Cutting Edge Communication

Nonmyeloablative Stem Cell Transplantation with CD8-Depleted or CD34-Selected Peripheral Blood Stem Cells

FRÉDÉRIC BARON,1 ETIENNE BAUDOUX,1 PASCALE FRÈRE,1 SORAYA TOURQUI,2 NICOLE SCHAAF-LAFONTAINE,3 ROLAND GREIMERS,4 CHRISTIAN HERENS,5 GEORGES FILLET,1 and YVES BEGUIN1

ABSTRACT

To decrease the incidence of graft-versus-host disease (GVHD) observed after nonmyeloablative stem cell transplantation (NMSCT), we studied the feasibility of CD8-depleted or CD34-selected NMSCT followed by CD8-depleted preemptive donor lymphocyte infusion (DLI) given in incremental doses on days 40 and 80. Fourteen patients with high-risk malignancies and an HLA-identical sibling (n = 8) or alternative donor (n = 6) but ineligible for a conventional transplant were included. Nonmyeloablative conditioning regimen consisted in 2 Gy total body irradiation (TBI) alone, 2 Gy TBI and fludarabine (previously untreated patients) or cyclophosphamide and fludarabine (patients who had previously received ≥12 Gy TBI). Patients 1–4 (controls) received unmanipulated peripheral blood stem cells (PBSC) and DLI and patients 5–14 CD8-depleted or CD34-selected PBSC followed by CD8-depleted DLI. Post-transplant immunosuppression was carried out with cyclosporine A (CsA) and mycophenolate mofetil (MMF). Initial engraftment was seen in all patients, but 1 patient (7%) later rejected her graft. The actuarial 180-day incidence of grades II–IV acute GVHD was 75% for patients 1–4 versus 0% for patients 5–14 (p = 0.0019). Five of 14 patients were in complete remission (CR) 180 days after the transplant and 6/14 had partial responses. The 1-year survival rate was 69%, and nonrelapse and relapse mortality rates were 16 and 18%, respectively. We conclude that CD8-depleted or CD34-selected NMSCT followed by CD8-depleted DLI is feasible and considerably decreases the incidence of acute GVHD while preserving engraftment and apparently also the graft-versus-leukemia (GVL) effect. Further studies are needed to confirm this encouraging preliminary report.

INTRODUCTION

ALLOGENEIC HEMATOPOIETIC STEM CELL (HSC) transplants are classically performed after myeloablative regimens consisting of high-dose chemotherapy with or without total body irradiation (TBI) (1,2). The aims of this conditioning are to eliminate host (1) hematopoiesis to “make space” for donor HSC; (2) immune system to prevent rejection; and (3) cancer or leukemic cells (3). However, it has been recently demonstrated that the graft itself, most likely through subclinical graft-versus-host disease (GVHD) reactions, is capable to create these marrow spaces without chemotherapy or bone marrow irradiation (4). In addition, tumor cells resistant to standard doses of chemotherapy are unlikely to be completely eliminated by tolerable higher-intensity doses of che-
radiotherapy alone (5). Moreover, donor alloreactivity against tumor cells has been recognized as a major factor in the success of HSC transplantation (6–10). This graft-versus-leukemia (GVL) effect may be so potent that leukemia relapses after standard allogeneic transplantation can be effectively [70% long-term complete remission (CR) in chronic myeloid leukemia (CML)] treated with donor lymphocyte infusions (DLI) (10–17). Taken together, these findings suggested that the potential for cure after allogeic HSC transplantation could be obtained by induction of host-versus-graft tolerance with low-dose highly immunosuppressive regimens, allowing in a second step the implantation of donor T and natural killer (NK) (18,19) cells (DLI) to eradicate host tumor cells (3,5,20–32).

The Seattle team has recently proposed an original approach to nonmyeloablative stem cell transplantation (NMSCT) with a conditioning regimen based on single-dose (2 Gy) TBI followed by post-transplant immunosuppression with cyclosporine A (CsA) and mycophenolate mofetil (MMF) (3,33,34). They observed a powerful GVL effect but also a significant incidence of GVHD (33). Recently, we have reported a prospective study of transplantation of CD34-selected allogeic peripheral blood stem cells (PBSC) after myeloablative conditioning, followed by preemptive CD8-depleted DLI (35). Compared to unmanipulated marrow transplantation, this approach significantly decreased the incidence of acute and extensive chronic GVHD without compromising the GVL effect. Therefore, we undertook to investigate the feasibility of applying such a strategy of CD8-depletion or CD34-selection of the graft followed by pre-emptive CD8-depleted DLI in the context of NMSCT after a conditioning and immunosuppression regimen based on the Seattle approach.

**PATIENTS AND METHODS**

**Patient eligibility**

Patients with hematologic malignancies were eligible for this program if they were deemed poor candidates for conventional myeloablative therapy because they were older than 55 years \(n=4\), had concurrent medical conditions \(n=0\), had failed a previous autograft \(n=4\), or for a combination of these factors \(n=3\). Patients with metastatic renal cell carcinoma refractory to interferon-α were also eligible \(n=3\). Written informed consent was obtained from patients and donors and our institution’s Ethical Committee approved the protocol.

**Patients and donors**

Fourteen patients with malignancies, 12 males and 2 females, aged 22–65 (median 58) years were included. Their clinical characteristics are summarized in Table 1. The number of previous lines of therapy ranged from 1 to 6. Eight patients had undergone a previous autotransplant with 6/8 failures. Only those with CML or renal cell carcinoma (RCC) did not receive a previous autograft. Eight patients had HLA-identical sibling and 6 alternative donors. Conditioning (Table 2) consisted of 2 Gy of single-dose TBI on day 0 \(n=6\). For patients not heavily pretreated or unrelated transplants, TBI was combined with 30 mg/m² per day fludarabine for 3 days \(n=5\). The first patient was an exception to this rule because the high rejection rate in CML patients with the original Seattle protocol was not yet published. Finally, 3 patients received a combination of fludarabine and cyclophosphamide 1 g/m² per day for 3 days (Fluda-Cy) because they had previously received 12 Gy TBI as conditioning regimen for an autotransplant (Table 1). Post-transplant immunosuppression was carried out orally with CsA (6 mg/kg b.i.d. from day −1 to day 120 or longer in case of alternative donor or chronic GVHD) and MMF (15 mg/kg b.i.d. from day −1 to day 28).

**Clinical management**

The trigger values for red blood cell (RBC) and platelet transfusions were 8.0 g/dl and 15 × 10⁹/L, respectively. Granulocyte colony-stimulating factor (G-CSF) (5 μg/kg/d) was administered when the granulocyte count was below 1.0 × 10⁹/L. The diagnosis and grading of acute and chronic GVHD was established as previously reported (36,37). Disease evaluation were routinely carried out on days 40, 100, 180, and 365. Polymerase chain reaction (PCR) for cytomegalovirus (CMV) was performed weekly until day 100 and every 2–4 weeks thereafter. Patients with a positive PCR received preemptive ganciclovir for a minimum of 4 weeks and generally up to day 100.

**Stem cell mobilization, collection, and selection**

Donors received human G-CSF (Neupogen®, kindly provided by Amgen, Brussels, Belgium) at 10 μg/kg from day −5 through day −1 before transplant. Collection of PBSC was carried out on days −1 and 0, using a continuous flow blood cell separator (CS3000+, Baxter-Fenwall Laboratories, Deerfield, IL, or Cobe Spectra, Lakewood, CO). The volume of blood processed was 12–16 liters. The PBSC from the first day of harvest were stored overnight in the patient’s own plasma. Patients 1–4 received unmanipulated PBSC. In patients 5–11, PBSC were CD8-depleted and in patients 12–14 PBSC were CD34-selected (Fig. 1). Immediately after the second harvest, PBSC from the first and second harvests were pooled. CD8 depletion as well as CD34⁺ cell selection were carried out using the Isolex 300i© magnetic cell sep-
<table>
<thead>
<tr>
<th>Number</th>
<th>Age/sex</th>
<th>Diagnosis</th>
<th>Status at transplant</th>
<th>Previous autograft</th>
<th>Previous regimens (other than autograft)</th>
<th>Donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58/F</td>
<td>CML</td>
<td>1st CP</td>
<td>No</td>
<td>Hydroxyurea, interferon-α</td>
<td>Sibling</td>
</tr>
<tr>
<td>2</td>
<td>64/M</td>
<td>RCC</td>
<td>Metastatic</td>
<td>No</td>
<td>Vinblastine, interferon-α</td>
<td>Sibling</td>
</tr>
<tr>
<td>3</td>
<td>65/M</td>
<td>NHL</td>
<td>RR</td>
<td>Yes</td>
<td>Chlor, CVP × 6, CHOP × 7, DHAP, E-Cy</td>
<td>Sibling</td>
</tr>
<tr>
<td>4</td>
<td>50/M</td>
<td>NHL</td>
<td>RR</td>
<td>Yes</td>
<td>CVP × 6, CHOP × 6, DHAP × 2, abdominal irradiation</td>
<td>Sibling</td>
</tr>
<tr>
<td>5</td>
<td>22/M</td>
<td>HD</td>
<td>2nd CR</td>
<td>Yes</td>
<td>MOPP-ABV × 8, E-Cy × 2, mediastinal irradiation</td>
<td>Sibling</td>
</tr>
<tr>
<td>6</td>
<td>58/M</td>
<td>NHL</td>
<td>RR</td>
<td>Yes</td>
<td>ACVBP × 4, VIM-Ara-C, E-Cy, CHOP × 2, abdominal irradiation</td>
<td>Sibling</td>
</tr>
<tr>
<td>7</td>
<td>49/M</td>
<td>RCC</td>
<td>Metastatic</td>
<td>No</td>
<td>Vinblastine, interferon-α</td>
<td>Sibling</td>
</tr>
<tr>
<td>8</td>
<td>62/M</td>
<td>CML</td>
<td>1st CP</td>
<td>No</td>
<td>Hydroxyurea</td>
<td>Sibling</td>
</tr>
<tr>
<td>9</td>
<td>57/F</td>
<td>AML</td>
<td>1st CR</td>
<td>Yes</td>
<td>Dnr + Ara-C × 2, HiDAC</td>
<td>Sibling</td>
</tr>
<tr>
<td>10</td>
<td>25/M</td>
<td>ALL</td>
<td>2nd CR</td>
<td>Yes</td>
<td>Dnr + Cy + V, HAM, Mtx + ASP × 2, VAD × 4, Mtx + Ara-C × 2</td>
<td>Unrelated</td>
</tr>
<tr>
<td>11</td>
<td>40/M</td>
<td>NHL</td>
<td>RR</td>
<td>Yes</td>
<td>CHOP × 6, E-Cy, PDN-Ri × 4, Mtx + Cy + Vds + PDN, DHAP</td>
<td>Unrelated</td>
</tr>
<tr>
<td>12</td>
<td>61/F</td>
<td>CLL</td>
<td>Refractory</td>
<td>No</td>
<td>Chlor + Pdn, CVP × 6, fludarabine × 6</td>
<td>Sibling</td>
</tr>
<tr>
<td>13</td>
<td>43/M</td>
<td>NHL</td>
<td>1st CR</td>
<td>Yes</td>
<td>MOPP-ABV × 7, DHAP × 2, E-Cy for HD; ACVBP × 4 for NHL</td>
<td>Sibling</td>
</tr>
<tr>
<td>14</td>
<td>64/M</td>
<td>RCC</td>
<td>Metastatic</td>
<td>No</td>
<td>Vinblastine, interferon-α</td>
<td>Child</td>
</tr>
</tbody>
</table>

Chlor, Chlorambucil; CVP, cyclophosphamide, vincristine, prednisone; CHOP, vincristine, Adriamycin, cyclophosphamide, prednisone; DHAP, dexamethasone, cytosine arabinoside, cisplatin; E, etoposide; Cy, cyclophosphamide; ACVBP, Adriamycin, cyclophosphamide, vincristine, Bleomycin, prednisone; VIM, etoposide, ifosfamide, mitoxantrone; MOPP, nitrogen mustard, vincristine, procarbazine, prednisone; ABV, Adriamycin, bleomycin, vinblastine; Dnr, daunorubicine; Mtz, mitoxantrone; V, vincristine; HAM, high-dose cytosine arabinoside, mitoxantrone; Mtx, methotrexate; Asp, asparaginase; Ara-C, cytosine arabinoside; HiDAC, high-dose cytosine arabinoside; NHL, non-Hodgkin's lymphoma; HD, Hodgkin's disease; CP, chronic phase; RR, refractory relapse; CR, complete remission; HLAid, HLA-identical; M, male; F, female.
<table>
<thead>
<tr>
<th>Number</th>
<th>Conditioning regimen</th>
<th>Cells collected</th>
<th>Manipulation of PBSC</th>
<th>Cells infused</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD34&lt;sup&gt;+&lt;/sup&gt; cells $\times 10^9$/kg</td>
<td>CD3&lt;sup&gt;+&lt;/sup&gt; cells $\times 10^9$/kg</td>
<td>CD4&lt;sup&gt;+&lt;/sup&gt; cells $\times 10^9$/kg</td>
<td>CD8&lt;sup&gt;+&lt;/sup&gt; cells $\times 10^9$/kg</td>
</tr>
<tr>
<td>1</td>
<td>TBI (2 Gy)</td>
<td>14.85</td>
<td>212</td>
<td>156</td>
</tr>
<tr>
<td>2</td>
<td>Fludarabine + TBI (2 Gy)</td>
<td>6.91</td>
<td>382</td>
<td>271</td>
</tr>
<tr>
<td>3</td>
<td>Fludarabine + Cyclophosphamide</td>
<td>10.57</td>
<td>323</td>
<td>223</td>
</tr>
<tr>
<td>4</td>
<td>TBI (2 Gy)</td>
<td>10.59</td>
<td>318</td>
<td>197</td>
</tr>
<tr>
<td>5</td>
<td>TBI (2 Gy)</td>
<td>4.59</td>
<td>270</td>
<td>154</td>
</tr>
<tr>
<td>6</td>
<td>TBI (2 Gy)</td>
<td>9.09</td>
<td>206</td>
<td>144</td>
</tr>
<tr>
<td>7</td>
<td>Fludarabine + TBI (2 Gy)</td>
<td>5.08</td>
<td>333</td>
<td>256</td>
</tr>
<tr>
<td>8</td>
<td>Fludarabine + TBI (2 Gy)</td>
<td>4.47</td>
<td>168</td>
<td>123</td>
</tr>
<tr>
<td>9</td>
<td>TBI (2 Gy)</td>
<td>5.09</td>
<td>432</td>
<td>309</td>
</tr>
<tr>
<td>10</td>
<td>Fludarabine + TBI (2 Gy)</td>
<td>15.30</td>
<td>753</td>
<td>424</td>
</tr>
<tr>
<td>11</td>
<td>Fludarabine + Cyclophosphamide</td>
<td>7.76</td>
<td>227</td>
<td>92</td>
</tr>
<tr>
<td>12</td>
<td>TBI (2 Gy)</td>
<td>2.64</td>
<td>374</td>
<td>195</td>
</tr>
<tr>
<td>13</td>
<td>Fludarabine + Cyclophosphamide</td>
<td>8.32</td>
<td>365</td>
<td>216</td>
</tr>
<tr>
<td>14</td>
<td>Fludarabine + TBI (2 Gy)</td>
<td>8.30</td>
<td>340</td>
<td>245</td>
</tr>
</tbody>
</table>
arator (Nexell International, Wemmel, Belgium), ac-
cording to the manufacturer's recommendations.

**Donor lymphocyte infusions**

Around day 40 post-transplantation, donors underwent 12- to 16-liter leukophereses on 2 consecutive days to col-
lect lymphocytes. The collection from the first day of har-
vest was stored overnight in the patient's own plasma and
pooled with the second harvest. Patients 1–4 (unmanipu-
lated PBSC) were assigned to receive unmanipulated DLI
(1 \times 10^7 and 2 \times 10^7 CD3^+ cells/kg recipient around
days 40 and 80, respectively) whereas patients 5–14 (ma-
nipulated PBSC) were assigned to receive CD8-depleted
DLI (1 \times 10^7 and 5 (2 in mismatched transplants) \times 10^7
CD3^+ cells/kg recipient around days 40 and 80, respec-
tively) (Fig. 1). CD8-depletion of DLI was carried out with Nexell Isolex 300i ©
as previously reported. The first DLI was infused fresh whereas the following ones were cryopreserved and thawed. DLI ... nor in recipients of unrelated transplants. Patients with mixed chimerism on day 100 received a third DLI around day 120.

**Laboratory analyses**

Aliquots of the pooled PBSC as well as the CD8-de-
pleted or CD34-selected fractions were incubated with phycoerythrin (PE)-conjugated anti-CD34, CD3, CD4,
CD8, and CD56 monoclonal antibodies (HPCA2; Bec-
ton-Dickinson, Palo-Alto, CA) for 20 min at 20°C,
washed, and fixed. A total of 1 \times 10^6 cells/condition was
analyzed using a FACS-scan analyzer (Becton-Dickin-
son). The percentage of CD34^+ cells was defined with
dot plot analysis using the whole nucleated cell popu-
lation. The percentage of positive cells in the isotype con-
trol was subtracted from the CD34^+ percentage to give
the final percentage of CD34^+ cells. Data acquisition was
performed with the Cellquest software (Becton-Dickin-
son). Donor lymphocytes (before and after CD8 deple-
tion) are well as patient peripheral white blood cells (on
days 28, 42, 60, 80, 100, 120, 180, and 365) were simi-
larly examined using double labeling with fluorescein
isothiocyanate (FITC)- and PE-conjugated antibodies af-
ter treatment with a lysing solution.

Chimerism among peripheral blood white blood cells
(WBC), T cells, and myeloid cells as well as in unfrac-
tionated marrow was assessed at days 28, 42, 60, 80, 100,
120, 180, and 365 after HCT using fluorescence in situ hy-
bridization (FISH) to detect X and Y chromosomes for re-
cipients of sex-mismatched transplants and PCR-based
analysis of polymorphic minisatellite or microsatellite re-
gions for recipients of sex-matched transplants (38). CD3
(T cells) and CD13 (myeloid cells) selection was carried
out with a FACStar Plus sorter (Becton-Dickinson). Mixed
chimerism (MC) was defined as between 1% and 94% donor cells and full chimerism (FC) as \geqslant 95% donor cells.

**Statistical analyses**

The probability of GVHD, transplant-related mortality
(TRM), and survival were studied by life-table analyses,
and Wilcoxon rank tests were used for comparisons be-
tween groups. Statistical analyses were carried out with
Graphpad Prism (Graphpad Software, San Diego, CA).

**RESULTS**

**Collection of PBSC, CD8 depletion, and CD34 selection**

A median of 8.0 (2.6–15.3) \times 10^6 CD34^+ cells/kg,
328 (168–753) \times 10^6 CD3^+ cells/kg, 207 (92–424) \times
10^6 CD4^+ cells/kg, and 108 (62–496) × 10^6 CD8^+ cells/kg were collected (Table 2). After CD8 depletion, a median of 4.54 (2.65–10.43) × 10^6 CD34^+ cells/kg, 136 (63–249) × 10^6 CD3^+ cells/kg, 121 (55–185) × 10^6 CD4^+ cells/kg, and 4 (1–22) × 10^6 CD8^+ cells/kg were infused (Table 2). The procedure reduced the number of CD3^+, CD4^+, and CD8^+ cells infused by 0.38, 0.22, and 1.7 log, respectively. After CD34 selection, a median of 5.71 (1.83–7.28) × 10^6 CD34^+ cells/kg, 0.08 (0.08–0.11) × 10^6 CD3^+ cells/kg, 0.04 (0.03–0.06) × 10^6 CD4^+ cells/kg, and 0.05 (0.03–0.06) × 10^6 CD8^+ cells/kg were infused (Table 2). The procedure reduced the number of CD3^+, CD4^+, and CD8^+ cells infused by 3.6, 3.7, and 3.6 log, respectively.

**Toxicities and engraftment**

None of the 14 patients developed grade 2 or higher regimen-related toxicities (39). Initial engraftment was observed in 100% of the patients, and nonfatal graft rejection occurred only in 1 CML patient (patient #1, whose conditioning did not include fludarabine). The actuarial 180-day incidence of graft rejection was 9%. The neutrophil nadir occurred on day 7 and was 0.72 (0.09 to 2.33) × 10^9/L (Fig. 2A). Ten out of 14 patients (75%) received a median of 4 (0–8) doses of G-CSF for treatment of neutropenia (Fig. 2A). The median platelet nadir was 88 (8–191) × 10^9/L (Fig. 2B), and only 3/14 patients (21%) required 1 single (n = 2) or 6 (n = 1) platelet transfusions (Fig. 2C). Finally, the median hemoglobin (Hb) nadir was 10.2 (7.3–11.8) g/dL and 4 patients (29%) required RBC transfusions (1, 2, 3, and 9 units, respectively) during the first month after HSCT (Fig. 2C).

**Infections, CMV reactivation, and hospitalization**

Six patients (that received only 2 Gy TBI as conditioning regimen) were eligible for outpatient transplantation. Four of the 6 patients did not require hospitalization within 100 days of HSCT. The other patients were hospitalized before transplant for administration of fludarabine with (n = 3) or without (n = 5) cyclophosphamide. They were discharged on day 1 (range 0–9) and 6 of them were rehospitalized within 100 days of HSCT for a median duration of 7 (range 0–35) days.

Forty-three percent of the patients experienced bacterial infections within 100 days of transplant that were successfully managed by antibiotics except in patient 10 who died of pseudomonas septic shock and lung aspergillosis on day 51. In addition, 1 patient died of (presumably viral) encephalitis on day 186. PCR CMV reactivation was detected in 8 of 11 (73%) CMV-seropositive patients and was successfully managed by preemptive ganciclovir in all of them (Table 3).
## Table 3. Clinical Data

<table>
<thead>
<tr>
<th>DLI</th>
<th>DLI#1 (d40)</th>
<th>DLI#2 (d80)</th>
<th>DLI#3 (d120)</th>
<th>Acute GVHD</th>
<th>Chronic GVHD</th>
<th>CMV</th>
<th>Other complications</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade</td>
<td>Grade (day)</td>
<td>Treatment</td>
<td>Status</td>
<td>Reactivation (PCR+)</td>
<td></td>
<td></td>
<td>Disease</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Don/Rec</td>
<td></td>
<td></td>
<td></td>
<td>status</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>10</td>
<td>—</td>
<td>0</td>
<td>None</td>
<td>—/—</td>
<td>Yes (d60)</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
<td>2</td>
<td>Extensive (210)</td>
<td>CsA, steroids</td>
<td>+/+</td>
<td>Yes (d39)</td>
</tr>
<tr>
<td>3</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
<td>2</td>
<td>Limited (155)</td>
<td>FK506</td>
<td>—/+</td>
<td>Yes (d36)</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>20</td>
<td>—</td>
<td>2</td>
<td>Extensive (158)</td>
<td>CsA, steroid, ECP</td>
<td>—/+</td>
<td>Yes (d74)</td>
</tr>
<tr>
<td>5</td>
<td>10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>0</td>
<td>Limited (180)</td>
<td>Steroids</td>
<td>—/-</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>0</td>
<td>Extensive (236)</td>
<td>CsA, steroids</td>
<td>—/+</td>
<td>Yes (d25)</td>
</tr>
<tr>
<td>7</td>
<td>Death</td>
<td>Death</td>
<td>—</td>
<td>0</td>
<td>None</td>
<td>—/—</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>130&lt;sup&gt;(c)&lt;/sup&gt;</td>
<td>0</td>
<td>None</td>
<td>—/—</td>
<td>Yes (d52)</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>0</td>
<td>Extensive (219)</td>
<td>—</td>
<td>+/+</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>—&lt;sup&gt;b&lt;/sup&gt;</td>
<td>—&lt;sup&gt;b&lt;/sup&gt;</td>
<td>—</td>
<td>0</td>
<td>None</td>
<td>—/—</td>
<td>Yes (d25)</td>
<td>Septic shock, aspergil.</td>
</tr>
<tr>
<td>11</td>
<td>—&lt;sup&gt;b&lt;/sup&gt;</td>
<td>—&lt;sup&gt;b&lt;/sup&gt;</td>
<td>—</td>
<td>1</td>
<td>Extensive (180)</td>
<td>CsA, steroids</td>
<td>—/-</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20</td>
<td>1</td>
<td>None</td>
<td>—/—</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>13</td>
<td>10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>120&lt;sup&gt;(c)&lt;/sup&gt;</td>
<td>0</td>
<td>None</td>
<td>—/-</td>
<td>No</td>
<td>Endocarditis</td>
</tr>
<tr>
<td>14</td>
<td>10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>—</td>
<td>0</td>
<td>None</td>
<td>—/—</td>
<td>Yes (d26)</td>
<td>No</td>
</tr>
</tbody>
</table>

<sup>a</sup> Active GVHD at that time  
<sup>b</sup> Unrelated transplant  
<sup>c</sup> CD8-depleted  

ECP, extracorporeal photopheresis; CR, complete response; PR, partial response; Maj cytog resp, major cytogenetic response; aspergil., aspergillosis; Don, donor; Rec, recipient.
The evolution of chimerism is shown in Fig. 3. Total white blood cell, CD3\(^+\) cell, CD13\(^+\) cell, and bone marrow (BM) chimerisms were 83\% (50–97), 90\% (30–98), 95\% (50–98), and 90\% (50–97), respectively, on day 28; 90\% (48–100), 94\% (37–100), 95\% (71–100), and 90\% (7–99), respectively on day 100; and 95\% (9–100), 78\% (18–98), 98\% (3–100), and 97\% (60–100), respectively, on day 180. The lowest figures were obtained in patient 1, who rejected her graft after day 100. Two of 3 patients who were full donor chimera (FC) before DLI remained FC after DLI (the third died before DLI). In addition, CD8-depleted DLI converted MC to FC in 5/9 evaluable patients. Thus, FC was achieved in 9/12 evaluable patients. For CD8-depleted or CD34-selected transplant recipients, WBC and BM chimerisms were 83\% (64–97) and 89\% (70–94), respectively, on day 28; 90\% (73–100) and 90\% (74–90), respectively, on day 100; and 95\% (73–96) and 95\% (60–100), respectively, on day 180.

**Acute and chronic GVHD**

The actuarial 180-day incidence of grade II–IV acute GVHD was 23\% (Fig. 4). Three out of 4 patients who received unmanipulated PBSC and DLI (patients 1–4) versus 0/10 patients who received CD8-depleted or CD34-selected PBSC and CD8-depleted DLI (patients

**FIG. 3.** Evolution of WBC chimerism on days 28 (black bars), 100 (white bars), and 180 (gray bars) after transplantation.

**FIG. 4.** (A) Actuarial 180-day incidence of grades II–IV acute GVHD. (B) Actuarial 1-year probability of extensive chronic GVHD.
5–14) experienced grade II acute GVHD (Table 3). The actuarial 180-day incidence of grade II–IV acute GVHD was 75% for patients 1–4 versus 0% for patients 5–12 ($p = 0.0019$) (Fig. 4). The actuarial 1-year probabilities of chronic GVHD and extensive chronic GVHD were 68 and 53%, respectively (Fig. 4). For patients 5–14, the figures were 57% and 43%, respectively. For patients 1–4, the figures were 100% and 100%, respectively.

**Immune recovery**

CD4+ T cells remained greater than 200/µl in 7/13 evaluable patients (Fig. 5). The CD8+ cell recovery was fast and 9/11 evaluable patients had more than 200 CD8+ T cells/µl on day 40. Patients conditioned with Fluda-Cy had slower CD4+ and CD8+ T cell recovery compared to other patients (Fig. 5A,B). On day 40, the median number of CD4+ T cells/µl was 94 (51–127) for Fluda-Cy recipients versus 315 (156–570) for other patients ($p = 0.0160$). For CD8+ T cells, the figures were 104 (76–283) and 369 (161–676), respectively ($p = 0.07$). On the other hand, PBSC manipulation apparently also impacted on immune recovery. Patients 1–4 (unmanipulated PBSC) had a higher CD8+ cell count than patients 5–14 (Fig. 5C,D). Finally, mean CD4+ and CD8+ cell counts remained low despite DLI for at least 180 days after CD34-selected transplants (Fig. 5C,D).

**Disease responses**

Five patients were grafted for refractory or poor prognosis non-Hodgkin’s lymphoma (NHL (see Fig. 6)). Four of them were in CR 6 months after the transplant. Patient 11 who developed NHL after an autologous HSCT for refractory Hodgkin’s disease was in CR before and remained in CR >6 months after the transplant. One pa-

---

**FIG. 5.** CD4+ T cell (A and C) and CD8+ T cell (B and D) reconstitution after transplantation. In A and B, patients receiving Fluda-Cy conditioning (broken line) and other patients (continuous line) are shown separately. In C and D, patients receiving unmanipulated PBSC (patients 1–4; continuous line and closed triangles), CD8-depleted PBSC (patients 5–11; broken line and open triangles), or CD34-selected PBSC (patients 12–14; broken line and stars) are shown separately. The horizontal line indicates the lower limit of normal values.
tient with refractory chronic lymphocytic leukemia (CLL) showed a partial response. The two CML patients achieved hematologic remission and major cytogenetic responses (11 and 14% \( \text{Ph}^+ \) BM cells on FISH analysis). Unfortunately, the first patient rejected her graft while developing blast crisis on day 141. The second one is currently treated with DLI after cyclosporine discontinuation. Three patients were treated for RCC. The first one (patient 2) achieved partial response on day 180 that further improved on day 365. The second one (patient 7 who had a 15-cm lung metastasis) died of disease progression on day 24. The third one is not yet evaluated for response. The 2 patients with acute leukemia in CR remained in CR 51+ and 180+ days after the transplant. Finally, a patient with Hodgkin’s disease (HD) in CR after autologous HSCT and mediastinal radiotherapy remains in CR more than 1 year after the NMSCT. The 1-year probability of relapse mortality was 18%.

Transplant-related mortality and survival

After a median follow-up of 230 (44-561) days, 10 of 14 patients (71%) were alive. The actuarial 1-year probability of survival was 69% (Fig. 7A). Two patients died of progressive disease, 1 of septic shock, and 1 of encephalitis. The actuarial 180-day and 1-year probabilities of transplant-related mortality (TRM) were 8 and 16%, respectively (Fig. 7B).

DISCUSSION

NMSCT is based on a two-step approach: first, induction of mixed chimerism and then, in a second step, eradication of malignant cells by the GVL effect (5,9). The Seattle group has developed a very-low-intensity conditioning regimen with only 2 Gy TBI and post-grafting immunosuppression with CsA and MMF (33,34). This approach allowed the transplant procedure to be performed in an outpatient setting in about 50% of the patients and clearly induced a strong GVL effect with a low TRM (34). However, a high rate of graft rejection was observed in patients who had not previously received intensive chemotherapy.

In our study, initial engraftment was seen in all patients. Chimerism analysis on day 42 in the first patient included [a CML patient in first chronic phase (CP)] evidenced poor chimerism (30% T cell and 50% myeloid as well as BM chimerism). She subsequently rejected her graft around day 100. This observation, as well as the report of similar cases by the Seattle team led us to add fludarabine in the conditioning regimen of patients not heavily pretreated, such as CML and RCC patients. This modified conditioning permitted to achieve durable engraftment in all other patients. To avoid radiation-induced organ damage, patients who had previously received \( \geq 12 \) Gy TBI had a non-myeloablative conditioning regimen consisting of cyclophosphamide 3 g/m\(^2\) and fludarabine 90 mg/m\(^2\). This later conditioning regimen also allowed us to conduct the immediate post-transplant course in an outpatient setting, although the degree of myelosuppression was higher. CD8-depletion or CD34-selection of the graft was not associated with initial graft failure or graft rejection, even in mismatched or unrelated transplant recipients. In such patients, initial as well as long-term chimerism was at least as good as in patients receiving unmanipulated PBSC. Although it was thought that a large dose of donor T lymphocytes (and particularly CD8\(^+\) lymphocytes) (40) was required to implant an allogeneic graft in the NMSCT setting (4,41), this study demonstrated for the first time that the absence of CD8 lymphocytes, or even of any lymphocytes, in the graft does not impair initial engraftment and sustained chimerism after a very mild nonmyeloablative regimen and post-transplant CsA and MMF.
Acute and chronic GVHD remained a substantial limitation of the Seattle approach: the incidence of grade II, III, and IV acute GVHD were 33%, 13%, and 4%, respectively (33), and the incidence of chronic GVHD was 74% (34). In animal models, it is now well demonstrated that donor lymphocytes given several weeks after the transplant in mixed chimera induce significantly less GVHD than a similar dose of donor T cells given together with the graft (41,42), without reducing their antitumor efficacy (21,41). More recently, we have reported that after a myeloablative conditioning regimen, the transplantation of CD34-selected allogeneic PBSC followed by pre-emptive CD8-depleted DLI significantly decreased the incidence of acute and severe chronic GVHD compared to transplantation of unmanipulated bone marrow (35). Therefore, we evaluated the feasibility of transplanting T cell-depleted PBSC (by CD8-depletion or by CD34-selection) followed by preemptive CD8-depleted DLI after a mild nonmyeloablative regimen.

Three of the 4 patients (including 2 mismatched transplants) who received unmanipulated PBSC and DLI developed grades II–IV acute GVHD and chronic GVHD. On the other hand, none of the 10 patients who received CD8-depleted or CD34-selected PBSC and pre-emptive CD8-depleted DLI experienced grades II–IV acute GVHD, although this group also included 2 mismatched and 2 unrelated transplants. We chose to give preemptive DLI under cyclosporine prophylaxis because two previous reports have shown that this strategy maintains the GVL effect (35,43). Moreover, in a preliminary report of the French transplant group, the duration of cyclosporine prophylaxis after NMSCT was strongly associated with increased overall survival (44). Unfortunately, despite the short follow-up of our patients, the incidence of extensive chronic GVHD in CD8-depleted transplants was still significant (43%), although lower than that reported by the Seattle team and that observed in our patients receiving unmanipulated PBSC and DLI (in 3 patients).

The small number of patients included as well as their heterogeneity does not allow an accurate estimate of the GVL effect. However, responses were observed in both the unmanipulated and CD8-depleted or CD34-selected groups, suggesting that these latter approaches did not impair the GVL effect, at least when preemptive CD8-depleted DLI are systematically given after the PBSC transplant.

Another approach (than CD8-depletion) to separate the GVL effect from GVHD would be to infuse specific cytotoxic T cells (CTL) instead of DLI. Donor-derived CTL have been successfully used for the treatment of CMV infections (45) or for prevention or treatment of Epstein-Barr virus (EBV)-associated lymphoma after allogeneic HSCT (46). Remarkably, no significant toxicity nor GVHD were observed with this early post-transplant cell immunotherapy. Recently, the Leiden group reported the achievement of CR by treatment with leukemia-reactive CTL in a patient with accelerated phase CML refractory to standard DLI (47,48). The infusion of donor-derived CTL against specific antigens such as minor histocompatibility antigen (mHA) preferentially expressed in the hematopoietic system (49,50), tumor-specific antigens (51), or antigens overexpressed in tumor cells (52,53) after CD8-depleted or CD34-selected PBSC may permit the GVL effect to be increased greatly while minimizing the risk of GVHD.

In conclusion, our study showed that CD8-depleted and CD34-selected PBSC can engraft after a light conditioning regimen consisting of 2 Gy TBI (and fludarabine for previously untreated patients). The preliminary results suggest that CD8 depletion or CD34 selection of the graft followed by CD8-depleted DLI strongly de-
crease the incidence of acute GVHD while apparently preserving the GVL effect. Further studies are needed to confirm this preliminary report, to assess the impact of our strategy on the incidence and severity of chronic GVHD, and to investigate the infusion of specific CTL instead of CD8-depleted DLI.

ACKNOWLEDGMENTS

Frédéric Baron is Research Assistant and Yves Beguin Research Director of the National Fund for Scientific Research (FNRS, Belgium). This work was supported by grants from “La Fondation Bonjean-Oleffe,” “L’Association sportive contre le Cancer,” “Le Fonds de Recherche Scientifique du CHU Sart-Tilman,” and the National Fund for Scientific Research (FNRS, Belgium).

REFERENCES


Address reprint requests to:
Dr. Yves Beguin
University of Liège
Department of Hematology
CHU Sart-Tilman
4000 Liège, Belgium

E-mail: yves.beguin@chu.ulg.ac.be

Received October 12, 2001; accepted October 27, 2001.
This article has been cited by:

1. P Frère, F Baron, C Bonnet, K Hafraoui, M Pereira, E Willems, G Fillet, Y Beguin. 2006. Infections after allogeneic hematopoietic stem cell transplantation with a nonmyeloablative conditioning regimen. Bone Marrow Transplantation 37:4, 411-418. [CrossRef]

2. Frédéric Baron, Nicole Schaaf-Lafontaine, Stéphanie Humblet-Baron, Nathalie Meuris, Emilie Castermans, Etienne Baudoux, Pascale Frère, Vincent Bours, Georges Fillet, Yves Beguin. 2004. T-cell reconstitution after unmanipulated, CD8-depleted or CD34-selected nonmyeloablative peripheral blood stem-cell transplantation. Transplantation 76:12, 1705-1713. [CrossRef]