Second Stem Cell Transplantation in Patients with Fanconi Anemia Using Antithymocyte Globulin Alone for Conditioning

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ABSTRACT
Despite the promising data on the outcome of allogeneic stem cell transplantation (SCT) in patients with Fanconi anemia (FA), a certain percentage of these patients still experience graft failure; some of these patients undergo second transplants, but the existing data on the outcome of the second SCT in FA patients are scarce, with no long-term follow-up provided in many of the publications addressing this issue. This is a review of our experience in 4 such patients who underwent second stem cell transplants using rabbit ATG only for conditioning. Three engrafted promptly and are alive and free of disease at 25, 23, and 21 months, respectively. We conclude, therefore, that the use of ATG alone for conditioning before a second SCT may offer a chance of long-term disease-free survival for FA patients who fail their first transplant.

INTRODUCTION
Presently, allogeneic stem cell transplantation (SCT) is the only known curative modality for bone marrow failure in patients with Fanconi anemia (FA) [1-10]; thus, when graft failure occurs after SCT, the prognosis is extremely grim, as the salvage options are limited. The use of growth factors (granulocyte-colony stimulating factor [G-CSF] or granulocyte macrophage colony stimulating factor [GM-CSF]) to enhance hematopoiesis after graft failure in general has been associated with variable degrees of success, but these cytokines are lineage specific and thus have no effect on the recovery of the red blood cells or the platelets [11,12]. Second transplantation may be considered in these patients, but extreme care must be exercised, as FA cells are known to be exceptionally sensitive to chemotherapy and radiation therapy; therefore, further cytotoxic conditioning may be detrimental. We report here our own experience in 4 patients who underwent second SCT after conditioning only with ATG and a review of the available literature on this subject.

PATIENTS AND METHODS
Using a conditioning regimen of cyclophosphamide 60 mg/kg in addition to rabbit ATG (Fresenius, Waltham, MA), 38 FA patients underwent matched related SCT at King Faisal Specialist Hospital and Research Center (KFSHRC) (35 from fully HLA-matched siblings, 2 from fully HLA-matched fathers, and 1 from a 1-antigen mismatched brother on the A locus) during the period from April 1999 through May 2007. All patients had received 12 or fewer blood product transfusions and had had no other therapy prior to SCT. Five (13%) of these patients experienced graft failure: 1 was primary, and 4 were secondary; all 5 underwent second SCT using bone marrows harvested from their same respective donors. One of the 5 patients (her donor was a fully HLA-matched father) was retransplanted 1.5 years after the 1st SCT using cyclophosphamide 20 mg/kg, ATG, and thoracoabdominal irradiation (TAI); she is currently alive with no evidence of disease 2.5 years after the second SCT, but is not included in this analysis. The remaining
Table 1. Patients’ Chimerism Results before and after the Second SCT

<table>
<thead>
<tr>
<th>Patient UPN</th>
<th>STR (% of donor cells lymphoid/Myeloid) before the 2nd SCT</th>
<th>Most Recent STR (% of Donor Cells Lymphoid/Myeloid) after the 2nd SCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1497</td>
<td>82%/67%</td>
<td>70%/100%</td>
</tr>
<tr>
<td>1635</td>
<td>83%/68%</td>
<td>100%/100%</td>
</tr>
<tr>
<td>1557</td>
<td>56%/78%</td>
<td>63%/99%</td>
</tr>
<tr>
<td>1870</td>
<td>6%/88%</td>
<td>N/A</td>
</tr>
</tbody>
</table>

SCT indicates stem cell transplantation.

4 patients (donors for all 4 patients were matched-related siblings) received uniform conditioning consisting of only rabbit ATG (Fresenius) 10 mg/kg/day i.v. for 3 days given on days $-4, -3, -2$ with cyclosporine (CSA) as GVHD prophylaxis, and they are the subject of this report.

Although the chimerism analysis (done by STR) before the second SCT on all 4 patients showed different degrees of mixed chimerism (Table 1), patients were considered to have graft failure because all 4 were blood and platelet transfusion dependent after the first transplant, and even though 1 patient was maintaining an absolute neutrophil count (ANC) around $500 \times 10^6/L$, the other 3 patients persistently had ANCs below $200 \times 10^6/L$ and were unresponsive to G-CSF therapy. The bone marrow examinations on all patients showed severe hypocellularity with no evidence of engraftment 1-2 weeks before the second SCT.

RESULTS

Three of the 4 patients engrafted promptly; the details of the second transplant are delineated in Tables 2 and 3. The conditioning was well tolerated. One patient (UPN 1635) had evidence of Mucor infection of the skin of her left arm before the second SCT; she had been started on antifungal therapy and her infection resolved fully after the second SCT. Another patient (UPN 1870) had sepsis after the first SCT and was continuously febrile; he also had pulmonary fungal infection, and despite the second SCT and support with G-CSF, his status continued to deteriorate and he died 41 days after the second SCT with no evidence of engraftment. GVHD did not develop in any patient.

DISCUSSION

Various definitions of graft failure exist in the literature. On a practical level, graft failure is defined as failure to achieve or maintain a peripheral blood neutrophil count of $500 \times 10^6/L$ after SCT. However, with the advent of more sophisticated technology, graft failure is viewed now as the inability to attain or sustain a certain level of donor cell engraftment. Thus, in practicality, what sometimes is considered a graft failure may actually reflect graft insufficiency, making the comparison of outcome in the different studies sometimes difficult [11].

After allogeneic SCT, the rate of graft failure is estimated to occur in around 5% of the cases [11,13], but higher rates have been reported in certain diseases such as severe aplastic anemia [14]. In FA patients undergoing allogeneic SCT, the incidence of graft failure varies from 1 study to another. Socié et al. [4] reported a total of 4 graft failures (3 primary and 1 secondary) in 49 evaluable patients who underwent matched-related SCT (8%); the conditioning consisted of CY 20 mg/kg, and in thoracoabdominal irradiation (TAI) at 500 cGy, all 4 patients were retransplanted; 1 remains alive and well 1.2 years at the time of the report. Farzin et al. [5] used cyclophosphamide 20 mg/kg and TAI at 400 cGy in addition to ATG; 2 of 30 patients transplanted for marrow aplasia (7%) developed graft failure (1 primary and 1 secondary). We used the same regimen of cyclophosphamide/TAI/ATG at our institution on 22 FA patients, all receiving matched-related SCT, and 2 patients (9%) developed delayed graft failure and died, 1 of sepsis and 1 after progression to AML [10].

Eliminating radiation therapy from the conditioning of patients with acquired aplastic anemia undergoing allogeneic SCT has been associated with increased graft failure [14,15], and here we report a graft failure rate of 13% in FA patients after conditioning with a nonradiation containing regimen (cyclophosphamide 60 mg/kg and ATG only). Similarly, Bonﬁn et al. [2] reported a graft failure rate of 12% in 42 FA patients conditioned with cyclophosphamide 60 mg/kg alone without ATG or radiation (5 graft failures: 1 primary and 4 secondary).

Fludarabine has been used increasingly in conditioning FA patients in an effort to reduce the dose and therefore the toxicity of cyclophosphamide normally.

Table 2. Types of Graft Failure and Engraftment Details of the Patients at the Second SCT

<table>
<thead>
<tr>
<th>Patient UPN</th>
<th>Type of Failure</th>
<th>CD34 Dose 1st SCT ($10^6$/kg)</th>
<th>CD34 dose 2nd SCT ($10^6$/kg)</th>
<th>Time to Graft Failure (Months)</th>
<th>Time from 1st SCT to 2nd SCT (Months)</th>
<th>Days to ANC &gt;0.5 $\times 10^9/L$</th>
<th>Days to Platelets &gt;20 $\times 10^9/L$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1497</td>
<td>Secondary</td>
<td>7</td>
<td>6.0</td>
<td>7</td>
<td>10.3</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td>1635</td>
<td>Primary</td>
<td>6</td>
<td>6.3</td>
<td>N/A</td>
<td>1.25</td>
<td>17</td>
<td>32</td>
</tr>
<tr>
<td>1557</td>
<td>Secondary</td>
<td>3</td>
<td>2.7</td>
<td>3</td>
<td>9.4</td>
<td>N/A</td>
<td>13</td>
</tr>
<tr>
<td>1870</td>
<td>Secondary</td>
<td>12</td>
<td>11</td>
<td>One week</td>
<td>1.5</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

SCT indicates stem cell transplantation.
used in the conditioning of FA patients [3,6]; it has been shown to be associated with better engraftment and improved survival both in the unrelated cord blood transplant and in the unrelated donor SCT settings [16,17].

Many factors may increase the rate of rejection after allogeneic SCT, including repeated blood products transfusions before SCT and a low dose of stem cells [11,15,18,19]. We and others have additionally observed that the presence of myelodysplasia or clonal abnormalities in FA patients pre-SCT may be an adverse risk factor, predisposing patients to graft failure [8]. In the series reported by Bonfim et al. [2], 4 of the 5 patients with graft failure had marrow dysplasia and/or abnormal cytogenetic analysis prior to SCT. Furthermore, the 1 graft failure that occurred in the series reported by Bitan et al. [6] was in a patient with MDS features and 18% blasts prior to SCT, and 1 of the 2 graft failures in the report by Tan et al. [3] was in a patient with myelodysplasia and abnormal cytogenetic studies before SCT. At our institution, all FA patients with myelodysplasia and/or clonal abnormality pre-SCT receive total-body irradiation (TBI) as part of the conditioning regimen, and we recently reported our experience in 11 such patients who received conditioning with cyclophosphamide 20 mg/kg and TBI at 450 cGy; no graft failures were noted at a median follow-up of 4 years [8].

The outcome of the second SCT is generally poor and depends on multiple factors, and the results are even more dismal in malignant disorders [11,20-22]. Guardiola et al. [21] reported on 82 consecutive second SCT in patients with acute leukemia, aplastic anemia, or chronic myeloid leukemia; the 100-day transplant-related mortality (TRM) was 53%, and the 3-year overall survival (OS) was 30%. Only 1 of the 4 patients transplanted for malignancies survived. Others have also corroborated these findings [13,15,20,22]. Guardiola et al. [21] also showed that a longer interval between the 2 transplants (>80 days) was associated with a remarkably lower 100-day TRM. Therefore, second allogeneic SCT may be a viable option for many FA patients with graft failure, particularly if enough time has elapsed since the first SCT, but further cytotoxic conditioning for these patients may be harmful in light of the increased sensitivity that FA patients demonstrate to chemotherapy and radiation therapy. The data on the toxicity patterns in FA patients following a second SCT are sketchy and scarce. Bonfim et al. [2] used a combination of cyclophosphamide/fludarabine or cyclophosphamide alone on 4 FA patients undergoing a 2nd SCT; 2 had a successful outcome, 1 died, and 1 required a 3rd SCT and was reported well 33 months after the first SCT. Tan et al. [3] performed a second SCT on 1 patient on day 33 after the first SCT using cyclophosphamide and TBI, but he succumbed to progressive disease (MDS). Bitan et al. [6] used a combination of busulfan/fludarabine/alemtuzumab to successfully condition 1 patient for the second SCT.

The use of anti-T cell serotherapy (ATG) alone in the conditioning for the second SCT is not novel [19], but this has not been widely studied in FA patients. Farzin et al. [5] successfully used rabbit ATG alone before the infusion of marrow cells in 1 patient who failed to engraft 27 days after matched sibling cord blood transplantation; the same sibling was used as the source of marrow cells.

Evidently, there are various approaches to condition FA patients for second SCT, and the outcome appears to be better in those transplanted for secondary graft failure [15]. In our series, the use of ATG alone has led to a successful outcome in 3 out of the 4 patients who underwent the procedure; 2 of them had the second SCT because of secondary graft failure. Despite the normalization of peripheral cell blood counts in our 3 patients, the short tandem repeats (STR) analysis continued to show mixed chimerism in 2 of them (Table 1). Because full donor chimerism is preferable in allografting FA patients to obviate later hematologic risks of leukemia in the residual host cells, it may be beneficial in some patients to add other agents such as fludarabine to the ATG regimen to ensure complete donor chimerism.

### REFERENCES

2. Bonfim CM, de Medeiros CR, Bitencourt MA, et al. HLA-matched related donor hematopoietic cell transplantation in 43 patients with Fanconi anemia conditioned with 60 mg/kg of

### Table 3. Length of Follow-up of Patients after Second SCT and Their Status at Last Contact

<table>
<thead>
<tr>
<th>Patient UPN</th>
<th>Follow-up after 2nd (Months)</th>
<th>ANC at Last Contact</th>
<th>Hb at Contact</th>
<th>Platelets at Last Contact</th>
<th>Status upon Last Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1497</td>
<td>25</td>
<td>$1.07 \times 10^9$/L</td>
<td>142 g/L</td>
<td>$226 \times 10^9$/L</td>
<td>Alive, with no evidence of disease</td>
</tr>
<tr>
<td>1635</td>
<td>23</td>
<td>$2.23 \times 10^9$/L</td>
<td>142 g/L</td>
<td>$208 \times 10^9$/L</td>
<td>Alive, with no evidence of disease</td>
</tr>
<tr>
<td>1557</td>
<td>21</td>
<td>$7.52 \times 10^9$/L</td>
<td>137 g/L</td>
<td>$220 \times 10^9$/L</td>
<td>Alive, with no evidence of disease</td>
</tr>
<tr>
<td>1870</td>
<td>1</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>Failed, deceased</td>
</tr>
</tbody>
</table>

ANC indicates absolute neutrophil count; SCT, stem cell transplantation.


