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Allogeneic stem cell transplantation with alemtuzumab-based conditioning for patients with advanced chronic myelogenous leukemia

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Abstract
Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the treatment of choice for patients with chronic myelogenous leukemia (CML) who have failed or are intolerant to tyrosine kinase inhibitors (TKI). Myeloablative conditioning regimens have been associated with high treatment-related mortality (TRM) rate in such patients, and reduced-intensity conditioning (RIC) regimens are often preferred but have high rates of disease recurrence and graft-versus-host-disease (GVHD). We report our experience with nine CML patients (four chronic phase and five with accelerated phase or blast crisis) who failed TKI and underwent allo-HSCT using an alemtuzumab-based RIC regimen. The conditioning regimen was well tolerated and induced engraftment in all patients, and complete cytogenetic remission (CCyR) in eight of nine. Four patients, all with a history of accelerated phase or blast crisis, died. Four of the five remaining patients had a cytogenetic relapse a median of 10 months after transplantation. Donor lymphocyte infusion (DLI), TKI or both induced a CCyR in all cases. With a median follow-up of 47 months, five patients, including all those transplanted in first or second chronic phase, are alive and in remission. Allo-HSCT with an alemtuzumab-based conditioning regimen induces remission in patients with CML that have failed TKI therapy and has a low incidence of GVHD. Disease recurrence is frequent but responds to DLI. In some cases, restoration of susceptibility to TKI was observed. Outcomes may improve with the routine administration of post-transplant TKI.

Keywords: Chronic myelogenous leukemia, reduced-intensity conditioning, alemtuzumab, tyrosine kinase inhibitors

Introduction
Although allogeneic hematopoietic stem cell transplantation (allo-HSCT) is still considered the only curative therapy in chronic myelogenous leukemia (CML), it is currently mainly used for patients who failed, or are intolerant to imatinib or other tyrosine kinase inhibitors (TKI) [1,2]. Such patients tend to have higher complication rates and decreased survival after myeloablative transplantation [3–5]. Reduced-intensity conditioning (RIC) regimens were developed to allow allo-HSCT in older patients and those with co-morbidities. CML patients treated with RIC have acceptable treatment-related mortality (TRM), and overall survival but a high incidence of graft-versus-host-disease (GVHD) as well as higher relapse rate [6,7]. Alemtuzumab is a humanised monoclonal antibody directed against CD52 antigen, expressed on T and B lymphocytes, as well as other non-target cells [8]. Widely used in chronic lymphocytic leukemia, there is now mounting evidence of its benefits in stem cell transplantation, particularly in the prevention of acute and chronic GVHD [7,9–12]. Here, we report our experience with alemtuzumab-based RIC regimens in nine consecutive patients with CML.
Patients and results

Between March 2002 and December 2007, nine patients with CML underwent allo-HSCT using an alemtuzumab-based conditioning at the University of Chicago Medical Center. One additional patient left the hospital after allo-HSCT against medical advice on Day 18 and explicitly refused follow-up. He is not included in this analysis. All patients were enrolled on treatment protocols approved by the Institutional Review Board and had provided written informed consent.

Interval from diagnosis to transplantation ranged from 10 to 156 months. As shown in Table I, most patients were extensively pretreated and had a high comorbidity score and decreased performance status at the time of transplantation. All had received prior imatinib. Seven of the nine were resistant to imatinib and two (Patients 2 and 8, Table I) were intolerant; Patient 2 developed diarrhea and Patient 8, liver toxicity. They both lost their response upon discontinuation of imatinib. Four patients (Patients 1, 3, 4 and 6, Table I) received dasatinib after failure of imatinib; only Patient 6 achieved a major cytogenetic response to dasatinib.

At the time of initial transplant recommendation, three patients (Patients 1–3, Table I) had blast crisis, three (Patients 4–6, Table I) were in accelerated phase and three (Patients 7–9, Table I) were in first chronic phase. One patient with accelerated phase (Patient 6, Table I) achieved a second chronic phase with dasatinib; the five other patients with blast crisis or accelerated phase received cytoreductive therapy before transplantation with cladribine 15 mg/m² given intravenously daily for 5 days [13]. This was followed by a course of high-dose cytarabine and mitoxantrone in one patient. Patients 1–4 underwent transplantation during the nadir of chemotherapy-induced cytopenia. Patients 5 and 6 were transplanted in a second chronic phase.

Conditioning and GVHD prophylaxis

All patients received alemtuzumab 20 mg daily for five consecutive days before transplant. In one patient (Patient 5, Table I), this was combined with busulfan 0.8 mg/kg/day intravenously for 4 days and cyclophosphamide 60 mg/kg/day for 2 days. In four patients (Patients 1, 3, 4 and 6, Table I), it was combined with fludarabine 30 mg/m² daily for 5 days and area under the curve (AUC)-targeted busulfan daily for 4 days [14]. In the remaining four patients (Patients 2, 7, 8 and 9, Table I), it was combined with fludarabine 30 mg/m² daily for 5 days and melphalan 140 mg/m² for 1 day [7]. Post-transplant GVHD prophylaxis consisted of tacrolimus in all patients. Transplant supportive care was as previously described [12,13] and included high-dose acyclovir instead strategy [17]. All patients received peripheral blood stem cell transplantation; seven had Human leukocyte antigen (HLA)-identical sibling donors and two had unrelated donors. No prophylactic TKI therapy or donor lymphocyte infusion (DLI) was given after engraftment.

Regimen-related toxicity was graded according to the common toxicity criteria (CTC) criteria [18]. White blood cell (WBC) engraftment was defined as the first of three consecutive days with an absolute neutrophil count of at least 0.5 × 10⁹/L. Acute and chronic GVHD were graded according to standard criteria [19,20]. Post-transplantation donor–recipient chimerism was assessed by means of DNA microsatellite analysis as previously described [21] and/or by fluorescent in situ hybridization (FISH) when a sex mismatch was present. Hematologic response was defined as complete if all peripheral blood counts had normalised. For molecular and cytogenetic response, blood and bone marrow were analysed for BCR-ABL by RT-PCR or t(9;22) using FISH [22]. Most of the patients were managed before mutational analysis of BCR-ABL was routinely done.

Toxicity, treatment-related mortality, engraftment

Life threatening toxicities occurred only in patients with advanced disease and/or a high comorbidity score (HCT-CI). Three patients (Patients 1, 2 and 4, Table I) required intensive care admissions with prolonged hospitalisations and ventilation assistance in two of them, but recovered. No treatment-related deaths occurred in the immediate post-transplant period. WBC engraftment occurred in all patients between Day +9 and Day +18. At the time of the first disease assessment on Day +30, eight of the nine patients showed full engraftment with more than 95% donor chimerism in unfractionated cells. T-cell chimerism on Day +30 was available in three patients and showed more than 95% donor chimerism in unfractionated cells. T-cell chimerism in two patients and 82% donor chimerism in Patient 4. But Patients 2, 3 and 6 had persistent poor hematopoietic function and continued to require growth factor and transfusion support with blood and platelets, despite persistent donor chimerism. No further stem cell infusion was administered.

Outcomes

On Day 30, Patients 2–9 were in complete cytogenetic response by FISH or conventional karyotype
Table I. Patients, disease, and transplant.

<table>
<thead>
<tr>
<th>Age at transplantation/sex</th>
<th>37/F</th>
<th>63/F</th>
<th>61/M</th>
<th>61/F</th>
<th>54/M</th>
<th>64/M</th>
<th>44/F</th>
<th>55/F</th>
<th>64/M</th>
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<td>0</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0–1</td>
<td>1</td>
<td>1</td>
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<td>HCT CI</td>
<td>≥3</td>
<td>&lt; 3</td>
<td>≥3</td>
<td>≥3</td>
<td>&lt; 3</td>
<td>&lt; 3</td>
<td>&lt; 3</td>
<td>&lt; 3</td>
<td>≥3</td>
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<tr>
<td>Number of prior therapies</td>
<td>4</td>
<td>1–2</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>1–2</td>
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<td>Imatinib;</td>
<td>Imatinib;</td>
<td>Imatinib;</td>
<td>Imatinib;</td>
<td>Imatinib;</td>
<td>Imatinib;</td>
<td>Imatinib;</td>
<td>Imatinib;</td>
</tr>
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<td>Resistance</td>
<td>Intolerance</td>
<td>Resistance</td>
<td>Resistance</td>
<td>Resistance</td>
<td>Resistance</td>
<td>Intolerance</td>
<td>Resistance</td>
<td></td>
</tr>
<tr>
<td>Disease status at AlloSCT</td>
<td>MyBC</td>
<td>MyBC</td>
<td>LyBC</td>
<td>AP</td>
<td>2d CP</td>
<td>2d CP</td>
<td>CP</td>
<td>CP</td>
<td>CP</td>
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<tr>
<td>Cytoreduction before SCT</td>
<td>Cladribine</td>
<td>Cladribine</td>
<td>Cladribine</td>
<td>Cladribine</td>
<td>Cladribine</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<td>Interval from diagnosis</td>
<td>10 m</td>
<td>18 m</td>
<td>10 m</td>
<td>116 m</td>
<td>29 m</td>
<td>156 m</td>
<td>14 m</td>
<td>15 m</td>
<td>36 m</td>
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<td>Conditioning regimen</td>
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<td>Flu/Mel/Camp</td>
<td>Bu/Flu/Camp</td>
<td>Bu/Flu/Camp</td>
<td>Bu/Flu/Camp</td>
<td>Flu/Mel/Camp</td>
<td>Bu/Flu/Camp</td>
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<td>Flu/Mel/Camp</td>
</tr>
<tr>
<td>Donor</td>
<td>Sibling</td>
<td>Sibling</td>
<td>Sibling</td>
<td>Sibling</td>
<td>MUD 8/8</td>
<td>MUD 7/8</td>
<td>Sibling</td>
<td>Sibling</td>
<td>Sibling</td>
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<tr>
<td>Time to ANC &gt; 500</td>
<td>D + 12</td>
<td>D + 9</td>
<td>D + 15</td>
<td>D + 11</td>
<td>D + 15</td>
<td>D + 18</td>
<td>D + 10</td>
<td>D + 9</td>
<td>D + 9</td>
</tr>
<tr>
<td>D 30 cytogenetic response</td>
<td>NoCyR</td>
<td>CCyR</td>
<td>CCyR</td>
<td>CCyR</td>
<td>CCyR</td>
<td>CCyR</td>
<td>CCyR</td>
<td>CCyR</td>
<td>CCyR</td>
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<tr>
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<td>FDC</td>
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<td>FDC</td>
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<td>No</td>
<td>No</td>
<td>4 m</td>
<td>No</td>
<td>12 m</td>
<td>12 m</td>
<td>11 m</td>
</tr>
<tr>
<td>GVHD</td>
<td>No</td>
<td>Skin</td>
<td>Skin</td>
<td>Skin</td>
<td>Skin</td>
<td>No</td>
<td>No</td>
<td>Skin + liver</td>
<td>Skin + liver</td>
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<tr>
<td>Salvage therapy</td>
<td>Nilotinib</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Imatinib + DLI; dasatinib</td>
<td>NA</td>
<td>Imatinib</td>
<td>DLI</td>
<td>DLI</td>
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<tr>
<td>Response to salvage</td>
<td>NR</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>CCyR</td>
<td>NA</td>
<td>CCyR</td>
<td>CCyR</td>
<td>CCyR</td>
</tr>
<tr>
<td>Last follow-up</td>
<td>Died +4 m</td>
<td>Died +15 m</td>
<td>Died +6 m</td>
<td>Died +9 m</td>
<td>Alive +52 m</td>
<td>Alive +30 m</td>
<td>Alive +49 m</td>
<td>Alive +54 m</td>
<td>Alive +48 m</td>
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<tr>
<td>Cause of death</td>
<td>BC CML</td>
<td>Infection</td>
<td>Infection</td>
<td>CV event</td>
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<td>NA</td>
<td>NA</td>
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<tr>
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<td>Good</td>
<td>Poor</td>
<td>Poor</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
</tr>
</tbody>
</table>

CML, chronic myelogenous leukemia; M, male; F, female; HCT CI, hematopoietic cell transplantation-specific comorbidity index; TKI, tyrosine kinase inhibitor; CP, first chronic phase; 2dCP, second chronic phase; AC, accelerated phase; MyBC, myeloblast crisis; LyBC, lymphoid blast crisis; m, months; D, day; MUD, matched unrelated donor; CCyR, complete cytogenetic response; NoCyR, no cytogenetic response; NR, no response; FDC, full donor chimerism; MDC, mixed donor chimerism; GVHD, graft-versus-host disease; DLI, donor lymphocyte infusion; NA, not applicable; CV, cardiovascular.
analysis. Patient 1 never achieved a cytogenetic response after transplantation despite salvage therapy with nilotinib and died 4 months later. Among the 8 patients who achieved a complete cytogenetic response after transplant, Patients 2–4, all transplanted in blast crisis, died 15, 6 and 9 months after transplant, without evidence of CML. Patient 2 died from respiratory failure secondary to a septic shock. Patient 3 developed CMV pneumonia with subsequent multiorgan failure, despite high-dose valacyclovir prophylaxis. These two events occurred in the setting of persistent poor hematopoietic function. Patient 4 who had a long history of severe renal and cardiac illness died suddenly at home from a presumed cardiovascular event. Four of the remaining five other patients relapsed. Three had a durable response to DLI. Their course is briefly described. Patient 5, who was resistant to imatinib prior to transplant and who was transplanted in second chronic phase, relapsed 4 months after transplant with 10% Philadelphia positive cells on cytogenetic analysis and still 94% donor chimerism in the bone marrow. He was started on imatinib 400 mg per day resulting in complete response by cytogenetic analysis and still 94% donor chimerism. She was started on imatinib 400 mg per day resulting in complete response by FISH within 30 days. Five months later, ~9 months after transplant, 34% of cells were Philadelphia positive and donor chimerism decreased to 70%. Imatinib was increased to 800 mg per day but without further response. Ten months after transplant and while continuing imatinib, he received a DLI containing a dose of $2 \times 10^7$ CD3+ /kg. A second DLI containing $8 \times 10^7$ CD3+ /kg was given 1 month later. He developed limited GVHD of the skin and dry eyes. Thirteen months after transplant he obtained a durable complete cytogenetic response with more than 95% donor chimerism. BCR-ABL remained however detectable in the blood for 36 months after achieving cytogenetic remission, he was recently switched to dasatinib. Two recent molecular assays have been negative for BCR/ABL.

Patient 7, who was resistant to imatinib before transplant and who was transplanted in chronic phase, relapsed 12 months after transplant with detection of 10% Philadelphia positive cells on cytogenetic analysis and positive BCR/ABL RT-PCR. At that time she still had 92% donor chimerism. She was started on imatinib 400 mg per day and obtained a durable hematological response and complete cytogenetic remission (CCyR) that is currently ongoing 4 years after transplant despite persistent minimal residual disease detectable by BCR/ABL RT-PCR.

Patient 8 who was intolerant to imatinib and transplanted in chronic phase, relapsed 12 months after transplant with detection of 10% Philadelphia positive cells on cytogenetic analysis and 87% donor chimerism. She received a first DLI containing $2 \times 10^7$ CD3+ /kg followed 2 months later by a second DLI containing $5 \times 10^7$ CD3+ /kg. She achieved a durable complete cytogenetic response but developed skin and liver GVHD after the second infusion. She is currently alive and in complete molecular remission 54 months after the transplant.

Patient 9 who was resistant to imatinib before transplant and transplanted in chronic phase, lost his cytogenetic response almost 1 year after transplant when he presented with 18% Philadelphia positive cells by FISH and 65% donor chimerism. Fifteen months after transplant he received a DLI containing $20 \times 10^6$ CD3+ /kg. He developed limited GVHD of skin, liver, gut and lungs but achieved a durable complete cytogenetic response and complete molecular remission.

At the time of the last follow-up in May 2008 five of the nine patients were alive, all of them in CCyR or better. All these patients were transplanted in first or second chronic phase (Figure 1).

GVHD

Grade II acute GVHD mainly involving skin occurred after transplantation in four patients and responded well to treatment with steroids. Two more patients developed GVHD only after DLI infusion. Patient 8 who had no previous history of GVHD, developed skin and liver GVHD after DLI and required treatment with prednisone and tacrolimus for approximately 1 year. Patient 9 had a previous history of GVHD when he received DLI 1 year after transplant. After DLI he developed Grade 2 skin and liver GVHD that was refractory to prednisone, tacrolimus and mycophenolate. He subsequently was treated with extracorporeal photopheresis and finally received one course of rituximab resulting in a slow improvement. At the time of the last follow-up, he was still taking prednisone.

Discussion

Allo-HSCT remains an important salvage therapy for patients with CML who are resistant or intolerant to TKI. Patients with CML in chronic phase who have failed prior TKI have acceptable rates of disease control after myeloablative transplantation, but are prone to toxicities and TRM. Oehler et al. reported that patients who underwent transplantation in chronic phase with a suboptimal response to TKI had a significant worse overall survival when compared with chronic phase patients who were in complete cytogenetic response [3]. RIC are being increasingly used in high-risk patients with comorbidities as well as older patients, and rely mainly
on GvL (graft-versus-leukemia) effects to induce remissions [23]. Studies of RIC in CML show feasibility, but increased relapse rate and GVHD [6,7]. In vivo alemtuzumab, a monoclonal antibody directed against CD52, allows depletion of recipient and donor T cells and significantly reduces the risk for acute and chronic GVHD without much increasing the risk of graft rejection [24,25]. Nevertheless, the T-cell depletion causes a delay in immune reconstitution, and an impairment of GVL effect.

Here we report our experience with alemtuzumab-based GVHD prophylaxis regimen in nine patients with CML with both myeloablative and RIC. Seven had become resistant to TKI and two were intolerant. More than half the patients were in accelerated phase or blast crisis before transplantation and received cytoreductive therapy with cladribine in the weeks leading up to transplant [13]. Several patients had a decreased performance status and three had a high HCT-CI. They represent therefore a very high risk group for TRM and relapse. We hypothesised that the tolerability of alemtuzumab-based conditioning might be to their advantage despite the increase risk of disease recurrence.

Most patients achieved rapid neutrophil engraftment and eight out nine patients achieved a complete cytogenetic response with more than 95% donor chimerism on Day 30. The incidence of severe acute and chronic GVHD was also quite low. Of the four patients transplanted in blast crisis or accelerated phase, one relapsed, one died of unrelated cardio-vascular event and two, despite full donor chimerism, had persistent poor hematopoietic function contributing to late, fatal opportunistic infections. The reason for the incomplete hematopoietic reconstitution is unclear, is extremely uncommon after fludarabine alemtuzumab melphalan conditioning in patients with other hematologic malignancies, and may be related to stromal dysfunction perhaps related to exposure to cladribine in the weeks leading up to the transplant.

Four of the five patients transplanted in first or second chronic phase relapsed after a median time of 10 months. These data are consistent with those of Crawley et al. who reported outcomes of RIC in CML patients using different regimens and noticed a higher relapse rate after the use of alemtuzumab than after the use of ATG (anti-thymocyte globulin) [6]. Two other reports about the use of alemtuzumab in myeloablative transplant in CML patients showed a high relapse rate as well [26,27]. More intensive conditioning therefore does not seem to overcome the impairment of GvL effect by alemtuzumab, but interestingly additional therapy with DLI is quite effective in this setting. All our relapsed patients responded to salvage therapy with DLI and/or imatinib and all are currently alive and in ongoing remission. We also observed one transient and one durable response to imatinib in Patients 1 and 3, respectively, both of whom were resistant to imatinib before transplantation.

In summary, for patients with CML who failed imatinib, but are in chronic phase at the time of allo-HSCT, alemtuzumab-based conditioning is well
tolerated, leads to excellent engraftment, a high percentage of remissions with a low risk of GVHD and excellent long-term survival. For patients with more advanced disease, the results were disappointing. Because of a very high recurrence rate for patients transplanted in chronic phase, additional prophylactic treatment is necessary. Prophylactic low dose DLI is the most effective approach but puts patients at risk of pancytopenia and GVHD [28]. Maintenance treatment with a TKI appears safer and well tolerated and should therefore be routinely considered [28–31]. Because several non-cross resistant TKIs are available, we propose a systematic maintenance therapy during 1 year with dasatinib or nilotinib in imatinib failures. DLI could then be given in case of molecular or cytogenetic relapse as was suggested by Olavarria et al [32]. It is likely that increasingly patients will be referred who have failed all available TKIs. For such patients our observation of restoration of sensitivity to TKI justifies their reintroduction after transplant. One could also consider combination of DLI with TKI as suggested by Savani et al. [31], combination of TKI to overcome resistance, or the use of aurora kinase inhibitors or other experimental drugs [33–35]. Close disease monitoring usually by monitoring BCR-ABL levels in the peripheral blood is particularly important. Mutation analysis of BCR-ABL should be helpful as well.

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References


