse rate and poorer leukemia-free survival. Jiang et al found an association between lower CD8 and natural killer cell numbers after allogeneic BMT in patients with chronic myeloid leukemia and an increased relapse rate.

Hancock et al in a retrospective study of children with acute lymphoblastic leukemia receiving T-cell–depleted marrow from unrelated donors, found that mixed chimerism on day 21 (persistent recipient hematopoiesis) was associated with a significantly higher probability of relapse. Although no data were available from their group of patients, it is possible that complete elimination of recipient hematopoiesis, and consequently a lower risk of relapse, may have been correlated with adequate lymphocyte reconstitution.

**Fig 3.** The effect of the day 29 lymphocyte count on relapse in 188 patients (13 of 201 patients died before day 29 or did not have lymphocyte counts available).

**Table 3. Correlation Between GVHD Prophylaxis and Lymphocyte Counts on Day 29**

<table>
<thead>
<tr>
<th>GVHD Prophylaxis</th>
<th>Lymphocytes &lt;0.2 × 10^9/L</th>
<th>Lymphocytes ≥0.2 × 10^9/L</th>
<th>Day 29 Lymphocytes (10^9/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-cell depletion</td>
<td>4 (33)</td>
<td>8 (67)</td>
<td>0.34 (0-1.52)</td>
</tr>
<tr>
<td>Cyclosporine alone</td>
<td>25 (20)</td>
<td>100 (80)</td>
<td>0.48 (0.8-5.87)</td>
</tr>
<tr>
<td>Cyclosporine- methotrexate</td>
<td>6 (12)</td>
<td>45 (88)</td>
<td>0.53 (0.08-2.82)</td>
</tr>
<tr>
<td>P</td>
<td>.18</td>
<td>(.Chi squared)</td>
<td>.14 (TCD v Cy-MTX)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>.19 (Cy v Cy-MTX) (ANOVA)</td>
</tr>
</tbody>
</table>

Thirteen patients had either died by day 29 (n = 9) or had no lymphocyte counts available for that day (n = 4). None of the differences is significant.
It would be interesting to look at lymphocyte subset data to see if there was any correlation between specific subsets and the observed outcome. Unfortunately, these investigations were either not available or were not routinely performed. A higher nucleated cell dose would be expected to contain a higher number of lymphocytes, and thus, possibly a lower relapse rate. Thus, the observation of a higher relapse rate with higher nucleated cell doses is difficult to explain. Although the difference is significant in the Cox analysis, it is not significant in univariate analysis (16 of 101 vs 9 of 100; 3-year probabilities 24.5% vs 13.4%; \( P = .11 \)). Unlike those between marrow and blood,26 the differences between lymphocyte numbers at various nucleated marrow cell doses are likely to be rather modest and may not have any impact on immune recovery.

The risk of relapse as a result of slow lymphocyte recovery has to be interpreted in the context of other factors, which are known to (or would be expected to) increase relapse rates such as adverse chromosomal abnormalities.14 Thus, in a patient with a high-risk chromosomal abnormality, a low lymphocyte count could be a clear indication for action. In a patient without such abnormalities, it may simply call for careful monitoring for the earliest evidence of recurrence. It would be worthwhile to see if our data can be duplicated by other centers and in other diseases.

The potential therapeutic options in patients identified as being at a high risk of relapse a month after allografting could include administration of interferon-\( \alpha \), interferon-\( \tau \), interleukin-2 or more donor leukocytes, or modification (curtailment) of immunosuppression. Administration of interferon-\( \alpha \) (in fact given for its potential antiviral effects) has been shown to reduce relapse rates,21 but has also been reported to increase the incidence and severity of GVHD.27 Interferon-\( \tau \) can sometimes increase the CD4 count in patients with lymphopenia (unpublished observations). The administration of interleukin-2 after T-cell–depleted BMT has been shown to increase the natural killer cell numbers and reduce relapse without any effect on the lymphocyte numbers.22 Administration of graded increments of donor lymphocytes to acute leukemia patients after T-cell–depleted BMT decreased relapse of acute leukemia, but was associated with clinically significant GVHD.20 Curtailing the immunosuppression can reduce relapse rates,23 but may also give rise to severe GVHD, if tapered too rapidly.38 This could also be an appropriate setting to test active specific immunotherapy using inactivated malignant cells,39 possibly in combination with stimulatory cytokines.

In conclusion, our data suggest that poor lymphocyte recovery a month after allogeneic BMT is associated with an increased risk of relapse. Because the relapse risk is a combined function of the conditioning regimen and GVHD prophylaxis, it may be best for each transplant team to define their own criteria for identifying patients at risk of relapse. Patients identified on the basis of this could be candidates for prophylactic immunotherapy to enhance graft-versus-leukemia.

REFERENCES


Fig 3. The effect of the day 29 lymphocyte count on survival in 188 patients (13 of 201 patients died before day 29 or did not have lymphocyte counts available).

Fig 4. The relation between the day 29 lymphocyte count and the cumulative probability of acute or chronic GVHD. The lower count is associated with a higher risk of GVHD.


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