Bone Marrow Transplantation for Constitutional Pure Red Cell Aplasia

By Carl Lenarsky, Kenneth Weinberg, Eva Guinan, Peter P. Dukes, Yigal Barak, Jorge Ortega, Stuart Siegel, Kenneth Williams, Jack Lazerson, Howard Weinstein, and Robertson Parkman

Constitutional pure red cell aplasia (CPRCA) is a syndrome of failed erythropoiesis usually diagnosed within the first year of life. Four patients with CPRCA received transplants with marrow from their HLA-identical, mixed lymphocyte culture-nonreactive siblings. All patients were resistant to corticosteroid therapy and were dependent on regular red cell transfusions for at least 5 years. Three patients were conditioned with procarbazine, antithymocyte globulin, cyclophosphamide, and busulfan, and one was conditioned with antithymocyte serum, cyclophosphamide, and busulfan. Three patients promptly had successful engraftments with establishment of donor hematopoiesis. One patient initially rejected his graft but received a successful retransplant. All patients are currently alive with Karnofsky performance scores of 100 and normal erythropoiesis of donor origin. Despite a history of multiple transfusions, bone marrow transplantation is a potentially curative therapy for patients with CPRCA.

Bone marrow transplantation (BMT) is the treatment of choice for patients with a variety of hematologic, immunologic, and oncological disorders. The utilization of BMT for diseases of the erythroid compartment has been limited. The balance between the risks of long-term transfusion therapy and BMT have tended to favor the former. However, recent encouraging results with BMT for patients with thalassemia major have led to a renewed interest in BMT for patients with erythroid disorders.

It is preferable to perform BMT before patients have received multiple red cell transfusions to avoid the problems associated with sensitization to minor histocompatibility antigens as well as organ damage secondary to iron deposition. However, patients with erythroid disorders who have been repeatedly transfused might still potentially benefit from BMT.

We have treated four patients with constitutional pure red cell aplasia (CPRCA) with allogeneic BMT. CPRCA is a syndrome of failed erythropoiesis, with the onset of anemia usually within the first year of life. The cumulative evidence indicates that the anemia is due to a defective erythroid stem cell, thereby suggesting the possibility of permanent correction with BMT. All 14 Iriondo et al have reported one child with CPRCA who was alive with normal hematopoiesis 650 days after allogeneic BMT.

All four patients in our study were dependent on regular red cell transfusions for at least 5 years and were resistant to corticosteroid therapy. To achieve sustained hematopoietic engraftment in the multiply transfused immunocompetent host, transplant conditioning must achieve both adequate immunosuppression and the ablation of host hematopoiesis. We sought to determine whether an intensive conditioning regimen would be safe and efficacious in multiply transfused patients.

MATERIALS AND METHODS

The diagnosis of CPRCA was established in all four patients by the presence of a transfusion-dependent anemia starting in infancy that was associated with reticulocytopenia and an absence or decrease in erythroid precursors upon morphological examination of bone marrow. In addition, the number of erythroid progenitors present in peripheral blood and bone marrow demonstrable by culture in a methylcellulose-containing medium was low to nondetectable (progenitor assays were not performed on patient no. 4). Patient no. 3 had a syndrome of CPRCA associated with unusual physical anomalies and a history of exposure to anagyrine in utero. This case has been previously reported. All patients were referred for BMT after a minimum of 5 years of requirement for red cell transfusions (Table 1). All donors were HLA-identical, mixed lymphocyte culture (MLC) nonreactive siblings. The lymphocytes of patients no. 1 and 2 were not sensitized to non-MHC antigens of their donors as determined by antibody-independent, cell-mediated lysis (CML) assays. CML assays were not performed on patients no. 3 or 4.

Cytogenetics (karyotype with banding) was performed on donor mitogen-stimulated peripheral blood lymphocytes before BMT. Similar studies were performed on recipient peripheral blood lymphocytes as well as unstimulated bone marrow cells before and after BMT. Erythroid progenitor assays of peripheral blood were performed on patients no. 1, 2, and 3 post-BMT (Table 3).

The conditioning regimen used for patients no. 1, 2, and 3 was as follows: horse antithymocyte globulin, 20 mg/kg/d on days 9, 7, 5, and 3; busulfan, 3.0 to 3.5 mg/kg/d on days 9, 8, 7, and 6; procarbazine, 12.5 mg/kg/d on days 8, 6, 4, and 2; and cyclophosphamide, 50 mg/kg/d on days 5, 4, 3, and 2. Patient no. 4 received antithymocyte serum, 0.2 mL/kg/d on days 9 and 7; busulfan, 3 mg/kg/d on days 9, 8, 7, and 6; and cyclophosphamide, 50 mg/kg/d on days 5, 4, 3, and 2.

Bone marrow was harvested and infused as previously described. The total number of nucleated marrow cells infused was a minimum of $3 \times 10^7$/kg recipient body weight.

For graft-vs-host disease (GVHD) prophylaxis, patients 1, 2, and 3 initially received methotrexate, 10 mg/m² intravenously on days +3, +6, +11, and +18 post-BMT. Patient 4 received the same dose of methotrexate on day +1, +3, +6, and +11. GVHD was graded according to previously published criteria. Patient performance...
status was assessed by using the Karnofsky performance scale. Informed consent was obtained for all patients.

RESULTS

The clinical course and outcome for all patients is summarized in Table 2. Patients no. 1, 2, and 4 had restoration of normal hematopoiesis of donor origin within 2 months. Patient 3 had evidence of donor hematopoiesis within 1 month post-BMT but rejected his graft with a return to recipient hematopoiesis 2 months post-BMT. He received a transplant a second time with a similar four-drug regimen plus 3-Gy total lymphoid irradiation; no methotrexate was administered after he received his second transplant. He had sustained hematopoietic engraftment of donor origin within 6 weeks of the second transplant.

Table 3 provides data documenting the establishment of donor hematopoiesis post-BMT. For patients no. 1, 3, and 4 the uniform presence of donor-type sex chromosomes in marrow cells post-BMT demonstrated allogeneic engraftment; the presence of a Q band chromosome polymorphism of the donor type post-BMT demonstrated engraftment in patient no. 3. Further evidence of functional donor hematopoiesis post-BMT was provided by the presence of normal numbers of erythroid progenitors in the peripheral blood of patients no. 1, 2, and 3.

All patients are alive 520 to 1,320 days post-BMT. Grade II acute GVHD was observed in all four patients. Patients no. 1 and 3 had mild to moderate chronic GVHD requiring prolonged immunosuppressive therapy; the chronic GVHD has resolved, and treatment with all immunosuppressive agents has been discontinued. Karnofsky performance status is 100 for all patients.

DISCUSSION

There are two prerequisites for successful BMT for diseases whose expression is restricted to hematopoietic cells. The defective hematopoietic stem cell population must be sufficiently eradicated, and there must be adequate immunosuppression of the potentially sensitized recipient immune system. For hematopoietic stem cell ablation, busulfan and dimethylbusulfan have been most frequently used in BMT for genetic diseases. For untransfused patients, cyclophosphamide is the best single agent for adequate immunosuppression. Although total-body irradiation may be an effective modality for assisting in stem cell ablation and immunosuppression, we wished to avoid the potential side effects of irradiation in young patients. Because our patients were heavily transfused, antithymocyte globulin and procarbazine were added for further immunosuppression (except for patient no. 4, who received no procarbazine).

Our series of four patients confirms the efficacy of BMT for CPRCA. The establishment of donor hematopoiesis was documented by cytogenetic analysis of bone marrow cells as well as the presence of normal numbers of peripheral blood erythroid progenitors post-BMT. Several hematopoietic disorders have been successfully treated by BMT. For some diseases the risk of severe morbidity and mortality early in life is high, and the indication for BMT is clear. For diseases where the primary defect is limited to erythroid differentiation and/or function, chronic red cell transfusion has been the only means to avert early morbidity. Nevertheless, many risks are associated with chronic red cell transfusions, including hemosiderosis, transmission of infectious agents, and isoimmunization to both cellular and plasma antigens. Iron

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age at Diagnosis</th>
<th>Age at Transplant</th>
<th>Response to Steroids</th>
<th>Approximate No. Previous Transfusions</th>
<th>Cardiac Function</th>
<th>Liver Function</th>
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<tr>
<td>1</td>
<td>1</td>
<td>5½</td>
<td>No response</td>
<td>100</td>
<td>Mildly reduced left ventricular function</td>
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<td>2</td>
<td>6</td>
<td>16</td>
<td>Steroid-dependent remission until age 9; no response thereafter</td>
<td>150</td>
<td>Mildly reduced left ventricular function</td>
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<tr>
<td>3</td>
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<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>5</td>
<td>No response</td>
<td>100</td>
<td>Mildly reduced left ventricular function</td>
<td>Normal (biopsy specimen with moderate periportal fibrosis, mild hemosiderosis)</td>
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</table>

Table 2. Clinical Outcome

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Days to Engraftment*</th>
<th>WBC</th>
<th>RBC</th>
<th>Platelet</th>
<th>GVHD Acute/Chronic</th>
<th>Days Post-BMT</th>
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<td>13</td>
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<td>19</td>
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<td>++/+/- Resolved</td>
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<td>100</td>
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</table>

*Engraftment is defined as follows: WBC, absolute neutrophil count greater than 500/µL for three consecutive days; RBC, last required red cell transfusion; Platelet, last required platelet transfusion.
chelation therapy has the potential to prevent hemosiderosis, but its long-term efficacy and safety are not established. The major drawbacks to chelation are the requirement for strict compliance as well as the cost, which varies between $5,000 and $20,000 per patient per year.\(^2\) Theoretically, patients dependent on red cell transfusions should have a better chance of a successful outcome with BMT if it is performed before they have developed complications. Thomas et al hypothesized that, for the best chance of success in patients with thalassemia, BMT should be done before the patient has been multiply transfused so that iron toxicity is unlikely and the risk of graft rejection is low.\(^1\) Some centers have reported experience with BMT for these “early” thalassemic patients and success rates of 70% to 80%.\(^2\)\(^9\)\(^\text{29}\)\(^\text{30}\) Recently, Lucarelli et al have reported 40 patients with thalassemia from late childhood to early adolescence who underwent BMT and have a probability of disease-free survival of 69%.\(^4\)

In contrast to thalassemia where dependence on red cell transfusions is unremitting for all patients, patients with CPRCA have the potential for a more variable course. BMT may not be a reasonable alternative for CPRCA patients at diagnosis. For those patients who remain dependent on transfusions or on chronic high-dose corticosteroid therapy, BMT is a reasonable treatment alternative. Our results demonstrate that a decision to perform BMT for patients with CPRCA can be safely delayed until the patient has demonstrated a requirement for red cell transfusions for 5 years.

Although the requirement for RBC transfusions can be eliminated with a successful marrow transplant, the impact of BMT on the long-term survival for patients with CPRCA or other erythroid disorders is unknown. It remains to be determined whether the iron accumulated in vital organs as a result of prior transfusions can be mobilized and excreted. The transplant studies by Weiden et al in dogs with congenital hemolytic anemia secondary to pyruvate kinase deficiency suggest that abnormal liver accumulations of iron are excreted after BMT, although the dogs in the study were not transfused before receiving the transplants.\(^3\) Politi et al have reported preliminary evidence that iron overload in patients with thalassemia might be gradually reduced after BMT.\(^3\)\(^2\) Post-BMT chelation therapy or phlebotomy may be indicated to hasten the removal of iron.

In summary, BMT is a potentially curative therapy for patients with CPRCA despite a history of prolonged red cell transfusions. These results suggest that patients with disorders of erythropoiesis who have been multiply transfused should be considered for BMT.

### REFERENCES

13. Dukes PP, Ma A, Clemons GK, Meytes D: Measurement of

### Table 3. Laboratory Evidence of Donor Hematopoiesis Post-BMT

<table>
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<tr>
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<td>Bone Marrow</td>
<td>Peripheral Blood</td>
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<td>4 F</td>
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<td>Not Done</td>
<td>Not Done</td>
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*Normal values, median (range)/10⁶ nucleated cells: peripheral blood BFU-E, 17 (2 to 38); bone marrow BFU-E, 81 (37 to 134).
†Karyotype/banding analysis pre-BMT revealed the presence of a distinct polymorphic variant appearing on both no. 3 donor chromosomes but not recipient ones. This polymorphic variant was present in 100% of recipient marrow cells post-BMT.
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