Relapse of Chronic Myeloid Leukemia After Allogeneic Bone Marrow Transplant: The Case for Giving Donor Leukocyte Transfusions Before the Onset of Hematologic Relapse

By Frits van Rhee, Feng Lin, Jonathan O. Cullis, Andrew Spencer, Nicholas C.P. Cross, Andrew Chase, Bernardo Garicochea, Julie Bungey, John Barrett, and John M. Goldman

Fourteen patients with chronic myeloid leukemia (CML) relapsing after allogeneic bone marrow transplant (BMT) were treated with leukocyte transfusions from the original marrow donor (sibling, n = 9; volunteer unrelated, n = 5). The relapse was defined at the molecular level in two cases, cytogenetically in five cases and hematologically in seven cases. Ten patients responded, seven of seven patients with cytogenetic/molecular relapse compared with three of seven with hematologic relapse (P < .03). All five recipients of cells from unrelated donors responded. Eight of the 10 responders have achieved polymerase chain reaction-negative status and this persisted in three patients for more than 2 years; no responder has shown sign of relapse. Reversible marrow aplasia occurred in two patients, both treated in hematologic relapse. Severe graft-versus-host disease occurred in four patients and was fatal in one. We confirm previous reports that donor leukocyte transfusions are effective in the management of CML in relapse after BMT. In this series, therapeutic intervention before the onset of hematologic relapse was associated with an increased likelihood of response and no marrow aplasia.

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Patients and Methods

Patients. Fourteen patients with relapsed CML after BMT received donor leukocytes from their original marrow donor (sibling, n = 9; volunteer, n = 5). Thirteen patients were treated for relapse of Philadelphia chromosome-positive (Ph+) CML. The fourteenth patient (unique patient number [UPN] 236) had been transplanted for Ph− CML, but leukemia-specific BCR-ABL mRNA was detected by RT-PCR. Twelve patients were in first chronic phase at the time of BMT, whereas the remaining two had accelerated phase disease. Two patients in hematologic relapse were deemed to have disease acceleration because both had multiple cytogenetic abnormalities associated with disease progression and a platelet count of more than 1,000 $\times 10^9$/L. Five patients were in cytogenetic relapse at the time of leukocyte transfusion. Two patients (UPN 43 and 349) in complete cytogenetic remission had evidence of molecular relapse with high numbers of BCR-ABL transcripts detected by competitive PCR (5,000 and 3,000 transcripts/μg RNA, respectively). UPN 43 was in cytogenetic remission after previous IFN therapy.

Management of relapse. Treatment of relapse before DLT consisted of hydroxyurea (n = 3), IFNα (n = 3) and withdrawal of cyclosporin A (n = 4). The remaining 10 patients were not on cyclosporin A therapy at the time of relapse. Donor leukocytes were collected on a continuous flow cell separator (Cobe Spectra; Cobe Laboratories, Gloucester, UK) and immediately transfused to the recipient without plasma or red blood cell depletion. Informed consent was provided according to the Declaration of Helsinki. Donor leukocytes were obtained from the United Kingdom in 13 cases; in one case (UPN 334) leukocytes were obtained from a donor in the National Marrow Donor Program in the United States, transported to the United Kingdom at room temperature in acid citrate dextrose and administered to the recipient 36 hours after collection. Each donor was leukapheresed on an average of 2 occasions (range: 1 to 4) with a maximum interval of 16 days between collections. The nucleated cell and lymphocyte doses administered are shown in Table 1.

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RESULTS

Response. The median times from BMT to cytogenetic and hematologic relapse were 18 and 29 months, respectively (overall range: 3 to 108). Immunosuppressive therapy with cyclosporin A was discontinued with no response in 4 patients before transfusion of donor leukocytes. Three patients were treated with IFNα before DLT and a cytogenetic response was seen in only 1 patient (UPN 43). The median time from relapse to leukocyte transfusion was 13 months (range: 0 to 65). A complete response to transfusion of donor leukocytes with reversion to Ph or PCR negativity was observed in 10 of 14 (71%) patients. All 7 patients in cytogenetic or molecular relapse at the time of transfusion responded, but a complete cytogenetic response was noted in only 3 of 7 (43%) patients with hematologic relapse \( (P = 0.022, \text{ Fisher’s exact test).} \) One patient with hematologic relapse (UPN 202) responded to DLT only after addition of IFNα therapy. This patient had previously been treated for 13 months with IFNα with no cytogenetic response and IFNα had been discontinued 3 months before transfusion of donor leukocytes. The median follow-up after DLT was 8 months (range: 4 to 37). There was no significant difference in the numbers of nucleated cells administered to responders and nonresponders. Neither of the patients with relapsed CML in accelerated phase responded and a further nonresponder (UPN 263) developed disease acceleration 5 months after infusion of donor leukocytes. None of the three patients who received a second course of donor leukocytes responded.

Cytogenetic and PCR data. Eight of 12 patients with hematologic or cytogenetic evidence of relapse went into complete cytogenetic remission. Five patients were in cytogenetic relapse and had a mean of 58% Ph+ metaphases in the marrow at the time of treatment (range: 16.7% to 83%). The median time to complete cytogenetic remission in the responders was 4 months (range: 2.5 to 11). Complete molec-
Table 2. Response to DLTs

<table>
<thead>
<tr>
<th>UPN</th>
<th>Criteria for Relapse</th>
<th>Relapse to DLT (mos)</th>
<th>Cell Dose x10^8/kg*</th>
<th>No. of Infusions</th>
<th>Cytogenetics</th>
<th>GVHD (acute/chronic)</th>
<th>Other Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>116</td>
<td>Cytogenetic</td>
<td>21</td>
<td>1.8 (0.81)</td>
<td>1</td>
<td>CR</td>
<td>I/nil</td>
<td>—</td>
</tr>
<tr>
<td>197</td>
<td>Cytogenetic</td>
<td>11</td>
<td>2.7</td>
<td>2</td>
<td>CR</td>
<td>III/ext</td>
<td>—</td>
</tr>
<tr>
<td>241</td>
<td>Cytogenetic</td>
<td>2</td>
<td>6.5 (2.6)</td>
<td>4</td>
<td>CR</td>
<td>I/nil</td>
<td>—</td>
</tr>
<tr>
<td>112</td>
<td>Hem CP</td>
<td>65</td>
<td>2.3 (1.4)</td>
<td>2</td>
<td>NR†</td>
<td>I/nil</td>
<td>—</td>
</tr>
<tr>
<td>202</td>
<td>Hem CP</td>
<td>13</td>
<td>6.0</td>
<td>2†</td>
<td>CR</td>
<td>IV/nil</td>
<td>—</td>
</tr>
<tr>
<td>239</td>
<td>Hem CP</td>
<td>8</td>
<td>3.3</td>
<td>3</td>
<td>CR</td>
<td>nil/nil</td>
<td>Aplasia</td>
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<tr>
<td>180</td>
<td>Hem AP</td>
<td>16</td>
<td>3.6 (2.0)</td>
<td>2</td>
<td>NR†</td>
<td>nil/nil</td>
<td>—</td>
</tr>
<tr>
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<td>Hem AP</td>
<td>7</td>
<td>1.5 (0.7)</td>
<td>2</td>
<td>NR</td>
<td>I/IV/Ext</td>
<td>PCP Pneumonia</td>
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<tr>
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<td>2</td>
<td>CR</td>
<td>I/nil</td>
<td>Aplasia</td>
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<td>1</td>
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<td>—†</td>
<td>4.6 (2.4)</td>
<td>1</td>
<td>MR</td>
<td>I/IV/Ext</td>
<td>—</td>
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<tr>
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<td>1</td>
<td>CR</td>
<td>I/nil</td>
<td>—</td>
</tr>
<tr>
<td>334</td>
<td>Cytogenetic</td>
<td>4</td>
<td>4.7 (3.1)</td>
<td>2</td>
<td>CR</td>
<td>I/nil</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviations: Hem CP, hematologic relapse in chronic phase; Hem AP, hematologic relapse in accelerated phase; CR, complete cytogenetic response; MR, molecular response (see text); NR, no response; nil, no acute or chronic GVHD; ext, extensive chronic GVHD; PCP, pneumocystis carinii.

* Total nucleated cell dose. Lymphocyte dose in parentheses.
† UPN 180, 263, and 112 received a second course of donor leukocytes after not responding. UPN 180 and 263 received two further infusions with respective MNC doses (x10^8/kg) of 3.2 (1.8) and 6.8 (2.3). UPN 180 received three transfusions with MNC dose of 8.7 (2.8).
§ Patient 349 had only molecular evidence of relapse at the time of DLT.
Patient 43 had only molecular evidence of relapse at the time of DLT after previous IFN therapy for hematologic relapse.
‡ Patient 297 had Ph^+ metaphases detectable in the marrow for the first time on the day of DLT.

ular remissions with no detectable BCR-ABL transcripts were documented in 8 of 10 responders, but the remaining 2 both have reducing transcripts levels and may become PCR negative on longer follow-up. The median time to achieve PCR negativity was 6 months (range: 2.5 to 15). The remissions in the responder group appear to be durable and no second relapses have been seen. The three longest responders remained PCR negative 24, 24, and 32 months after transfusion of donor leukocytes (Fig 1A). Four responders were studied by competitive PCR, while in cytogenetic remission and reducing or low numbers of BCR-ABL transcripts, could still be demonstrated by competitive PCR (Fig 1B).

Five patients were studied by competitive PCR before DLT and cytogenetic relapse was preceded by high or increasing numbers of BCR-ABL transcripts in four cases (Fig 1C). The decision to give a single DLT was taken on the basis of competitive PCR data in three cases. The first patient (UPN 349) relapsed early after BMT from a volunteer donor and was still in cytogenetic remission at the time of treatment. Prophylactic cyclosporin A was administered after DLT and initially an increase in BCR-ABL transcript numbers was observed with simultaneous evidence of cytogenetic relapse. Acute GVHD developed after discontinuation of cyclosporin A and a complete molecular remission was subsequently observed. The second patient (UPN 297) had a gradual increase in transcript levels after transplant and marrow examination on the day of leukocyte transfusion from an unrelated donor showed 17% Ph^+ cells. A response was only observed after prophylactic cyclosporin A had been withdrawn. A further patient (UPN 43) was in complete cytogenetic remission after previous IFNα therapy, but high numbers of BCR-ABL transcripts were still detectable by competitive PCR. After responding to DLT, a reduction in the number of transcripts was noted and this patient subsequently became PCR negative (Fig 1B).

The type of BCR-ABL transcript (b3a2, n = 10; b2a2, n = 4) expressed did not correlate with response (data not shown).

T-lymphocyte chimerism before leukocyte transfusion. Cytogenetic evaluation of PHA-blasts in six sex-mismatched donor/recipient pairs showed that in all cases, the T lymphocytes were 100% of donor origin at the time of relapse.

GVHD. Acute GVHD occurred in 7 of the 10 responders and was confined to skin and gut (grade 1) in 4. Two patients developed acute liver GVHD (grade 4) that responded to therapy with systemic corticosteroids. One of these patients (UPN 202) initially showed no response to transfusion of donor leukocytes and was treated with IFNα 6 months later. This patient developed severe liver GVHD 1 month after commencing IFNα and was later documented to be in complete cytogenetic remission. Extensive chronic GVHD occurred in 3 responders and proved steroid responsive in 2.

GVHD was observed in two nonresponders. One patient (UPN 112) developed skin GVHD (grade 1) after which a transient reduction in peripheral white blood cells was observed. The remaining nonresponder (UPN 321) who was in accelerated phase at the time of relapse developed pneumocystis carinii pneumonia 4 weeks post-DLT, and responded to appropriate therapy. No response was observed to transfu-
sion of donor leukocytes and IFNα therapy was commenced 2 months later after which severe acute liver GVHD occurred. This patient died after 7 months because of liver failure, sepsis, and hemorrhage from a liver biopsy site. Histology of the liver showed the typical features of GVHD with complete obliteration of small bile ducts.

There was no correlation between cell dose transfused and the occurrence of GVHD or between GVHD after DLT and after the original transplant.

**Marrow aplasia.** Two patients with hematologic relapse developed severe pancytopenia requiring blood support and marrow aplasia that was confirmed by trephine biopsy. One
patient (UPN 239) developed pancytopenia 2 months after leukocyte transfusion and GVHD was not observed. He recovered normal hemopoiesis shortly after a second marrow infusion from his sibling donor. The other patient (UPN 236) had received a sex-mismatched transplant from a volunteer donor, and pancytopenia occurred 4 months after treatment with donor leukocytes. BM myeloid cells obtained during pancytopenia were shown to be of donor origin by fluorescence in situ hybridisation (FISH) with Y-chromosome and BCR-ABL probes. This patient was managed with granulocyte colony-stimulating factor and marrow function recovered slowly over several months. No aplasia was observed in any of the patients who were in cytogenetic or molecular relapse at the time of DLT.

**Leukocyte transfusion from volunteer unrelated donors.** Five patients were treated with leukocytes from their original volunteer marrow donor (hematologic relapse n = 1, cytogenetic relapse n = 3 and molecular relapse n = 1). A complete cytogenetic or molecular response was seen in all five patients compared with five of nine (44%) patients in the sibling donor group (P = .10 Fisher’s exact test). Prophylactic cyclosporin A was administered for 4 to 12 weeks to four patients receiving cells from a volunteer donor and a response was only observed after cyclosporin A had been withdrawn (Fig 1, B C). GVHD was seen in all five patients. One patient (UPN 349) developed acute GVHD (grade 4) of the liver that resolved with systemic steroid therapy. Extensive chronic GVHD of the skin was observed in 2 patients and was poorly responsive to immunosuppressive measures in one (UPN 197). Severe GVHD was seen in two of nine patients in the sibling donor group, both of whom had received additional therapy with IFNa after transfusion of donor leukocytes.

**DISCUSSION**

Relapse of CML after allogeneic marrow transplant is an important cause of treatment failure and the optimum strategy for the management of relapse remains to be defined. Administration of IFNa can improve short-term survival by inducing a cytogenetic remission in 10% to 15% of patients that delays disease progression. A second transplant may salvage a minority of patients and result in long-term disease-free survival, but at the cost of increased morbidity and mortality. Several reports have now described the use of DLTs from the original marrow donor as an alternative to a second transplant. We report here cytogenetic or molecular responses in 10 of 14 patients (71%) treated for recurrence of CML after transplant. The median time to restoration of Ph negativity was 4 months. However, reducing numbers of BCR-ABL transcripts could still be shown by competitive PCR in four responders in cytogenetic remission, indicating that the elimination of the leukemic clone after donor cell infusion was gradual. Eight of 10 responders eventually achieved complete molecular remissions and long-term follow-up in 3 shows that these remissions are durable.

The evolution of relapse of CML post transplant is in general slow, and hematologic relapse is often preceded by a gradual reappearance of Ph+ cells in the marrow that is referred to as cytogenetic relapse. We have previously reported that the detection of BCR-ABL transcripts more than 1 year after BMT identifies a group of patients at increased risk of relapse, but is not predictive for any particular individual. Serial measurement of residual disease by competitive PCR can identify patients with increasing numbers of leukemia-specific transcripts who may be progressing to clinical relapse. However, it has not yet been established whether administration of donor leukocytes before overt hematologic relapse is desirable. In this series, patients with only cytogenetic or molecular evidence of disease recurrence responded better to transfusion of donor leukocytes than those who were in hematologic relapse. Molecular data were used to initiate therapy in three patients with high levels of BCR-ABL transcripts, all of whom responded to therapy. However, it must be remembered that Ph+ cells identified in the marrow may disappear spontaneously, but such reversal is rare if the number of leukemic cells in the marrow exceeds 40%. Thus early treatment has the disadvantage that it may unnecessarily expose some patients with transient chromosomal relapse to the adverse effects associated with transfusion of donor leukocytes.

Marrow aplasia is a potentially fatal complication of DLT and a recent review of published data suggests that pancytopenia develops in 54% of patients treated in hematologic relapse. Persistent marrow aplasia could occur if the residual donor stem cell pool were insufficient to repopulate the BM after elimination of the leukemic cell population. A second stem cell infusion from the donor would then be required for full hematopoietic reconstitution. We observed marrow aplasia only in patients treated in hematologic relapse; no aplasia was seen in the seven responders who were transfused with donor leukocytes during cytogenetic or molecular relapse. Therefore, it is reasonable to speculate that administration of donor leukocytes before progression to frank hematologic relapse may prevent aplasia. We showed in one patient residual donor hematopoiesis in the myeloid compartment during pancytopenia using FISH analysis that appeared to predict for recovery from aplasia.

The high response rate to donor leukocytes underlines the powerful antileukemic effect mediated by allogeneic lymphocytes and is strong support for the existence of a graft-versus-leukemia effect (GVL) after allogeneic marrow transplant. It is notable that two patients who did not develop GVHD after DLT achieved complete remission, suggesting that GVL is not always dependent on clinically manifest GVHD. It is not clear why GVL may fail to eliminate small numbers of residual leukemic cells after the initial transplant and yet transfusion of further donor cells is a highly effective treatment of relapse in patients in whom complete donor T-cell chimerism can be shown. There has been considerable variation in the number of mononuclear cells transfused in various studies and although more lymphocytes are present in a leucapheresis collection compared with a marrow harvest, a clear relation between cell dose transfused and thus far, response has not been apparent. Three nonresponders in our series did not benefit from a second course of DLTs. However, we did observe a complete response to a single transfusion of only 0.6 × 10⁶ nucleated cells/kg and the minimum cell dose required for the induction of GVL may be lower than has hitherto been assumed. Qualitative factors
other than cell dose such as expression of minor histocompatibility antigens on leukemic cells, and therefore, GVHD prophylaxis could also be important determinants of GVL. GVHD prophylaxis after transplant could predispose some patients to relapse by deleting or suppressing lymphocyte populations mediating GVL. Withdrawal of cyclosporin A has been reported to restore remission in a few patients with relapsed CML after transplant.24 We treated four patients who received donor leukocytes from volunteer donors with prophylactic cyclosporin A and a response was only observed after cessation of cyclosporin A therapy. These observations suggest that immunosuppressive therapy can indeed abrogate GVL.

Five patients received leukocytes from a volunteer donor and all responded to therapy. This has important implications for volunteer donor registries because requests for leukocyte donation are likely to increase and may obviate the need for a second marrow harvest in case of relapse. Severe acute or extensive chronic GVHD occurred in three patients in the volunteer donor group and was only seen in the sibling group in patients treated with IFNα after transfusion of donor leukocytes. Despite close phenotypic matching volunteer donor-recipient pairs may differ for both major and minor histocompatibility antigens; this could not only increase the incidence of GVHD, but also enhance GVL. However, it should be emphasized that in this series there was no significant difference in response between patients receiving cells from volunteer donors compared with those transfused with cells from HLA-identical siblings. The observation that the use of IFNα may be associated with severe GVHD after DLT can be explained by upregulation of class I expression that may increase presentation of minor histocompatibility antigens.25 Most investigators have combined the use of IFNα with DLTs, but in our series only one responder required additional administration of IFNα. These findings suggest that concomitant IFNα therapy is not mandatory. Therefore, it seems prudent to reserve the combination of IFNα and leukocyte transfusions to nonresponders.

Adoptive immunotherapy for relapse of CML is an important new development and offers the prospect of using donor leukocytes to restore GVL after T-cell-depleted BMT. The introduction of a reliable competitive PCR assay for BCR-ABL transcripts permits the early prediction of relapse and could be used to time the transfusion of a targeted, small T-cell dose that would minimize GVHD and prevent marrow aplasia. However, ultimately, new treatment strategies will probably be required to separate the lymphocyte populations causing GVHD from those mediating GVL. GVL reactivity does not appear to be confined to a particular lymphocyte subset and recent experimental work suggests that it may be feasible to achieve a functional separation of GVL and GVHD by selectively deleting donor lymphocytes responding to host-specific minor histocompatibility antigens presented by patient PHA-lymphoblasts.26-28

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REFERENCES


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