Autologous Stem Cell Transplantation with PCR-Negative Graft Would Be Associated with a Favorable Outcome in Core-Binding Factor Acute Myeloid Leukemia

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Although core-binding factor acute myeloid leukemia (CBF-AML) is generally considered to be a low-risk form of AML, the survival rate is still 50% to 60%. To evaluate the effectiveness of autologous stem cell transplantation (ASCT) with a PCR-negative graft we analyzed a series of consecutive CBF-AML patients. Between 1997 and 2006, 18 patients aged <60 years were referred under a diagnosis of CBF-AML. Peripheral blood stem cells (PBSC) were collected after a second or further course of postremission therapy. When \( >2.0 \times 10^6 \text{kg CD34-positive cells with minimal residual disease (MRD) undetectable by nested polymerase chain reaction (PCR)} \) had been collected, ASCT was performed with busulfan, etoposide, and cytarabine combined with granulocyte colony-stimulating factor. Event-free survival (EFS) and complications of ASCT were then assessed. Fourteen of the 18 patients received ASCT. The median observation period was 4.4 years. The 5-year EFS was 93% for ASCT patients, despite the presence of adverse factors. In 8 of 10 patients who had detectable MRD in the bone marrow before ASCT, MRD became undetectable after ASCT. Neutrophils recovered promptly within 2 weeks, but platelets recovered relatively slowly. Half of the patients suffered from varicella zoster virus infection. Although 1 case of myelodysplastic syndrome occurred, there was no case of relapse. ASCT with a PCR-negative graft was associated with excellent EFS. For patients with CBF-AML, especially with adverse factors or remnant MRD in the bone marrow, this strategy is the treatment of choice.


KEY WORDS: Core binding factor acute myeloid leukemia, Autologous stem cell transplantation, Minimal residual disease, Polymerase chain reaction

INTRODUCTION

Translocation (8;21) (q22;q22) or inversion (16) occurs in approximately 7% to 8% of patients with de novo acute myeloid leukemia (AML) [1]. These leukemia entities are associated with aberration of core-binding factors (CBF), which are heterodimeric transcriptional regulators containing a common \( \beta \) (CBF\( \beta \)) and 1 of 3 \( \alpha \) (CBF\( \alpha \)) subunits. Translocation fuses the AML1 (CBF\( \alpha \)2) gene located on chromosome 21 to the ETO (MTG8) gene located on chromosome 8. The CBF\( \beta \) gene located at 16q22 fuses with the MYH11 gene located at 16p13. The AML1-ETO or CBF\( \beta \)-MYH11 fusion protein represses and alters the function of CBF during normal differentiation [2].

Both cytogenetic groups (referred to as CBF-AML) have a relatively favorable prognosis compared with most other forms of adult AML [1,3-5]. In younger patients, repeated cycles of high-dose cytarabine (HDAC) therapy can prolong survival [6,7].

Prognostic factors of CBF-AML have been evaluated in several studies. In (8;21) AML patients, inferior outcome has been associated with a high white blood cell (WBC) count [8], a low platelet count [8,9], a high WBC index [10], loss of sex chromosomes [8], expression of CD56 antigen [11], extramedullary disease [12], non-White race [9], and older age [9]. In inv(16) AML patients, a high WBC count [13,14],
older age [9,15], a low platelet count [9,15], and absence of the additional aberration of trisomy 22 [8] have been considered to be adverse factors. In addition, c-KIT mutations have recently been identified as adverse factors for CBF-AML [16]. Although opinion about these adverse factors varies, disease-free survival (DFS) is estimated to be <50% [8-15] when patients with CBF-AML have at least 1 adverse factor. In addition, response rate and survival after first relapse are low and short in t(8;21) AML [8,9].

The European Group for Bone and Marrow Transplantation and the Japanese Society of Hematopoietic Stem Cell Transplantation have recommended autologous stem cell transplantation (ASCT) for selected AML patients achieving first complete remission (CR1) [17,18]. Based on these guidelines, ASCT has been performed at our center for younger AML patients with favorable risk other than acute promyelocytic leukemia or with intermediate risk without a human leukocyte antigen (HLA)-matched sibling. In addition, we have infused peripheral blood stem cells (PBSC) in which minimal residual disease (MRD) is undetectable using a nested polymerase chain reaction (PCR) (ASCT with a PCR-negative graft).

We have retrospectively analyzed a series of younger consecutive CBF-AML patients and evaluated ASCT using a PCR-negative graft.

**MATERIALS AND METHODS**

**Patients**

The analysis included all consecutive patients aged <60 years with CBF-AML diagnosed and treated at our institution between October 1997 and November 2006. In this survey, CBF-AML was defined by the presence of either t(8;21)(q22;q22) or inv(16)(p13;q22)/t(16;16)(p13;q22) chromosomal rearrangement, or by the presence of the AML1-ETO (MTG8) fusion gene or the presence of the CBFβ-MYH11 fusion gene confirmed by PCR. Although this study was not a formal clinical trial, all events and information were systematically recorded and available. This analysis was approved by our institutional review board.

**Therapy**

Induction therapy for younger patients aged <60 years was started with idarubicin (12 mg/m² days 1-7), etoposide (100 mg/m², days 1-5), daunorubicin (50 mg/m², days 1-3) and mercaptopurine (70 mg/m², days 1-7); third therapy, mitoxantrone (10 mg/m², days 1-2), etoposide (100 mg/m², days 1-4), and cytarabine (1 g/m², every 12 hours, days 1-4); fourth to seventh therapy, cytarabine (3 g/m², every 12 hours, days 1,3,5).

If a patient had neither active infection nor sepsis, PBSC were mobilized by granulocyte colony-stimulating factor (G-CSF), and a collection of PBSC was attempted in the phase of recovery from myelosuppression after second or further postremission chemotherapy. One cycle of PBSC collection was defined as a sequential collection course after 1 chemotherapy course. PBSC collection was repeatedly attempted within a maximum of 3 cycles. When more than 2.0 × 10⁶/kg CD34-positive cells in which MRD was undetectable by nested PCR had been collected, ASCT was attempted after a third or further session of postremission chemotherapy. Although the ideal doses were >2.0 × 10⁶/kg CD34-positive cells, in fact, ASCT was performed when at least 1.5 × 10⁶/kg CD34-positive cells were collected after 3 cycles of PBSC collections had been attempted.

The conditioning regimen for ASCT was G-CSF combined with BEA [19] as follows: busulfan (4 mg/kg/day for 4 days as 1 mg/kg four times a day for 16 doses on days −9−3), etoposide (20 mg/kg on days −5−4), cytarabine (100 mg/m² on days −10−4, 3 g/m² every 12 hours on days −3−2), and filgrastim 200 μg/m² on days −12−4). PBSC were administered on day 0. Filgrastim (300 μg) was started on day 1 until recovery of granulocytes. Prophylactic levofloxacin or tosufloxacin 300 mg/day, fluconazole 200 mg/day, and acyclovir 1000 mg/day were administered from day −7 until neutrophils had recovered to >0.5 × 10⁹/L.

**Minimal Residual Disease Monitoring**

We evaluated the bone marrow (BM) and harvested PBSC for assessment of MRD. From 2001, we used quantitative real-time reverse transcriptase PCR (RQ-PCR) for detection of MRD in BM. Before 2000, MRD in BM was evaluated by fluorescence in situ hybridization or nested reverse transcriptase PCR (nested RT-PCR). MRD in PBSC were evaluated by nested RT-PCR.

For PCR, total RNA was extracted from mononuclear cells in BM and transcribed to cDNA in accordance with the manufacturer’s instructions. RT-PCR assay [20-23] was performed by Taqman technology using the following primers: for AML1/MTG8 chimeric mRNA, forward 5’…GAG CCA TCA AAA TCA TTC TTG GAG CTC CTT…3’, reverse 5’…ATG AAC TGG TTC TTG GAG CTC CT’…3’, and probe 5’ FAM (6-carboxylfluorescein)…CAC CTG TGG
ATG TGA AGA CGC AAT CTA GGC TG…TAMRA (6-carboxy-tetramethyl-rhodamine) 3′; for CBFB/MYH11 chimeric mRNA, forward 5′…CTC CAA AGA GTG GATG ATG GGC …3′, reverse 5′…CTT GGA CTT TCT CAG CTC ATG G …3′; and probe 5′ FAM...TCT GGA GTT TGA TGA GGA GCG AGC CC...TAMRA 3′. Nested RT-PCR was performed in accordance with previous reports [21-23]. For RQ-PCR, the number of transcript copies was normalized relative to glycer-aldehyde 3-phosphate dehydrogenase, and converted into molecules/µg RNA. The detection threshold of RQ-PCR was 50 copies/µg RNA and the sensitivity was 10⁻⁴. The threshold of nested RT-PCR was 10⁻⁵.

Mutational Analysis of c-KIT

Mutational analysis of the extracellular (EC) domain (exons 8 and 9), transmembrane (TM) domain (exon 10), juxtamembrane (JM) domain (exon 11), and the second intracellular kinase (TK) 2 domain (exons 17 and 18) of the c-KIT gene was performed with PCR followed by direct sequencing. The genomic DNA from Wright-Giemsa-stained or unstained blood smears was extracted with a Gentra Puregene Tissue Kit (Qiagen, Hilden, Germany). TaKaRa LA Taq DNA polymerase (Takara, Shiga, Japan) was used to amplify the genes from genomic DNA. PCR products were purified using the QIAquick PCR purification kit (Qiagen) and sequenced bidirectionally using the Big Dye Termination 3.1 kit and the ABI Prism 310 system (Perkin-Elmer Cetus, Norwalk, CT). Specific sequences of primers used for PCR and sequencing are available upon request. To validate the sequencing results, PCR products were inserted into the pCR2.1-TOPO vector using a TOPO TA cloning kit (Invitrogen, Carlsbad, CA). Recombinant plasmids isolated from 8 to 12 white colonies were sequenced.

Statistical Analysis

The major indicator of outcome was event-free survival (EFS), defined as the period from initial diagnosis to relapse (failure), secondary malignancy (failure), death because of any cause (failure), and alive at last follow-up (censored). Overall survival (OS) was also assessed, and was defined as the period from initial diagnosis to death because of any cause (failure), and alive at last follow-up (censored). Estimations of EFS and OS distributions were performed by the Kaplan-Meier method. Comparisons of patient characteristics were performed by χ² test for categorical variables and by the Mann-Whitney U test for continuous variables. A Cox hazard model was used for univariate analysis of prognostic factors. Estimates of hazard ratios (HR) and corresponding 95% confidence intervals (CIs) were obtained for each of the following variables: gender, age, WBC, hemoglobin, platelets, lactate dehydrogenase (LDH), percentage of blasts in peripheral blood or BM, WBC index, karyotype aberration, CD56 positivity, and total dose of cytarabine. WBC index was derived as the product of WBC and the ratio of marrow blasts at diagnosis [10]. To assess the impact on EFS of a cytarabine dosage exceeding 1 g/m², the actual cumulative dosage was calculated and entered as a continuous variable for univariate analysis. For all analyses, statistical significance was defined as a 2-sided value of P < .05. Statistical analyses were performed with StatView version 5.0.

RESULTS

Patient Characteristics

Between October 1997 and November 2006, 25 patients were diagnosed as having CBFB-AML. Of these patients, 18 were <60 years old and eligible for ASCT. Sixteen had t(8;21) AML and 2 had inv(16) AML (Table 1). Fourteen patients actually received ASCT. The remaining 4 who did not receive ASCT included 2 with relapse and secondary myelodysplasia during postremission chemotherapy, 1 with poor mobilization of PBSC, and 1 who withdrew consent to treatment. The relapsed and MDS patients received allogeneic transplantation at other hospitals and were lost to follow-up.

Between patients with and without ASCT, there were no significant differences in additional chromosome aberrations, CD56 positivity or WBC index. However, patients with ASCT had moderately lower platelet counts as well as more blasts in their peripheral blood and BM at diagnosis, and received a lower cumulative dose of cytarabine, although the differences were not statistically significant.

Survival Analysis

The median period of observation of survivors was 4.4 years. All of 18 patients achieved complete remission after induction therapy. The estimated 5-year EFS for these patients and the patients with ASCT was 83.0% ± 9.0% (±standard error) and 92.9% ± 6.9%, respectively (Figure 1). The estimated 5-year OS for the 18 patients was 100%, although follow-up details were lost for relapsed and MDS patients who later underwent allogeneic transplantation at other hospitals. Univariate analysis of prognostic factors for EFS showed that ASCT was the only significant factor (HR 12.9 [CIs: 1.05-157, P = .045]), and that age, WBC, cumulative dose of cytarabine, CD56 positivity, loss of sex chromosomes, and lower platelet count had no prognostic value for EFS. Analysis of OS was not done because none of the patients died.
Fourteen patients received ASCT using an identical conditioning regimen and PBSC with undetectable MRD. The median period from diagnosis to ASCT was about 9 months (Table 2). A median of 5 chemotherapy courses were given before ASCT. Neutrophil counts recovered to more than 0.5 \times 10^9/L within a median period of 13 days (range: 11–36 days). Platelet counts recovered to >20 \times 10^9/L within a median period of 27.5 days (range: 12–217 days) and to >50 \times 10^9/L within a median period of 4 months (range: 15–378 days). Infusion of more CD34 cells was associated with prompter platelet recovery (P = .027 for platelets >20 \times 10^9/L and P = .038 for platelets >50 \times 10^9/L, calculated by Pearson’s correlation coefficient). As late adverse events, 5 patients suffered varicella zoster virus (VZV) reactivation, which was promptly resolved with acyclovir. These infections occurred at a median of 130 days after ASCT (range: 85–567 days). Although 1 case of secondary myelodysplasia was observed after ASCT, no relapse occurred.

### Details of PBSC Collection Among ASCT Patients

In total, 23 cycles of PBSC collection were performed in 14 patients. A median of 1.85 \times 10^6/kg CD34-positive cells were collected in each cycle. Eight cycles of PBSC collection were performed after the fourth session of postremission chemotherapy, 4 cycles after the fifth and sixth sessions, and 3 cycles after the second and third sessions. In 1 cycle, no harvest of PBSC was performed.

### ASCT and Clinical Features

Fourteen patients received ASCT using an identical conditioning regimen and PBSC with undetectable MRD. The median period from diagnosis to ASCT was about 9 months (Table 2). A median of 5 chemotherapy courses were given before ASCT. Neutrophil counts recovered to more than 0.5 \times 10^9/L within a median period of 13 days (range: 11–36 days). Platelet counts recovered to >20 \times 10^9/L without transfusion within a median period of 27.5 days (range: 12–217 days) and to >50 \times 10^9/L within a median period of 4 months (range: 15–378 days). Infusion of more CD34 cells was associated with prompter platelet recovery (P = .027 for platelets >20 \times 10^9/L and P = .038 for platelets >50 \times 10^9/L, calculated by Pearson’s correlation coefficient). As late adverse events, 5 patients suffered varicella zoster virus (VZV) reactivation, which was promptly resolved with acyclovir. These infections occurred at a median of 130 days after ASCT (range: 85–567 days). Although 1 case of secondary myelodysplasia was observed after ASCT, no relapse occurred.

### Table 1. Patient Characteristics and Clinical Features

<table>
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<tr>
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<th>Total</th>
<th>With ASCT</th>
<th>Without ASCT</th>
<th>P Value</th>
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<td>Number of patients</td>
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<td>14</td>
<td>4</td>
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<td>Gender</td>
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<td>male</td>
<td>15</td>
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<td>4</td>
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<td>female</td>
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<td>3</td>
<td>0</td>
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<td>Age (years) median</td>
<td>44</td>
<td>44</td>
<td>51</td>
<td>.12</td>
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<tr>
<td>range</td>
<td>20–59</td>
<td>20–59</td>
<td>43–53</td>
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<tr>
<td>WBC (x10^9/L) median</td>
<td>5.5</td>
<td>5.5</td>
<td>6.8</td>
<td>.91</td>
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<td>range</td>
<td>1.7–82</td>
<td>1.7–82</td>
<td>2.5–37</td>
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<tr>
<td>Hb (g/L) median</td>
<td>85.5</td>
<td>78</td>
<td>99</td>
<td>.46</td>
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<td>range</td>
<td>38–131</td>
<td>38–131</td>
<td>77–126</td>
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<td>Plt (x10^9/L) median</td>
<td>35</td>
<td>24</td>
<td>58</td>
<td>.089</td>
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<td>7.0–101</td>
<td>7.0–101</td>
<td>44–81</td>
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<td>LDH (IU/L) median</td>
<td>661</td>
<td>872</td>
<td>644</td>
<td>.75</td>
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<tr>
<td>range</td>
<td>245–6090</td>
<td>245–6090</td>
<td>420–1865</td>
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<tr>
<td>PB blast (%) median</td>
<td>47</td>
<td>49.5</td>
<td>32.8</td>
<td>.22</td>
</tr>
<tr>
<td>range</td>
<td>13–90</td>
<td>24.5–90</td>
<td>18–82.5</td>
<td></td>
</tr>
<tr>
<td>BM blast (%) median</td>
<td>67.3</td>
<td>71.2</td>
<td>61.25</td>
<td>.24</td>
</tr>
<tr>
<td>range</td>
<td>26.4–84.4</td>
<td>26.4–84.4</td>
<td>50.4–64.3</td>
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<tr>
<td>WBC index Low</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>.80</td>
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<tr>
<td>Intermediate</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td></td>
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<tr>
<td>High</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Karyotype t(8;21)</td>
<td>16</td>
<td>5</td>
<td>2</td>
<td>&lt;.50</td>
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<tr>
<td>only t(8;21)</td>
<td>7</td>
<td>5</td>
<td>2</td>
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<tr>
<td>-X or -Y</td>
<td>8</td>
<td>7</td>
<td>1</td>
<td></td>
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<td>1</td>
<td></td>
</tr>
<tr>
<td>inv(16)</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td></td>
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<tr>
<td>only inv(16)</td>
<td>2</td>
<td>2</td>
<td>0</td>
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<td>trisomy 22</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>CD56 +</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>&lt;.67</td>
</tr>
<tr>
<td>–</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td></td>
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<tr>
<td>Extramedullary</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>involvement Cumulative AC (g/m²)</td>
<td>48 (12.114)</td>
<td>48 (12.78)</td>
<td>60 (30.114)</td>
<td>1.3</td>
</tr>
</tbody>
</table>

ASCT indicates autologous stem cell transplantation; WBC, white blood cell; Hb, hemoglobin; plt, platelet; LDH, lactate dehydrogenase; PB, peripheral blood; BM, bone marrow; cumulative AC, the actual cumulative dosage of cytarabine exceeding 1 g/m²; WBC index, WBC x [% of marrow blast]; low index <2.5, intermediate index between 2.5 and 20; high index 20 or more.

P-value was calculated by x² exact test(*) or U exact test of Mann Whitney†. P < .05 was considered as significant value. ‡ CD56 expression was investigated in 12 of total 18 patients.

### Table 2. Clinical Features of Patients with ASCT

<table>
<thead>
<tr>
<th></th>
<th>Total cases</th>
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</thead>
<tbody>
<tr>
<td>Conditioning regimen Bu+VP16+AraC</td>
<td>14 cases</td>
</tr>
<tr>
<td>Median time to stem cell transplantation</td>
<td>273 days (167–375)</td>
</tr>
<tr>
<td>Median number of prior chemotherapy sessions</td>
<td>5 times (3–8)</td>
</tr>
<tr>
<td>Median number of cycle of PBSCH</td>
<td>2 times (1–3)</td>
</tr>
<tr>
<td>Median number of infused CD34 positive cells</td>
<td>2.6 \times 10^9/kg (1.5–4.4)</td>
</tr>
<tr>
<td>Recovery of neutrophil count (&gt;0.5 \times 10^9/L)</td>
<td>13 days (11–36)</td>
</tr>
<tr>
<td>Recovery of platelet count (&gt;20 \times 10^9/L)</td>
<td>27.5 days (12–217)</td>
</tr>
<tr>
<td>Recovery of platelet count (&gt;50 \times 10^9/L)</td>
<td>123 days (15–378)</td>
</tr>
<tr>
<td>Median observation duration after transplantation</td>
<td>4.3 years (1.3–8.9)</td>
</tr>
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</table>

Late adverse events

<table>
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<tr>
<th>Event</th>
<th>Frequency</th>
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</thead>
<tbody>
<tr>
<td>MDS/sAML</td>
<td>1 case</td>
</tr>
<tr>
<td>CBF-AML</td>
<td>5 cases</td>
</tr>
<tr>
<td>pneumonia</td>
<td>1 case</td>
</tr>
<tr>
<td>meningitis</td>
<td>1 case</td>
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</table>

PBSCH indicates harvest of peripheral blood stem cell; MDS/sAML, myelodysplastic syndrome/secondary acute myeloid leukemia.

* Ranges are shown in parentheses.
PCR sample was obtained just before the start of each postremission chemotherapy and conditioning of ASCT; detectable by nested PCR.

Table 4. Time Course of Minimal Residual Disease (MRD) among Those with Autologous Stem Cell Transplantation (ASCT)

<table>
<thead>
<tr>
<th>Pt</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
<th>7th</th>
<th>Actually infused CD34 Cell Dose</th>
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<tr>
<td>1</td>
<td>1.0 x 10⁶/kg</td>
<td>1.9 x 10⁹/kg</td>
<td>0.28 x 10⁹/kg</td>
<td>2.9 x 10⁹/kg</td>
<td>1.6 x 10⁹/kg</td>
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<tr>
<td>2</td>
<td>0.4 x 10⁹/kg</td>
<td>2.9 x 10⁹/kg</td>
<td>1.5 x 10⁹/kg</td>
<td>2.2 x 10⁹/kg</td>
<td>1.5 x 10⁹/kg</td>
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<tr>
<td>3</td>
<td>2.4 x 10⁹/kg</td>
<td>0.62 x 10⁹/kg</td>
<td>3.7 x 10⁹/kg</td>
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<tr>
<td>4</td>
<td>0.12 x 10⁹/kg</td>
<td>2.0 x 10⁹/kg</td>
<td>3.5 x 10⁹/kg</td>
<td>4.4 x 10⁹/kg</td>
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<tr>
<td>5</td>
<td>0.37 x 10⁹/kg</td>
<td>0.88 x 10⁹/kg</td>
<td>0.15 x 10⁹/kg</td>
<td>1.6 x 10⁹/kg</td>
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<td>6</td>
<td>2.4 x 10⁹/kg</td>
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<tr>
<td>8</td>
<td>0.37 x 10⁹/kg</td>
<td>1.3 x 10⁹/kg</td>
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<tr>
<td>9</td>
<td>0.37 x 10⁹/kg</td>
<td>1.3 x 10⁹/kg</td>
<td>2.0 x 10⁹/kg</td>
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Table 3. Details of Timings and Doses of CD34 Positive Cells in Peripheral Blood Stem Cell Collections

<table>
<thead>
<tr>
<th>CD34 Doses/days of Collection in Each Cycle</th>
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<tbody>
<tr>
<td>Post-remission chemotherapy</td>
</tr>
</tbody>
</table>

Table 4. Time Course of Minimal Residual Disease (MRD) among Those with Autologous Stem Cell Transplantation (ASCT)

<table>
<thead>
<tr>
<th>Pt</th>
<th>AML type</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
<th>7th</th>
<th>After ASCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>t(8;21)</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>1 year</td>
</tr>
<tr>
<td>2</td>
<td>t(8;21)</td>
<td>NA</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>2 year</td>
</tr>
<tr>
<td>3</td>
<td>t(8;21)</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>3 year</td>
</tr>
<tr>
<td>4</td>
<td>t(8;21)</td>
<td>NA</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>4 year</td>
</tr>
<tr>
<td>5</td>
<td>t(8;21)</td>
<td>NA</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>t(8;21)</td>
<td>NA</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>t(8;21)</td>
<td>NA</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>t(8;21)</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>t(8;21)</td>
<td>NA*</td>
<td>NA*</td>
<td>NA*</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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</tr>
<tr>
<td>10</td>
<td>t(8;21)</td>
<td>NA</td>
<td>NA*</td>
<td>NA*</td>
<td>—</td>
<td>—</td>
<td>N.A*</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>t(8;21)</td>
<td>NA</td>
<td>NA*</td>
<td>NA*</td>
<td>—</td>
<td>—</td>
<td>N.A*</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>t(8;21)</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>inv(16)</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>inv(16)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td></td>
</tr>
</tbody>
</table>

Table 3 indicates patient number; (X) means that MRD was detected in collected peripheral blood stem cells.

were performed for median 2 days (range: 1–4 days) (Table 3).

The PBSC in 3 cycles (patients 5 and 6) were inappropriate as grafts because MRD was detected by nested RT-PCR, and the PBSC were therefore discarded. For collection of PBSC with undetectable MRD, 1 cycle of PBSC collection was sufficient for 7 patients, and 2 cycles were needed in 5 patients (Table 3). In the remaining 2 patients, 3 cycles were performed but no more than 2.0 x 10⁹/kg CD34-positive cells could be collected.

MRD in BM was assessed by RT-PCR before chemotherapy in each of 20 cycles (Table 4). MRD remained in BM in 18 of the investigated 20 cycles. However, PBSC with undetectable MRD were collected in 15 of the 18 cycles. Finally, all 14 patients received PBSC with undetectable MRD.

MRD Monitoring of ASCT Patients

Table 4 shows the time courses of MRD and the timing of PBSC collections among ASCT patients. All of the patients sustained complete remission after ASCT, and MRD in BM remained at <100 copies/μgRNA in all 14 patients and became undetectable by RT-PCR in 12 patients (patients 1-3 and 6-14). MRD in BM became undetectable by RT-PCR after ASCT in 8 patients (1-3, 6, 8, 11, 13, and 14) of 10 patients (patients 1-6, 8, 11, 13, and 14) whose MRD in BM before ASCT was detectable by RT-PCR.
Interestingly, in patients 2 and 6, molecular disappearance of AML1/ETO(MTG8) was confirmed by RT-PCR 2 and 3 years after each ASCT, respectively (Table 4). In the other 6 patients [patients 1, 3, 8, 11, 13, and 14] MRD was undetectable by RT-PCR 1 year after ASCT.

**Analysis of c-KIT Mutations and MRD**

c-KIT mutations were analyzed in 3 patients (patients 1-3). Pt.2 had c-KIT mutations on exon 17, and the others had no mutations (Table 5). All 3 patients remained in CR1. AML1/MTG8 chimera was undetectable by RQ-PCR in all 3 patients.

**DISCUSSION**

We analyzed the survival of 18 consecutive young patients with CBF-AML treated between 1997 and 2006 at our center, and revealed that EFS of ASCT with a PCR-negative graft was 93% with no incidence of relapse.

Neutrophils recovered promptly within 2 weeks, but platelets tended to recover more slowly, although severe hemorrhage was not a complication. After ASCT, half of the patients suffered late infections, especially VZV reactivation, at a median of 4 months after ASCT. Prolonged prophylaxis with acyclovir is reportedly effective for prevention of VZV reactivation [24]. The high rate of VZV reactivation in the present series may have been because of the short duration of prophylaxis.

Until more than $2.0 \times 10^7$/kg CD34-positive cells were collected, 1 cycle of PBSC collection was sufficient for half of the patients and 3 cycles were necessary for only 2. This number of collections seems average in comparison with other studies (median 2 times) [25,26]. Although MRD remained in the BM in most patients, a PCR-negative graft was obtainable, except in 3 cycles. This was consistent with other studies [27,28] in which MRD was observed less frequently in PBSC than in BM. Also in an animal model, leukemic contamination was reportedly not enhanced by G-CSF mobilization, and a different mechanism for mobilization of leukemic cells into peripheral blood was suggested [29].

Previous studies of ASCT in CBF-AML have indicated a survival of about 45% to 66% [8-15], which was not superior to that achieved with chemotherapy alone [8,9]. Our present result was excellent in comparison with previous studies. This may have been because of the characteristics of the patients; our series might include only patients without adverse factors [8-15] or c-KIT mutations [16]. In fact, however, those with ASCT had at least 1 adverse prognostic factor other than non-White race (Table 1), including a case showing c-KIT mutation of exon 17 (Table 5), which is associated with a high rate of relapse, although the number of cases analyzed was too small to allow any conclusion to be drawn. The main reason for the good outcome in our series was probably because our ASCT strategy was based on MRD in PBSC (ASCT with a PCR-negative graft). A gene-marking study has suggested that relapse after autologous bone marrow transplantation originates from the graft [30]. In addition, graft contamination of leukemic cells is associated with rapid relapse and poor prognosis [31]. We employed grafts in which absence of MRD was confirmed by nested RT-PCR, and this would have contributed to the good outcome.

As our analysis was retrospective and involved a very small population, it might have included variable bias. Although the present study included truly consecutive patients, there might have been an institutional bias because of the discrepancy in the number of patients between t(8;21)-AML and inv(16)-AML and because only 2 patients as yearly average were referred as having CBF-AML in our institution. In addition, in general, ASCT was used for patients with good performance status and good control of leukemia, which would have contributed to the good outcome. However, the actual OS and EFS for ASCT with a PCR-negative graft were surprisingly good in our series (100% and 93%, respectively), and EFS for patients overall exceeded 80%. Therefore, a prospective trial will be needed to investigate further confirmation of ASCT with a PCR-negative graft.

MRD in BM before ASCT was detectable in 10 of the 12 investigated patients. In 8 of these, MRD in BM became undetectable by RT-PCR after ASCT. Interestingly, in patients 2 and 6 (Table 4), the AML1/ETO(MTG8) fusion transcript disappeared 2 and 3 years after each ASCT, respectively, without further therapy. Although the reason for the late disappearance of MRD was unclear, 1 possibility was an enhanced and reconstructed immune response after ASCT. The myeloablative conditioning regimen would also have eradicated leukemic stem cells with
self-renewal potential, so that the latent AML1/ETO fusion transcript may have been correlated with daughter leukemic cells without self-renewal potential [27].

The significance of MRD in CBF-AML has not yet been precisely evaluated because of the persistence of AML1/ETO and CBFβ/MYH11 in long survivors [13,32,33]. However, a lower frequency of gene fusion, especially undetectable MRD, is reportedly associated with long relapse-free survival (RFS) [13,34-36]. Therefore, our results suggest that ASCT with a graft that is PCR-negative for CBF-AML could be indicated not only for patients with adverse factors but also those with persistent MRD detectable by RQ-PCR after postremission therapy.

In conclusion, we have analyzed a series of consecutive CBF-AML patients, and found that those with ASCT had excellent EFS. Even if MRD was detectable in BM, it was possible to harvest a PCR-negative graft. Our ASCT strategy was based on graft MRD, and this was thought to have contributed to the excellent EFS and overcome other adverse factors. A large trial of ASCT with a PCR-negative graft is warranted for CBF-AML, especially in patients with adverse factors or with remnant MRD in BM after postremission therapy.

CONFLICT OF INTEREST

The authors report no potential conflicts of interest.

REFERENCES

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survival compared with bone marrow cells. Bone Marrow Transplant. 1999;24:467-472.


